

Distribution and Characterization of Herbicide-Resistant Italian ryegrass and
Palmer amaranth in Virginia

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ABSTRACT

Weed infestation is the major reason for economic losses in agriculture. Italian ryegrass and Palmer amaranth are some of the most troublesome weed species in Virginia. These species are strong competitors with crops for growth resources which eventually leads to significant yield losses in absence of adequate control. One of the challenges for the control of these species is development of herbicide-resistant populations.

There is a limited knowledge about the distribution of resistant populations of Italian ryegrass and Palmer amaranth in Virginia. Palmer amaranth resistance to glyphosate was confirmed in 2011 and Italian ryegrass resistance to diclofop was confirmed in 1993. These are the only two confirmed cases of herbicide resistance in Virginia but based on control failure reports, resistance to these species is suspected to be more widespread in Virginia. To investigate the distribution and levels of resistance in populations from Virginia there is a necessity for more updated surveys.

A total of 32 populations of Italian ryegrass were collected. Plants were grown in the greenhouse to test for sensitivity to herbicides commonly used for burndown or in-crop control of Italian ryegrass: pinoxaden, diclofop, glyphosate, mesosulfuron, pyroxulam, and pyroxasulfone. At 21 days after the herbicide treatments, visible injury ratings were recorded on a scale 0 to 100%, where 0 indicates no control and 100

represents complete plant necrosis. Populations exhibiting $\leq 49\%$ control were suspected to be resistant. Based on this criteria, 10, 27, 0, 14, 0, and 7 populations were found to be resistant to pinoxaden, diclofop, glyphosate, mesosulfuron, pyroxasulfone, and pyroxsulam, respectively. Following the initial screening, dose-response assays with pinoxaden, diclofop, mesosulfuron and pyroxsulam were conducted. Resistance indices (R/S ratios), calculated based on GR_{50} (herbicide dose that reduced biomass by 50%) values for resistant and susceptible populations, were 20 for pinoxaden, 87 for mesosulfuron, and 161 for pyroxsulam. The R/S value for diclofop could not be determined because even the highest tested dose could not achieve 50% growth reduction in the resistant population. Cross and multiple resistance was observed in this study and 6% of populations were found resistant to pinoxaden, diclofop-methyl, mesosulfuron, and pyroxsulam.

A total of 68 Palmer amaranth populations were collected from corn, soybean and cotton fields across Virginia. Palmer amaranth seedlings grown in the greenhouse were treated with: trifloxysulfuron, 2,4-D, fomesafen, atrazine, mesotrione, glyphosate, glufosinate and dicamba. Visible control ratings were recorded on a 0 to 100% scale, where populations with up to 49% injury were considered resistant. Upon testing the populations, resistance was found in 46, 1, 3, 7, 3, 50, 0 and 0 populations to trifloxysulfuron, 2,4-D, fomesafen, atrazine, mesotrione, glyphosate, glufosinate and dicamba, respectively. Dose-response assay for glyphosate revealed that GR_{50} value for resistant population was $1,238 \text{ g ae ha}^{-1}$, however R/S value could not be calculated as susceptible population was not available. The R/S values for trifloxysulfuron, fomesafen and atrazine were 47, 14 and 18, respectively. Approximately 69% of the populations

showed multiple resistance to two or more herbicide sites of action. Overall, findings from these statewide surveys provide critical insights into the current herbicide resistance status for both Italian ryegrass and Palmer amaranth in Virginia. This information will help growers better understand the effectiveness of commonly used herbicides and make more informed management decisions.

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GENERAL AUDIENCE ABSTRACT

Weeds are unwanted plants that grow in close proximity to crops and compete with them for nutrients, water, light and space. This competition affects crop growth, quality and yield, ultimately increasing production costs and decreasing profit for farmers. Growers use multiple strategies to control weeds, but herbicides remain one of the most cost-effective tools. However, repeated use of the same herbicide led to the evolution of herbicide resistance.

Two weed species of particular interest to growers in Virginia are Palmer amaranth, a broadleaf species, and Italian ryegrass, a grass weed species. Limited information is available about the level of herbicide resistance in these two species in Virginia. To address this knowledge gap, a survey was conducted to collect weed samples that had survived field application of herbicides and evaluate their sensitivity to different herbicides under greenhouse conditions.

During the summer of 2021 and 2024, Italian ryegrass populations were collected from 32 fields in Virginia. Seeds from each field were sown in greenhouse and plants were sprayed with several commonly used herbicides. Results indicated that Italian ryegrass in Virginia can be successfully controlled by pyroxasulfone, a soil-applied herbicide used after crop planting but prior to weed emerge, and glyphosate, applied as herbicide treatment to kill existing weeds before wheat planting. The other herbicides

used in this experiment including pinoxaden, mesosulfuron, and pyroxsulam did not control all tested populations. Moreover, for some populations, even herbicide rates substantially higher than recommended as per the label failed to provide satisfactory control of Italian ryegrass, indicating the presence of resistance.

In the fall of 2022 and 2024, 68 Palmer amaranth populations were collected from fields across Virginia. Seeds from collected plants were planted in greenhouse and used for testing the efficacy of commonly used herbicides. The results demonstrated that dicamba and glufosinate could effectively control all Palmer amaranth populations. Other herbicides including trifloxysulfuron, 2,4-D, fomesafen, atrazine, mesotrione and glyphosate could not control all the populations. Glyphosate failed to control all the tested populations and showed high resistance levels while in the case of other herbicides like atrazine, fomesafen, mesotrione, 2,4-D, and trifloxysulfuron, high level of resistance was observed in certain populations. Also the results showed that the herbicide effectiveness is location dependent. The outcomes of this resistance survey will help growers make educated and informed decisions on how to effectively control both Italian ryegrass and Palmer amaranth in their fields.

Dedication

I dedicate my thesis to my family, who always believed in me without ever placing pressure on me. Instead, they encouraged me to pursue what truly makes me happy. Following that advice led me to the United States, where I had opportunity to pursue the education. I always dreamed of enrolling in the discipline I love agriculture at a university that I admire. To my parents Jagoda and Andjeljko, sister Marijana and nephews Bogdan and Vojin, thank you for your endless support, love, and encouragement. I am deeply grateful for everything you have done for me. Pursuing my dreams meant moving far from home, and I appreciate your understanding and strength during that time.

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1. Introduction and Literature Review

Crop production is consistently challenged by a wide range of agricultural pests, among which weeds are the most problematic due to their ability to cause greater yield losses by competing with crops for essential resources (Chauhan, 2020; Oerke, 2006). To address this challenge, a range of weed management strategies are used, including physical, mechanical, biological, and chemical methods. Integrated weed management, which combines multiple approaches, has become increasingly important for achieving sustainable and effective weed control (Gao & Su, 2024). Herbicides have been used by growers for weed management for over five decades, mainly because of their effectiveness, lower cost and operational flexibility compared with traditional weed control methods (Powles & Yu, 2010; Chauhan et al., 2012). However, the broader adoption and application of synthetic herbicides have inflicted a strong selection pressure on weed populations, leading to development of herbicide resistance (HR) in numerous weed species worldwide (Boyd et al., 2022; Gaines et al., 2012). The Weed Science Society of America (WSSA) defined herbicide resistance as “The inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type”.

Weeds develop resistance to herbicides using different mechanisms. Herbicide resistance mechanisms are broadly categorized into target-site resistance (TSR) and non-target site resistance (NTSR). Target-site resistance is caused by different mutations in the target site which disrupts or disables herbicide binding ability. Non-target site resistance encompasses single or multiple mechanisms combined and affects the amount of herbicide reaching to the target site either by enhancing metabolic detoxification and sequestration, decreasing absorption, impairing

translocation or reducing retention (Gaines et al., 2020; Nandula et al., 2019; Riechers et al., 2024; Roe et al., 1997; Yu & Powles, 2014).

The first confirmed case of herbicide-resistant weed was reported in 1957 in spreading dayflower (*Commelina diffusa* Burm. f.) in Hawaii, followed by wild carrot (*Daucus carota* L.) in Canada, both exhibiting resistance to 2,4-D (Hilton, 1957; Switzer, 1957; Whitehead & Switzer 1963). Since then, global HR cases have expanded to more than 500 unique cases, with the United States having the greatest number of resistant populations (Heap 2025). Recent surveys from the United States and Canada identified Palmer amaranth (*Amaranthus palmeri* S. Watson), common lambsquarters (*Chenopodium album* L.), kochia (*Bassia scoparia* (L.) A.J. Scott), nutsedge species (*Cyperus* spp.), and waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) as among the most troublesome weeds in dicot cropping systems (Wyche L, 2022). In contrast, *Bromus* species (*Bromus* spp.), foxtail species (*Setaria* spp.), kochia, bluegrass species (*Poa* spp.), and Canada thistle (*Cirsium arvense* (L.) Scop.) were categorized as the most troublesome weeds in monocot crops, pasture and turf (Wyche L, 2023). Among crops, wheat (*Triticum durum* Desf.) is the one with highest number of resistant weed species (81), followed by maize (*Zea mays* L.) (63), rice (*Oryza sativa* L.) (52) and soybean (*Glycine max* (L.) Merr.) (50) (Heap, 2025). The greater number of herbicide-resistant weed species in monocot (grass) crops such as wheat can be attributed to the dominance of grass weeds from the same family (*Poaceae*) as the crop, which restricts the range of selective herbicides available, and consequently, increases selection pressure, accelerating the evolution of herbicide resistance (Heap, 2025; Nakka et al., 2019). Mutation breeding contributed to expanding herbicide tolerance in crops such as wheat, and rice, the impact of this approach has been overshadowed by the development of transgenic herbicide-tolerant crops. Since their introduction in the 1990s,

herbicide-resistant crops, particularly glyphosate-resistant varieties have transformed weed management by simplifying crop protection strategies and improving profitability for growers (Duke, 2015). Excellent performance in terms of non-selective weed control provided by this herbicide called for its excessive use, which eventually led to a huge spread of glyphosate-resistant (GR) biotypes (Duke & Powles, 2008). Currently, there are about 62 weed species resistant to glyphosate (Heap, 2025).

Following glyphosate-tolerant crops, additional transgenic crop traits were commercialized, conferring tolerance to herbicides such as glufosinate, 2,4-D, dicamba and isoxaflutole as well as stacked traits combining multiple herbicide tolerance. These technologies were primarily introduced to diversify and increase the number of available weed control options to mitigate the increasing prevalence of GR weeds (Duke, 2015; Heap, 2014; Norsworthy et al., 2012). However, despite these advantages, widespread adoption of transgenic crops has raised concerns related to the spread of resistance traits from crops to compatible weed species via gene flow, increased herbicide drift incidents, and management issues associated with volunteer transgenic crops (Alms et al., 2016; Dale, 1994; Jones et al., 2019).

Herbicide resistance evolution in weeds is driven by several factors, including, (1) repeated use of herbicides with the same site of action, resulting in high selection pressure, (2) the prevalence of annual weeds characterized by high fecundity, and substantial genetic variability, and (3) intensive cropping systems with limited crop rotation, which favor a narrow group of highly competitive weed species and reduce opportunities to diversify herbicide sites of action (Beckie, 2006; Owen, 2001; Thill & Lemerle 2001). To date, weed species have evolved resistance to 21 of the 31 known herbicide sites of action, involving resistance to 168 individual herbicides, with confirmed cases reported in 102 crops in 75 countries (Heap, 2025). These

numbers are expected to continue rising, given the absence of new herbicide sites of action and the lengthy, costly process associated with discovery and commercialization of new chemistries (Beckie & Tardif 2012; Duke, 2012; Macías et al., 2007).

Herbicide resistance in Virginia was first recognized in 1976 with the detection of atrazine-resistant smooth pigweed (*Amaranthus hybridus* L.), followed by atrazine-resistant common lambsquarters in 1979 (Heap. (2025). The first peer reviewed survey documenting their distribution in this state happened in late 1980s (Vencil et al., 1988). With the introduction of ACCase-, ALS- and EPSPS-inhibiting herbicides, the resistance cases have been more commonly reported with resistance to these herbicides. The most recent cases reported in 2015 include goosegrass (*Eleusine indica*) resistant to the Group 14 herbicide oxadiazon reported by McElroy et al., (2017) and mouse-ear-cress (*Arabidopsis thaliana*) resistant to several ALS herbicides including, thifensulfuron-methyl, tribenuron-methyl, flucarbazone-Na, mesosulfuron-methyl and pyroxsulam (Randhawa et al., 2018). According to Heap (2025), currently, 13 herbicide-resistant weed species are confirmed in Virginia, with resistance documented to four different herbicide sites of action in crops such as corn, soybean, wheat, golf courses and turf. According to a survey conducted in Virginia about a decade ago, Palmer amaranth, common ragweed and horseweed were identified as the most problematic weed species. These species are a major concern for soybean, corn and small grain production in the state and herbicide resistance issues have increased weed management cost by approximately 20% (Haring & Flessner 2017). Although Palmer amaranth has evolved resistance to nine different herbicide sites of action in the United States but confirmed resistance in Virginia currently includes only glyphosate (Heap, 2025). For wheat producers, Italian ryegrass is the most troublesome weed and its populations resistant to ACCase- and ALS-inhibiting herbicides are well distributed

across Eastern Virginia, significantly limiting control options for this species (Cahoon, 2016). Overall, the continued expansion of herbicide resistance in Virginia reflects broader national and global trends, underscoring the urgent need for diversified, sustainable weed-management strategies. As resistance spreads across additional sites of action and cropping systems, reliance on herbicides alone is becoming increasingly untenable. Integrated weed management approaches incorporating cultural, mechanical, and chemical tactics, along with stewardship practices that delay resistance evolution will be essential to preserve the efficacy of existing herbicides and protect crop productivity. Therefore, continued monitoring, research, and grower outreach remain essential to support informed and effective weed management decisions.

1.1 References

- Ahmed, A. M. A. T. (2011). *Control, assessment and glyphosate resistance of Palmer amaranth (Amaranthus palmeri S. Wats) in Virginia* (Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.).
- Bailey, W. A., & Wilson, H. P. (2003). Control of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*) with postemergence herbicides. *Weed Technology*, 17(3), 534-542.
- Cahoon, C. (2016). *Distribution of herbicide-resistant Italian ryegrass in eastern Virginia*. Virginia Cooperative Extension, Virginia Polytechnic Institute and State University, Blacksburg, VA. <https://ag-pest-advisory.vaes.vt.edu/distribution-of-herbicide-resistant-italian-ryegrass-in-eastern-virginia/>
- Ellis, A. T., Steckel, L. E., Main, C. L., De Melo, M. S., West, D. R., & Mueller, T. C. (2010). A survey for diclofop-methyl resistance in Italian ryegrass from Tennessee and how to manage resistance in wheat. *Weed Technology*, 24(3), 303-309.
- Heap I (2025) International Survey of Herbicide Resistant Weeds. <https://www.weedscience.org/>
- Hilton, H. W. (1957). Herbicide tolerant strains of weeds. Hawaiian Sugar Plant. Assoc. Annu. Rep. Pg, 69.
- Hite, G. A., King, S. R., Hagood, E. S., & Holtzman, G. I. (2008). Differential response of a Virginia common lambsquarters (*Chenopodium album*) collection to glyphosate. *Weed Science*, 56(2), 203-209.
- Hofer, U., Muehlebach, M., Hole, S., & Zoschke, A. (2006). Pinoxaden for broad-spectrum grass weed management in cereal crops. *Journal of Plant Diseases and Protection*, 113(Suppl.), 989-996.
- Jones, E. A., Taylor, Z. R., & Everman, W. J. (2021). Distribution and control of herbicide-resistant Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] in winter wheat (*Triticum aestivum* L.) in North Carolina. *Frontiers in Agronomy*, 2, 601917.
- Kuk, Y. I., & Bugos, N. R. (2007). Cross-resistance profile of mesosulfuron-methyl-resistant Italian ryegrass in the southern United States. *Pest Management Science* 63(4), 349-357.
- Kuk, Y. I., Burgos, N. R., & Scott, R. C. (2008). Resistance profile of diclofop-resistant Italian ryegrass (*Lolium multiflorum*) to ACCase-and ALS-inhibiting herbicides in Arkansas, USA. *Weed Science*, 56(4), 614-623.

- McElroy, J. S., Head, W. B., Wehtje, G. R., & Spak, D. (2017). Identification of goosegrass (*Eleusine indica*) biotypes resistant to preemergence-applied oxadiazon. *Weed Technology*, 31(5), 675-681.
- Randhawa, R. S., Westwood, J. H., Cahoon, C. W., & Flessner, M. L. (2018). Interference and control of ALS-resistant mouse-ear cress (*Arabidopsis thaliana*) in winter wheat. *Weed Technology*, 32(6), 671-677.
- Switzer, C. M. (1957). The existence of 2, 4-D-resistant strains of wild carrot. . *In Proc. Northeast Weed Contr. Conf.* (Vol. 11, pp. 315-318).
- Vencill, W. K., & Foy, C. L. (1988). Distribution of triazine-resistant smooth pigweed (*Amaranthus hybridus*) and common lambsquarters (*Chenopodium album*) in Virginia. *Weed Science*, 36(4), 497-499.
- Whitehead, C. W., & Switzer, C. M. (1963). The differential response of strains of wild carrot to 2, 4-D and related herbicides. *Canadian Journal of Plant Science*, 43(3), 255-262.

2. Distribution and Characterization of Herbicide-Resistant Italian ryegrass in Virginia

2.1 Abstract

Field surveys conducted in the summer of 2020 and 2024 were used to assess the distribution of herbicide-resistant Italian ryegrass infestations in winter wheat production areas across Virginia. Thirty-two field populations were collected and evaluated in the greenhouse for sensitivity to diclofop-methyl (516 g ai ha⁻¹), pinoxaden (59.4 g ai ha⁻¹), mesosulfuron (17.5 g ai ha⁻¹), pyroxulam (17.9 g ai ha⁻¹), glyphosate (867 g ae ha⁻¹), and pyroxasulfone (102.3 g ai ha⁻¹) at their respective 1X field rates. Then, the dose-response assays were conducted on putative resistant population observed during initial herbicide screenings at 1X rate. This population was treated with eight different rates (0.5, 1, 2, 4, 8, 16 and 32X), in comparison to a susceptible population at six rates (0.0625, 0.125, 0.25, 0.5, 1, and 2X). The experiment was conducted in a completely randomized design with three replications and two experimental runs. Survivors were characterized as resistant ($\leq 49\%$ injury) or less sensitive (50-79% injury) and susceptible ($\geq 80\%$ injury). Results showed a high level of resistance to individual ALS- and ACCase-inhibitor herbicides with two populations exhibiting cross- and multiple-herbicide resistance. R/S ratios indicated that the most resistant population had 20-, 87-, and 161-fold resistance to pinoxaden, mesosulfuron, and pyroxulam, respectively, compared to a susceptible population. The R/S ratio for diclofop-methyl-resistant population could not be estimated because none of the tested rates provided up to 50% control. None of the populations showed resistance to glyphosate and pyroxasulfone, but where multiple resistance to ALS- and ACCase-inhibitor herbicides occurs and there are no remaining post emergent options available in small grains. Given that Italian ryegrass

is an obligate outcrosser, the spread of multiple resistance via gene flow poses a significant threat to wheat production in Virginia and complicates the implementation of Integrated Weed Management strategies.

2.2 Introduction

Wheat (*Triticum aestivum* L.) is one of the oldest cultivated crops and remains among the world's three most important cereals, along with rice and maize (Awika, 2011). In Virginia, most wheat production is distributed primarily in the coastal plain and piedmont regions, with Accomack, Southampton, Essex, Northumberland, and Northampton counties representing the largest harvested acreage (USDA NASS, 2022). Soft red winter wheat is the predominant type grown in the state, planted in the fall and is usually double cropped with soybean (Schilling et al., 2003; Grey & Newsom 2017).

Weeds are widely recognized as the most damaging pests in wheat (Oerke, 2006). In the United States, winter wheat yield losses due to weeds ranges from 3% to 34% (Flessner et al., 2021). According to the 2023 Grass Crops Survey, *Bromus* spp., Italian ryegrass, horseweed, and bluegrass spp. were the most troublesome weed species in winter cereal grains (Wyche L, 2023). Wheat production is further challenged by a growing number of herbicide-resistant weeds. Worldwide, wheat ranks first among crops for number of total herbicide-resistant weeds, with 81 reported species and 140 distinct resistance cases, including 33 in winter wheat (Heap, 2025). Although herbicide-tolerant systems such as Clearfield® and CoAXium® have been developed, they are not widely used in Virginia (Nakka et al., 2019; Roberson, 2010). The state has the second-highest adoption of no-till practices in the US, with 73% of cropland under no-till (USDA NASS, 2022). Studies have shown that weed seedbank diversity is often greater under no-till systems

compared with that under conventional tillage (Sosnoskie et al., 2006). Reduced tillage increases reliance on herbicides (Kaya Altop et al., 2022) and has contributed to the rise in herbicide-resistant weeds, particularly Italian ryegrass (Preston et al., 2009).

Italian ryegrass, introduced from Europe as a cool-season forage, has become a major weed in various U.S. cropping systems (Elmore, 1986; Hulting et al., 2012). This species belongs to genus *Lolium* which is known for its ability to adapt to new environmental conditions and a variety of soil types (Hulting et al., 2012). It thrives in temperate climates and fertile soils, though it is poorly adapted to extreme heat, drought, or severe winters (USDA NRCS, 2002). Under optimal conditions, Italian ryegrass seedlings germinate in the fall, overwinter in a vegetative stage, and resume rapid growth in spring (McCullough, 2024). Although *Lolium* spp. are obligate cross-pollinated promoting genetic diversity and increasing and accelerating the risk of herbicide resistance evolution, however, recent research indicates potential for self-pollination under warmer conditions (Matzrafi et al., 2021). Italian ryegrass competes aggressively with wheat due to its rapid leaf expansion, nutrient uptake, photosynthesis, and high fecundity, therefore reducing wheat tillering (Carson et al., 1999; Stone et al., 1998; Sapkota et al., 2020).

Lolium spp. are among the most problematic grass weeds mostly due to rapid evolution of resistance to multiple herbicide modes of action (Campagna et al., 2001; Salas et al., 2013). Surveys conducted in 1994 and 2008 identified Italian ryegrass as the most troublesome weed in wheat in southern regions of the United States (Webster & Nichols 2012). Today Italian ryegrass is resistant to eight different herbicide modes of action with the first case of diclofop resistance reported in 1987 in Oregon, USA (Heap, 2025; Stanger & Appleby, 1989). Successful control of Italian ryegrass is challenging as resistance to all herbicides labeled for postemergence use in

winter wheat has been reported (Grey & Bridges 2003; Hoskins et al., 2005; Grey, et al., 2012; Jones et al. 2021; Heap, 2025). The postemergence control is limited only to ACCase (acetyl CoA carboxylase) inhibitors (Group 1) and Group 2 herbicides (Jones et al. 2021). For preemergence control of Italian ryegrass in winter wheat, available herbicide options include ALS (acetolactate synthase) inhibitors (Group 2), microtubule assembly inhibitors (Group 3), Photosystem II inhibitors (Group 5), PPO inhibitors (Group 14) and VLCFA (Very Long-Chain Fatty Acid) synthesis inhibitors (Group 15) (Jones et al., 2021).

ACCase inhibitors (Group 1) are selective grass herbicides, once highly effective and widely used, functioning by disrupting fatty acid biosynthesis in susceptible grasses and ultimately causing plant death (Kukorelli et al., 2013; Takano et al., 2020). Since their introduction, there has been extensive use of these herbicides for selective control of grass weeds in various crops worldwide (Konishi et al., 1996; Sasaki et al., 1995). Although ACCase inhibitors were initially highly effective in broadleaf crops, they are also used in cereals such as wheat due to crop selectivity. Typically, in cereals, selectivity is the attribute of cereal crops, occurs due to the use of safeners and the crop's ability to metabolize and detoxify these herbicides faster and more efficiently than the target grass weeds (Rangani et al., 2024; Rosinger et al., 2011). This metabolic capability of wheat has made these herbicides a valuable tool for selective weed control; however, their intensive and continuous use has resulted in rapid evolution of target-site resistance in grass weeds, diminishing their effectiveness (Gressel, 2009; Liu et al., 2007).

Diclofop-methyl (Hoelon®), a postemergence selective herbicide was widely used until Italian ryegrass populations developed resistance to this herbicide within 10 years of its initial deployment (Bailey & Wilson, 2003; Stanger & Appleby 1989). Resistance to diclofop in Italian

ryegrass was confirmed in Virginia in 1993 and remains the only documented case of resistance to this herbicide in the state (Heap, 2025). According to Bailey and Wilson (2003), more than half of the total wheat acreage in Virginia was infested with diclofop-resistant Italian ryegrass resulting in economic losses exceeding \$3.2 million (Bailey et al., 2003). Diclofop-methyl was phased out in the USA in 2014 and has been removed from the list of herbicides labeled for use in wheat (Singh et al., 2020). To date, Italian ryegrass resistance to diclofop-methyl has been reported in 10 US states (Heap, 2025).

Pinoxaden (Axial XL®), another Group 1 herbicide introduced in 2006, provided improved crop safety and enhanced weed-control efficacy (Hofer et al., 2006). Although both diclofop and pinoxaden inhibit ACCase (Group 1), they are from different chemical families: diclofop is an aryloxyphenoxypropionate (FOP), whereas pinoxaden is a phenylpyrazoline (DEN)- a distinction that helps explain differences in behavior, selectivity, and cross-resistance patterns (Kuk et al., 2008). Compared with earlier ACCase inhibitors, pinoxaden offers a broader application window and better compatibility with broadleaf herbicides (Bararpour et al., 2018). Despite belonging to different chemical families, cross-resistance between diclofop-resistant ryegrass and pinoxaden-resistant ryegrass has been documented (Kuk et al., 2008). In the United States, several cases of pinoxaden-resistant Italian ryegrass have been reported, with several populations showing reduced control under greenhouse or field conditions and, in some cases, cross-resistance as well (Chandi et al. 2011; Bararpour et al., 2018; Heap, 2025).

Failure of ACCase-inhibiting herbicides to control Italian ryegrass leaves ALS inhibitors as the only remaining postemergence option for wheat growers (Chandi et al., 2011; Salas et al., 2013). ALS inhibitors are effective on both monocot and dicot weed species through the inhibition

of the enzyme acetolactate synthase (Shaner et al., 1984; Dailey & Cronan, 1986). Wheat exhibits a strong tolerance to Group 2 herbicides such as mesosulfuron and pyroxsulam (Grey et al., 2012; Grey, Braxton, et al., 2012; Ellis et al., 2010). However, high selection pressure with this group of herbicides usually leads to quick resistance development. For instance, resistance to mesosulfuron was detected in an Arkansas population one year before the herbicide was commercially available (Kuk & Bugos 2007). Italian ryegrass populations resistant to pyroxsulam (Powerflex HL®) and mesosulfuron (Osprey®) have been reported in the states neighboring Virginia. Resistance to pyroxsulam was confirmed in North Carolina and Kentucky in 2007 and 2013 respectively (Chandi et al., 2011, Heap, 2025). Also, resistance to mesosulfuron has been documented in North Carolina in 2007 and Delaware in 2012. Overall, resistance to Group 2 herbicides is now widespread, and the number of resistant weed species continues to rise more quickly than for any other herbicide mode of action (Heap, 2025).

Despite its strong agronomic potential, glyphosate-resistant (GR) wheat has never achieved commercial level adoption due to limited consumer and poor market acceptance (Ogg Jr & Isakson, 2001). The widespread adoption of conservation tillage and no-till practices along with the increased use of genetically modified (GMO) crops resulted in heavy reliance on glyphosate as a primary tool for weed management (Baylis, 2000; Woodburn, 2000). Glyphosate is a nonselective, postemergence herbicide that belongs to Group 9 (EPSP synthase inhibitors). In non-GR crops such as wheat, its use is limited to only preplant and postharvest application although it is commonly used in rotation with GR crops (Franz et al., 1997). In the southern United States, glyphosate is often applied as an effective preplant burndown treatment in wheat to control Italian ryegrass (Singh, 2025; Bond, 2017; Hart, 2021). Although glyphosate-resistant Italian ryegrass

has not yet been reported in Virginia, but herbicide resistance reports from neighboring states such as North Carolina highlight the growing risk of resistance expansion (Singh 2025; Hart, 2021 ; Alsdorf 2024). Globally, glyphosate resistance in this species was first confirmed in Chile in 2001, followed by the first US case in Oregon in 2005. Currently, glyphosate-resistant Italian ryegrass populations are confirmed in eight states, particularly widespread distribution in the Southeast and Mid-South (Heap, 2025).

When postemergence herbicides lose effectiveness against Italian ryegrass, pyroxasulfone applied alone or in combination with flumioxazin offers a reliable preemergence weed control option in wheat (Hand et al., 2022). Pyroxasulfone, a Group 15 herbicide, inhibits very long-chain fatty acid elongases, thereby disrupting lipid biosynthesis in emerging seedlings (Busi et al., 2014; Tanetani et al., 2009; Trenkamp et al., 2004). Pyroxasulfone is labeled to control a wide range of grass and broadleaf weeds in several crops including wheat. Flexible application timing contributes to resistance-management strategies by helping delay the evolution of herbicide resistance in Italian ryegrass and providing greater operational flexibility for winter wheat growers (Hulting et al., 2012). Although, pyroxasulfone resistance has not yet been documented in Italian ryegrass, however, cases of resistance to flufenacet, another Group 15 herbicide with the same mode of action has been reported in the Pacific Northwest region of the United States (Heap, 2025).

Italian ryegrass infestations are often found in wheat fields of Virginia and North Carolina, often leading to field abandonment due to inadequate control (Bailey et al., 2003). A survey conducted in Eastern Virginia confirmed extensive distribution of Group 1 (ACCase inhibitors) and Group 2 (ALS inhibitors) resistant populations (Cahoon, 2016). Nearly ten years after the previous survey of herbicide resistance in Italian ryegrass in Virginia, a renewed assessment is

warranted to characterize the current resistance status in the state's wheat-producing regions. This survey expands previous efforts by including resistance screening for two additional herbicides and by conducting dose-response assays which would provide information about resistance levels and a clearer understanding of contemporary resistance dynamics in Virginia. Therefore, the objectives of this study are to (1) conduct a state-wide survey of Italian ryegrass populations to identify herbicide-resistant biotypes, and (2) conduct herbicide screenings and dose-response assays to evaluate the level of resistance when present. The results of this study would help wheat growers with an up-to-date assessment of herbicide resistance in Italian ryegrass and enabling more informed and effective weed management decisions.

2.3 Materials and Methods

2.3.1 Field surveys

Field surveys were conducted in the summers of 2021 and 2024 to collect Italian ryegrass (IR) samples from 36 wheat fields across the Coastal plain and Piedmont region of Virginia (Figure 2.1). Sample locations were selected based on reports of herbicide failures from extension agents and growers. Mature seed heads were collected from approximately 20 plants that had survived in-season herbicide applications to capture population-level variability. Plant samples were dried for 5 days at 55-60 °C, followed by threshing to obtain seeds, stored in a cold room at 5 °C until further use in greenhouse experiments.

2.3.2 Plant material preparation

For postemergence herbicide screening, 10 to 15 seeds were sown in 10 x 10 cm pots filled with Sunshine® Mix #1 (Sun Gro Horticulture, Agawam, MA, USA). After emergence, the

seedlings were thinned to maintain five seedlings per pot. Plants were grown in a greenhouse under a 12-hour photoperiod at 25/20 °C (day/night). For preemergence herbicide screening, Bojac sandy loam soil with pH 5.7 and soil organic matter <1% was used. Soil was watered to stimulate germination of the natural weed seedbank. Ten days after emergence, glyphosate was applied at 1100 g ai ha⁻¹ (Bayer CropScience) to eliminate the existing weeds. No irrigation was applied for 48 h to prevent herbicide movement into the soil. After the application of preemergence herbicide, watering was done uniformly by using fine droplets to allow slow infiltration, prevent overwatering as well as potential flow down on the sides of the pots.

2.3.3 *Herbicide screening*

Herbicide resistance screening was conducted in 2023 and 2024 at the greenhouse (GH) facility located at the Virginia Tech Eastern Shore Agricultural Research and Extension Center, Painter, Virginia (37°35'20.6" N, 75°49'34.7" W). All 36 accessions were evaluated for resistance to six herbicides (with recommended adjuvants for postemergence herbicides) and a nontreated check was included along with each treatment (Table 2.1). For postemergence herbicides, IR plants were treated at the three to four leaf stage. For preemergence screening, herbicides were applied after the seeds were sown but before the emergence of plants. Herbicide applications were made using a spray chamber equipped with a TeeJet® 8002 EVS spray nozzle calibrated to deliver 187 L ha⁻¹ at 276 kPa. Three weeks after application, visible injury was rated on a scale 0-100%, (0% = no injury; 100% = complete plant necrosis). The IR populations were classified into three categories based on the injury level susceptible (80-100% injury), moderately resistant (50-79%), or resistant (0-49%). For preemergence treatments, the emergence counts were made at 21 days

after application. The herbicide screening was arranged in a completely randomized design, with three replications and two experimental runs.

2.3.4 Dose-response assays

Based on initial screening results, the Populations with the lowest injury and susceptible population for each herbicide were selected for dose-response studies. In the initial herbicide resistance screening, two populations were observed to have multiple and cross resistance to pinoxaden, diclofop, mesosulfuron and pyroxsulam. Cross-resistance is referred as resistance to different herbicide families that target the same SOA. Multiple resistance is referred as resistance to minimum two unique herbicide SOA. Cross and multiple resistance were determined from each population's response to the 1X rate of each herbicide tested separately. Populations classified as resistant (0-49% injury) were subsequently grouped according to their resistance patterns as either cross-resistant or multiple-resistant. One population (IR-14) was resistant to all four herbicides and was included in all corresponding assays. Population IR-2 (mesosulfuron-resistant) and IR-1 (pyroxsulam-resistant) were also evaluated. Population IR-21 was selected as a susceptible standard for pinoxaden, mesosulfuron and pyroxsulam and IR-18 was the susceptible standard for diclofop. For resistant populations, eight doses (0, 0.5, 1, 2, 4, 8, 16, 32X of the labeled rate) and for susceptible populations, seven doses (0, 0.0625, 0.125, 0.25, 0.5, 1, and 2X of the labeled rate) were applied for dose response assays. Three weeks after the treatments were applied, above-ground biomass was recorded. Biomass samples were dried at 55-60 °C to a constant weight. The dose-response assays were conducted in a completely randomized design with four replications and two experimental runs.

2.3.5 *Statistical analysis*

For mapping of spatial distribution of Virginia Italian ryegrass populations sensitivity to selected herbicides ArcGis (version 10.8.2; ESRI, Redlands, CA). Implementation of interpolation analysis utilizing inverse-distance weighting method was conducted in order to visualize the distribution of 32 Italian ryegrass populations for sensitivity to selected herbicides across a spatial scale (Garetson et al., 2019). For herbicide screening data were analyzed by ANOVA in JMP (Version 18; SAS Inc., Cary, NC, USA). Since the population x run interaction was not significant, we pooled the data across runs. In order to classify populations and select representatives for dose-response experiments, the mean injury values were used. Biomass reduction as a response to different herbicide doses was analyzed by using a three-parameter logistic regression equation [Eqn (A)] in JMP (Version 18; SAS Inc., Cary, NC, USA) and SigmaPlot v16.0 (Grafiti LLC, 2024) was used to produce dose-response graphs. Model estimates were used to calculate growth reduction (GR_{50}) values, and resistance intensity was expressed as the GR_{50} ratio of resistant to susceptible populations (R/S).

$$Y = c / (1 + \exp(-a \times (\text{Dose} - b))) \quad (\text{A})$$

Y – Injury

a = Growth rate

b = Infection point

c = Asymptote

2.4 Results and Discussion

2.4.1 *Screening for herbicide resistance and dose response*

Initial greenhouse screenings were conducted using the 1X recommended field rate to evaluate the response of Italian ryegrass populations to selected herbicides (Table 2.1). Populations showing reduced sensitivity were subsequently subjected to dose–response assays to quantify resistance levels. Parameter estimates from the dose–response analyses are summarized in Table 2.4.

2.4.2 *Diclofop-methyl*

Across all the 32 populations screened, all exhibited resistance to diclofop-methyl. Among those, twenty-seven populations showed resistance (<50% injury), while the remaining 5 were less sensitive (50-79% injury) (Table 2.2). The result from our screening aligns with several previous regional assessments demonstrating that diclofop-methyl efficacy has been severely compromised across the Southeastern and Mid-Atlantic United States (Heap, 2025; Jones et al., 2021).

In a study conducted by Jones et al. (2021) in North Carolina, all of the tested Italian ryegrass populations were resistant to diclofop-methyl. Notably, several of the sampled sites in that study were near the Virginia–North Carolina border, reinforcing the geographic continuity of resistance. Diclofop-methyl resistance in Virginia is not a new phenomenon; the first confirmed case dates back more than 30 years (Heap, 2025). According to a report from 2003, over 50% of Virginia’s wheat acreage was already infested with diclofop-resistant Italian ryegrass (Bailey & Wilson, 2003). A subsequent statewide survey in 2016 found diclofop-methyl resistance in approximately 30% of the tested Italian ryegrass populations. Compared to those

earlier surveys, our study indicates a substantial increase in resistance frequency over the past decade reaching 100% highlighting both the persistence and rapid spread of resistance across production systems (Powles & Yu 2010; Beckie & Harker 2017). Dose-response experiments were conducted using the two most resistant populations, IR-15 and IR-14; no susceptible standard was available for comparison. For IR-15 the estimated GR_{50} was 3748.7 g ai ha⁻¹, far exceeding the labeled field rate. In contrast, IR-14 (Figure 2.4B) showed no measurable GR_{50} , (Table 2.3) as none of the tested doses were able to reduce biomass by 50%. A study conducted by Kuk et al. (2008) in Arkansas, reported that 81% percent of the Italian ryegrass populations were resistant to field rate of diclofop, with resistance levels reaching up to 400-fold although, the susceptible standard in that study was controlled at only 100 g ai ha⁻¹. However, several resistant populations survived rates as high as 40,000 g ai ha⁻¹, indicating profound resistance evolution.

Given that diclofop-methyl resistance in Virginia was confirmed as early as 1993, our findings further support the long-term and cumulative nature of selection pressure imposed by repeated ACCase inhibitor use (Délye, 2005; Powles & Yu, 2010). The continued escalation in resistance magnitude suggests not only maintenance of resistant biotypes in the seedbank but also potential stacking of target-site and non-target-site mechanisms over time (Vila-Aiub et al., 2009; Délye, 2013).

2.4.3 *Pinoxaden*

Pinoxaden is a relatively recent addition to the ACCase-inhibiting herbicide and compared with older aryloxyphenoxypropionate (FOP) herbicides such as diclofop and generally provides improved grass weed control (Hofer et al., 2006). In the present study, 31% (10 out of

32) of the populations were classified as resistant to pinoxaden (Table 2. 2). Among those 10 were pinoxaden-resistant populations, 9 populations also exhibited cross-resistance to diclofop. Half of the populations were susceptible, whereas 6 populations were in the less sensitive category. The Eastern Shore of Virginia comprising of Northampton and Accomack counties represents an important wheat producing area, since these two counties are among the top five counties in terms of wheat acreage harvested in Virginia (USDA, 2022). Out of the total 32 populations, 20 were collected from this region (Figure 2.1). Our studies showed that of those 20 populations, 45% had varied levels of resistance to pinoxaden (Figure 2.2). At the county level, 40% of the sampled populations from Northampton County were resistant, compared with 10% from Accomack County. In a similar survey of Eastern Virginia by Cahoon (2016) a decade ago, revealed that 23% of those samples (89 populations) showed resistance to pinoxaden at 1x rate. Even then, resistance was concentrated in Northampton County (64%), whereas no resistant populations were detected in Accomack County. The substantial increase in resistance frequency in both counties over the past decade underscores the accelerating selection pressure imposed by repeated ACCase inhibitor use in this region. Dose-response analysis in the present study revealed a high level of resistance, with a 79-fold increase in GR_{50} for resistant $505.5 \text{ g ai ha}^{-1}$ versus susceptible 6.4 g ai ha^{-1} (Table 2.3 and Figure 2.4 A). The GR_{50} for the resistant population exceeded the 1X field rate by more than eight-fold, indicating that field-level control failures are expected even under optimal application conditions. A 15-fold resistance in one of the tested populations to pinoxaden was also confirmed by Ellis et al. (2010). The GR_{50} values in that study were 4.5 and 69 g ai ha^{-1} for susceptible and resistant populations, respectively. The substantially higher level of resistance observed in the current study suggests either the accumulation of multiple resistance mechanisms or the selection of particularly robust target-site

mutations or enhanced metabolic pathways. This is particularly concerning because ACCase-inhibiting herbicides remain an important tool in wheat production systems, and resistance to this mode of action severely limits chemical control options. The complete loss of diclofop-methyl and pinoxaden efficacy observed here highlights the urgency of diversified, integrated weed management-rotating and mixing effective modes of action, deploying competitive crops, using residuals, and integrating harvest weed seed control and other cultural tactics-to slow further resistance evolution (Norsworthy et al., 2012; Beckie & Harker 2017).

2.4.4 *Mesosulfuron*

The results from mesosulfuron initial screening showed that approximately 44% of the tested Italian ryegrass populations were resistant. Among the tested populations, 9% were less sensitive, and 47% were susceptible (Table 2.2). This pattern is consistent with previous regional surveys documenting widespread mesosulfuron-resistant Italian ryegrass across Eastern Virginia. In that study, it was observed that 92% of the Italian ryegrass populations were resistant to mesosulfuron (Cahoon, 2016). In the current study, two resistant populations IR-2 and IR-14 were selected for dose-response analysis to understand the level of resistance. For IR-2, no tested rate including the highest 32X rate (499.2 g ai ha⁻¹) achieved 50% biomass reduction (Figure 2.4C). For IR-14 GR₅₀ value was 49.8 g ai ha⁻¹ while for the susceptible population IR-21 was 1.7 g ai ha⁻¹. Based on the given values the R/S ratio was 29.3-fold for IR-14 while for the IR-2, this ratio could not be calculated, since even the highest dose could not cause up to 50% growth reduction. However, this ratio would definitely be greater than 293, based on growth reduction caused by the highest dose tested (Table 2.3). The large disparity in resistance levels between IR-2 and IR-14 suggests multiple or differing resistance mechanisms, a

phenomenon well-documented in *Lolium* spp., where both target-site mutations at the ALS enzyme and non-target-site metabolic resistance frequently contribute to variable resistance phenotypes (Tehranchian et al., 2019). The high difference between the GR₅₀ values of these two resistant populations implies a potential difference in resistance mechanisms among them (Ellis et al. 2010). The first reported case of mesosulfuron-resistant Italian ryegrass in the southern United States was confirmed by Kuk & Bugos (2007), where population collected from Arkansas was 29-fold resistant comparing to susceptible population which is similar to R/S ratio that we observed comparing the GR₅₀ values of IR-14 and IR-21 populations.

2.4.5 *Pyroxsulam*

Approximately 22% of the screened populations were resistant to pyroxsulam, whereas 37% of the populations were less sensitive (Table 2.2). The remaining 13 pyroxsulam-susceptible populations were also susceptible to mesosulfuron. Cahoon (2016) reported that about 93% of the screened populations were resistant to pyroxsulam. About 44% and 22% of the populations had less than 50% injury for mesosulfuron and pyroxsulam, respectively. However, mesosulfuron also had higher number of populations (16%) with injury of 90% or more, as opposed to just 3.2% of populations for pyroxsulam. This means that populations were more widely spread from susceptible to resistant category for mesosulfuron, whereas, populations were more confined towards less sensitive category for pyroxsulam. For pyroxsulam-resistant population, IR-1 and IR-14 were selected for the dose-response assay. Results indicated that the estimated GR₅₀ value for the IR-1 population was 79 g ai ha⁻¹ and 5.8 g ai ha⁻¹ for IR-14 compared with GR₅₀ for susceptible populations was 0.1 g ai ha⁻¹ (Figure 2.4D). This indicates an R/S value of 98.8 in a case for IR-1 and R/S value of 7.3 for IR-14 (Table 2.3). A recent dose

response study from Alabama reported 90-fold resistance to pyroxsulam was observed in a population collected from a field border (Yadav et al., 2025). The high prevalence of mesosulfuron and pyroxsulam resistance in this study, combined with the documented heterogeneity in resistance levels, aligns with multi-state surveys showing extensive cross-resistance among ALS inhibitors.

2.4.6 *Glyphosate*

All the tested 32 populations (100%) were susceptible to glyphosate (Table 2.2). Although, glyphosate is not permitted for over-the-top application in wheat, but it is a very important tool for preplant burndown control of Italian ryegrass as well as controlling the weed prior to planting corn, soybean, and other crops commonly used in rotation with wheat in the region. Thus, farmers in Virginia can still rely on glyphosate for control of Italian ryegrass prior to crop planting and for post emergence control of this weed in labeled crops. To date, reports of resistance or reduced sensitivity to glyphosate in Virginia have been documented in some broadleaf weed species but not in grass species (Ahmed, 2011; Heap, 2025; Hite et al., 2008). However, glyphosate-resistant Italian ryegrass populations have been documented in several other states, including Arkansas, California, Louisiana, Mississippi, New York, North Carolina, Oregon, and Tennessee (Heap, 2025).

2.4.7 *Pyroxasulfone*

All 32 populations (100%) were susceptible to pyroxasulfone (Table 2.2). This was the only preemergence herbicide included in the screening because resistance to preemergence herbicides is uncommon, and there have been no reports of control failures of pyroxasulfone in Virginia. This screening confirms the strong and consistent efficacy of pyroxasulfone against

Italian ryegrass. Nonetheless, resistance to flufenacet, another group 15 herbicide has been reported in three states in the pacific northwest (Heap, 2025).

2.4.8 Cross resistance and multiple resistance

Across the 14 populations resistant to ALS-inhibiting herbicides, 7 were cross-resistant to both pyroxsulam and mesosulfuron (Table 2.4). Such results are consistent with single target-site mutations in the ALS gene that reduce herbicide binding across multiple ALS chemistries, thereby producing cross-resistance within the ALS mode of action. Minor differences in resistance expression between pyroxsulam and mesosulfuron likely reflect chemistry-specific binding interactions or background non-target-site metabolism that slightly modulates phenotypes without altering the overall cross-resistance pattern (Tranel & Wright 2002; Yu & Powles 2014).

Multiple resistance between ALS- and ACCase-inhibiting herbicides was also observed in our screening study, where 13 populations exhibited multiple resistance to diclofop and mesosulfuron, and 7 populations had multiple resistance to diclofop and pyroxsulam. Additionally, 7 populations showed multiple resistance to pinoxaden and mesosulfuron, whereas multiple resistance to pinoxaden and pyroxsulam was seen in 2 populations (Table 2.4). These pairwise outcomes indicate that a substantial fraction of populations carries resistance mechanisms to both targets i.e. independent target-site mutations in ALS and ACCase and/or non-target-site (metabolic) resistance mainly cytochrome P450-mediated detoxification, which is capable of reducing herbicide efficacy across distinct modes of action (Powles & Yu 2010; Délye, 2013; Gaines et al., 2020; Beckie & Tardif, 2012).

2.5 Conclusions

This survey showed that the limited number of herbicide options for managing Italian ryegrass in wheat has contributed to repeated use of the same herbicide modes of action. Such recurrent selection pressure has facilitated the evolution of herbicide-resistant Italian ryegrass biotypes in Virginia. However, all currently labeled wheat herbicides (ACCase and ALS) used in this experiment are still effective and presence of herbicide resistance or reduced control was highly population-specific. The surveyed populations were selected based on reports of poor herbicide performance, some level of resistance was anticipated. The findings of confirmed cross and multiple herbicide resistance in Italian ryegrass (Eastern Virginia) to ACCase- and ALS-inhibiting herbicides further narrows the already limited herbicide options for postemergence control of Italian ryegrass in wheat. The presence of highly resistant populations is a big concern, given that Italian ryegrass is an obligate outcrosser and the genes from this populations can further spread via pollen movement. This elevates the risk of resistance spreading across fields and farms. Given these constraints, reliance on preemergence herbicides becomes even more important. The only preemergence herbicide in our study provided excellent control in greenhouse conditions was pyroxasulfone, which can be continued as a viable option in the field. Likewise, glyphosate remains a valuable tool preplant burndown in wheat production systems in Virginia. None of the populations tested exhibited glyphosate resistance, suggesting that glyphosate can be an effective tool for effective Italian ryegrass control.

Although resistance to ACCase herbicides had been previously confirmed in Virginia, this survey provides additional confirmation of resistance to Group 2 herbicides as well. Future research will focus on molecular characterization of the underlying resistance mechanisms.

Understanding the prevalence, distribution, and molecular basis of resistance in Italian ryegrass will help growers in Eastern Virginia make more informed management decisions and may contribute to slowing the further spread of herbicide resistance.

2.6 References

- Ahmed, A. M. A. T. (2011). *Control, assessment and glyphosate resistance of Palmer amaranth (Amaranthus palmeri S. Wats) in Virginia* (Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.).
- Alsdorf, J. W. (2024). *Survey of Herbicide-Resistant Palmer Amaranth (Amaranthus palmeri) and Italian Ryegrass (Lolium perenne L Spp. multiflorum) Populations in North Carolina*. (Master's thesis, North Carolina State University, Raleigh, NC).
- Awika, J. M. (2011). Major cereal grains production and use around the world. In *Advances in cereal science: implications to food processing and health promotion* (pp. 1-13). *American Chemical Society*.
- Bailey, W. A., Hatzios, K. K., & Wilson, H. P. (2003). Responses of winter wheat and diclofop-methyl-sensitive and-resistant Italian ryegrass (*Lolium multiflorum*) to AE F130060 03. *Weed Science*, 51(4), 515-522.
- Bailey, W. A., & Wilson, H. P. (2003). Control of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*) with postemergence herbicides. *Weed Technology*, 17(3), 534-542.
- Bayer CropScience LP. (n.d.). Roundup PowerMAX® herbicide label (EPA Reg. No. 524-549). https://www3.epa.gov/pesticides/chem_search/ppls/0524-00549-20170615.pdf
- Bararpour, T., Korres, N. E., Burgos, N. R., Hale, R. R., & Tseng, T. M. P. (2018). Performance of pinoxaden on the control of diclofop-resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*) in winter wheat. *Agriculture*, 8(7), 114.
- Baylis, A. D. (2000). Why glyphosate is a global herbicide: strengths, weaknesses and prospects. *Pest Management Science: Formerly Pesticide Science*, 56(4), 299-308. Beckie, H. J., & Harker, K. N. (2017). Our top 10 herbicide-resistant weed management practices. *Pest management science*, 73(6), 1045-1052.
- Beckie, H. J., & Harker, K. N. (2017). Our top 10 herbicide-resistant weed management practices. *Pest Management Science*, 73(6), 1045-1052.
- Beckie, H. J., & Tardif, F. J. (2012). Herbicide cross resistance in weeds. *Crop Protection*, 35, 15-28.
- Busi, R., Gaines, T. A., Vila-Aiub, M. M., & Powles, S. B. (2014). Inheritance of evolved resistance to a novel herbicide (pyroxasulfone). *Plant Science*, 217, 127-134.
- Cahoon, C. (2016). *Distribution of herbicide-resistant Italian ryegrass in eastern Virginia*. Virginia Cooperative Extension, Virginia Polytechnic Institute and State University,

Blacksburg, VA. <https://ag-pest-advisory.vaes.vt.edu/distribution-of-herbicide-resistant-italian-ryegrass-in-eastern-virginia/>

- Campagna, C., Gasparetto, M. A., & Sattin, M. (2001). Situation and management of grass weeds resistant to ACCase inhibitors in Italy. *In Proceedings of the Brighton Conference on Weeds*. Farnham, UK: BCPC Registered Office (pp. 755-762).
- Carson, K. H., Cralle, H. T., Chandler, J. M., Miller, T. D., Bovey, R. W., Senseman, S. A., & Stone, M. J. (1999). *Triticum aestivum* and *Lolium multiflorum* interaction during drought. *Weed Science*, 47(4), 440–445.
- Chandi, A., York, A. C., Jordan, D. L., & Beam, J. B. (2011). Resistance to acetolactate synthase and acetyl Co-A carboxylase inhibitors in North Carolina Italian ryegrass (*Lolium perenne*). *Weed Technology*, 25(4), 659-666.
- Dailey, F. E., & Cronan Jr, J. E. (1986). Acetohydroxy acid synthase I, a required enzyme for isoleucine and valine biosynthesis in *Escherichia coli* K-12 during growth on acetate as the sole carbon source. *Journal of Bacteriology*, 165(2), 453-460.
- Délye, C. (2005). Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Science*, 53(5), 728-746.
- Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*, 29(11), 649-658.
- Ellis, A. T., Steckel, L. E., Main, C. L., De Melo, M. S., West, D. R., & Mueller, T. C. (2010). A survey for diclofop-methyl resistance in Italian ryegrass from Tennessee and how to manage resistance in wheat. *Weed Technology*, 24(3), 303-309.
- Elmore, C. D. (1986). Weed survey—southern states. Res. Rep. South. *Weed Sci. Soc*, 39, 136-158.
- Flessner, M. L., Burke, I. C., Dille, J. A., Everman, W. J., VanGessel, M. J., Tidemann, B., Manuchehri, M. R., Soltani, N., & Sikkema, P. H. (2021). Potential wheat yield loss due to weeds in the United States and Canada. *Weed Technology*, 35(6), 916–923.
- Franz, J. E., Mao, M. K., & Sikorski, J. A. (1997). Glyphosate: a unique global herbicide (pp. xv+-653).
- Gressel, J. (2009). Evolving understanding of the evolution of herbicide resistance. *Pest Management Science: formerly Pesticide Science*, 65(11), 1164-1173.
- Grey, T. L., & Bridges, D. C. (2003). Alternatives to diclofop for the control of Italian ryegrass (*Lolium multiflorum*) in winter wheat (*Triticum aestivum*). *Weed Technology*, 17(2), 219-223.

- Grey, T. L., Cutts III, G. S., Sosnoskie, L., & Culpepper, A. S. (2012). Italian ryegrass (*Lolium perenne*) control and winter wheat response to POST herbicides. *Weed Technology*, 26(4), 644-648.
- Grey, T. L., & Newsom, L. J. (2017). Winter Wheat Response to Weed Control and Residual. Wheat Improvement, *Management and Utilization*, 191.
- Hand, L. C., Randell, T. M., & Culpepper, A. S. (2022). Cereal rye response to eight commonly used wheat herbicides. *Weed Technology*, 36(4), 506-511.
- Hart, J. (2021, September 29). Resistant Italian ryegrass popping up across North Carolina. Farm Progress. <https://www.farmprogress.com/weeds/resistant-italian-ryegrass-popping-up-across-north-carolina>
- Heap, I. (2025). International Survey of Herbicide Resistant Weeds. <https://weedsociety.org>
- Hart, J. (2021, September 29). Resistant Italian ryegrass popping up across North Carolina. Farm Progress. <https://www.farmprogress.com/weeds/resistant-italian-ryegrass-popping-up-across-north-carolina>
- Hofer, U., Muehlebach, M., Hole, S., & Zoschke, A. (2006). Pinoxaden for broad-spectrum grass weed management in cereal crops. *Journal of Plant Diseases and Protection, Special Issue*, 20, 989–995.
- Hoskins, A. J., Young, B. G., Krausz, R. F., & Russin, J. S. (2005). Control of Italian ryegrass (*Lolium multiflorum*) in winter wheat. *Weed Technology*, 19(2), 261-265.
- Hulting, A. G., Dauer, J. T., Hinds-Cook, B., Curtis, D., Koepke-Hill, R. M., & Mallory-Smith, C. (2012). Management of Italian ryegrass (*Lolium perenne ssp. multiflorum*) in western Oregon with preemergence applications of pyroxasulfone in winter wheat. *Weed Technology*, 26(2), 230-235.
- Jones, E. A., Taylor, Z. R., & Everman, W. J. (2021). Distribution and control of herbicide-resistant Italian ryegrass [*Lolium perenne* L. *ssp. multiflorum* (Lam.) Husnot] in winter wheat (*Triticum aestivum* L.) in North Carolina. *Frontiers in Agronomy*, 2, 601917.
- Jones, G. T., Norsworthy, J. K., Barber, T., Gbur, E., & Kruger, G. R. (2019). Off-target Movement of DGA and BAPMA Dicamba to Sensitive Soybean. *Weed Technology*, 33(1), 51–65. <https://doi.org/10.1017/wet.2018.121>
- Kaya Altop, E., Erken Meral, S., Zandstra, B. H., & Mennan, H. (2022). Target-site point mutation conferring resistance to ALS herbicides in Italian ryegrass (*Lolium multiflorum* L.). *Phytoparasitica*, 50(5), 1133-1142.

- Kaundun, S. S. (2014). Resistance to acetyl-CoA carboxylase-inhibiting herbicides. *Pest Management Science*, 70(9), 1405-1417.
- Konishi, T., Shinohara, K., Yamada, K., & Sasaki, Y. (1996). Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. *Plant and Cell Physiology*, 37(2), 117-122.
- Kuk, Y. I., & Bugos, N. R. (2007). Cross-resistance profile of mesosulfuron-methyl-resistant Italian ryegrass in the southern United States. *Pest Management Science: formerly Pesticide Science*, 63(4), 349-357.
- Kuk, Y. I., Burgos, N. R., & Scott, R. C. (2008). Resistance profile of diclofop-resistant Italian ryegrass (*Lolium multiflorum*) to ACCase-and ALS-inhibiting herbicides in Arkansas, USA. *Weed Science*, 56(4), 614-623.
- Kukorelli, G., Reisinger, P., & Pinke, G. (2013). ACCase inhibitor herbicides—selectivity, weed resistance and fitness cost: a review. *International Journal of Pest Management*, 59(3), 165-173.
- Liu, W., Harrison, D. K., Chalupska, D., Gornicki, P., O'donnell, C. C., Adkins, S. W., ... & Williams, R. R. (2007). Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. *Proceedings of the National Academy of Sciences*, 104(9), 3627-3632.
- Matzrafi, M., Preston, C., & Brunharo, C. A. (2021). evolutionary drivers of agricultural adaptation in *Lolium* spp. *Pest Management Science*, 77(5), 2209-2218.
- McCullough, P. (2024). *Annual ryegrass control in Georgia hayfields* (UGA Extension Circular No. 1078). University of Georgia Cooperative Extension. <https://extension.uga.edu/publications/detail.html?number=C1078>
- Nakka, S., Jugulam, M., Peterson, D., & Asif, M. (2019). Herbicide resistance: Development of wheat production systems and current status of resistant weeds in wheat cropping systems. *The Crop Journal*, 7(6), 750-760.
- Preston, C., Wakelin, A. M., Dolman, F. C., Bostamam, Y., & Boutsalis, P. (2009). A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness, and agronomic management. *Weed Science*, 57(4), 435-441.
- Rangani, G., Langaro, A. C., Agrawal, S., Salas-Perez, R. A., Velásquez, J. C., Nelson, C. E., & Roma-Burgos, N. (2024). Resistance to Acetyl Coenzyme A Carboxylase (ACCase) Inhibitor in *Lolium multiflorum*: Effect of Multiple Target-Site Mutations. *Agronomy*, 14(10), 2316.

- Roberson 2, R. (2010, November 22). Herbicide-resistant wheat varieties tested in Virginia. Farm Progress. <https://www.farmprogress.com/corn/herbicide-resistant-wheat-varieties-tested-in-virginia>.
- Rosinger, C., & Schulte, W. (2019). Safeners for herbicides. *Modern Crop Protection Compounds*, 1, 425-450.
- Garetson, R., Singh, V., Singh, S., Dotray, P., & Bagavathiannan, M. (2019). Distribution of herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in row crop production systems in Texas. *Weed Technology*, 33(2), 355-365.
- Salas, R. A., Burgos, N. R., Mauromoustakos, A., Lassiter, R. B., Scott, R. C., & Alcober, E. A. (2013). Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. *multiflorum* in the United States.
- Sapkota, B., Singh, V., Neely, C., Rajan, N., & Bagavathiannan, M. (2020). Detection of Italian ryegrass in wheat and prediction of competitive interactions using remote-sensing and machine-learning techniques. *Remote Sensing*, 12(18), 2977.
- Sasaki, Y., Konishi, T., & Nagano, Y. (1995). The compartmentation of acetyl-coenzyme A carboxylase in plants. *Plant Physiology*, 108(2), 445.
- Schilling, A. S., Abaye, A. O., Griffey, C. A., Brann, D. E., Alley, M. M., & Pridgen, T. H. (2003). Adaptation and performance of winter durum wheat in Virginia. *Agronomy Journal*, 95(3), 642-651.
- Shaner, D. L., Anderson, P. C., & Stidham, M. A. (1984). Imidazolinones: potent inhibitors of acetohydroxyacid synthase. *Plant Physiology*, 76(2), 545-546.
- Singh, V., Viric, M., Brar, A., & Flessner, M. (2025, June 3). *Management of Italian ryegrass in wheat* (Virginia Cooperative Extension Publication SPES-697). Virginia Cooperative Extension, Virginia Tech. <https://www.pubs.ext.vt.edu/SPES/spes-697/spes-697.html>
- Sosnoskie, L. M., Herms, C. P., & Cardina, J. (2006). Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. *Weed Science*, 54(2), 263-273.
- Stanger, C. E., & Appleby, A. P. (1989). Italian ryegrass (*Lolium multiflorum*) accessions tolerant to diclofop. *Weed Science*, 37(3), 350-352.
- Stone, M. J., Cralle, H. T., Chandler, J. M., Miller, T. D., Bovey, R. W., & Carson, K. H. (1998). Above-and belowground interference of wheat (*Triticum aestivum*) by Italian ryegrass (*Lolium multiflorum*). *Weed Science*, 46(4), 438-441.

- Takano, H. K., Ovejero, R. F. L., Belchior, G. G., Maymone, G. P. L., & Dayan, F. E. (2020). ACCase-inhibiting herbicides: mechanism of action, resistance evolution and stewardship. *Scientia Agricola*, 78(1), e20190102.
- Tanetani, Y., Kaku, K., Kawai, K., Fujioka, T., & Shimizu, T. (2009). Action mechanism of a novel herbicide, pyroxasulfone. *Pesticide Biochemistry and Physiology*, 95(1), 47-55.
- Tehranchian, P., Nandula, V. K., Matzrafi, M., & Jasieniuk, M. (2019). Multiple herbicide resistance in California Italian ryegrass (*Lolium perenne* ssp. *multiflorum*): characterization of ALS-inhibiting herbicide resistance. *Weed Science*, 67(3), 273-280.
- Tranel, P. J., & Wright, T. R. (2002). Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed science*, 50(6), 700-712.
- Trenkamp, S., Martin, W., & Tietjen, K. (2004). Specific and differential inhibition of very-long-chain fatty acid elongases from *Arabidopsis thaliana* by different herbicides. *Proceedings of the National Academy of Sciences*, 101(32), 11903-11908.
- USDA NASS. (2022). 2022 census of agriculture. www.nass.usda.gov/AgCensus.
- United States Department of Agriculture, Natural Resources Conservation Service. (2015). Plant Chat: The lowdown on annual ryegrass (*Lolium perenne* ssp. *multiflorum*) (Plant Chat Summer 2015, Vol. 15, Issue 3). Bismarck Plant Materials Center. <https://www.nrcs.usda.gov/plantmaterials/ndpmonl12668.pdf>
- Vila-Aiub, M. M., Neve, P., & Powles, S. B. (2009). Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist*, 184(4), 751-767.
- Webster, T. M., & Nichols, R. L. (2012). Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. *Weed Science*, 60(2), 145-157.
- Woodburn, A. T. (2000). Glyphosate: production, pricing and use worldwide. *Pest Management Science: formerly Pesticide Science*, 56(4), 309-312.
- Wyche, L. V. (2023). Survey of the most common and troublesome weeds in grass crops, pasture & turf in the United States and Canada (*Weed Science Society of America National Weed Survey dataset*). <https://wssa.net/weed/surveys/>
- Yadav, A., Russell, D. P., Ganie, Z., Patel, J., Price, A. J., & Maity, A. (2025). Detection of herbicide resistance and a novel ALS-inhibitor mutation in Alabama Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) populations. *Weed Science*, 73(1), e66.

2.7 Figures & Tables

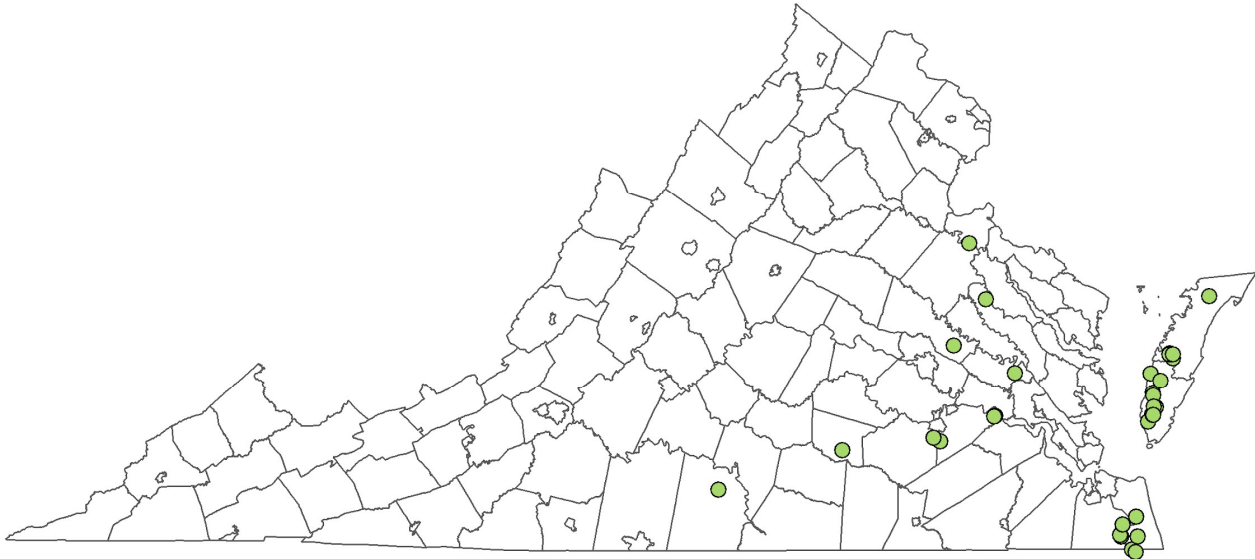


Figure 2.1. Geographical distribution of Italian ryegrass populations in Virginia sampled in 2021 and 2024.

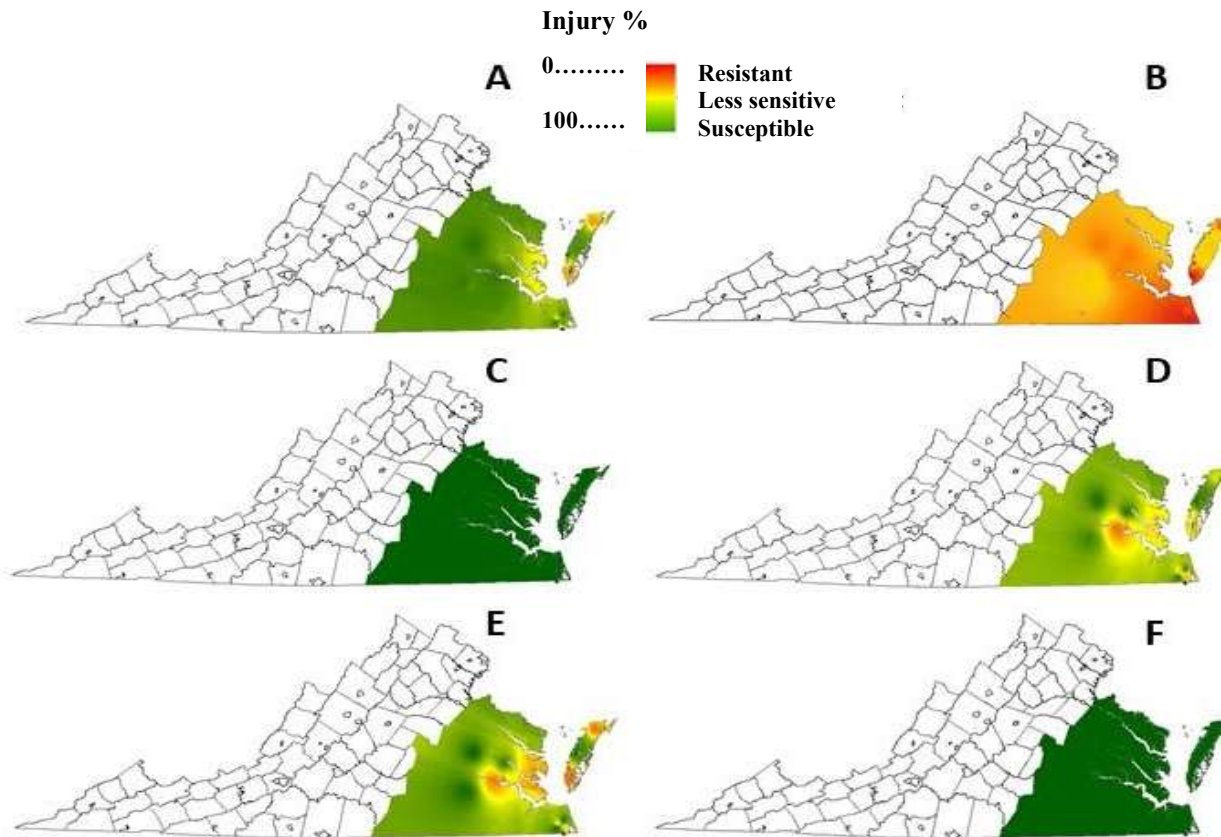


Figure 2.2. Spatial distribution of herbicide injury based on inverse distance weighted (IDW) interpolation of injury observations for six herbicides: (A) pinoxaden, (B) diclofop-methyl, (C) glyphosate, (D) mesosulfuron-methyl, (E) pyroxsulam, and (F) pyroxasulfone.

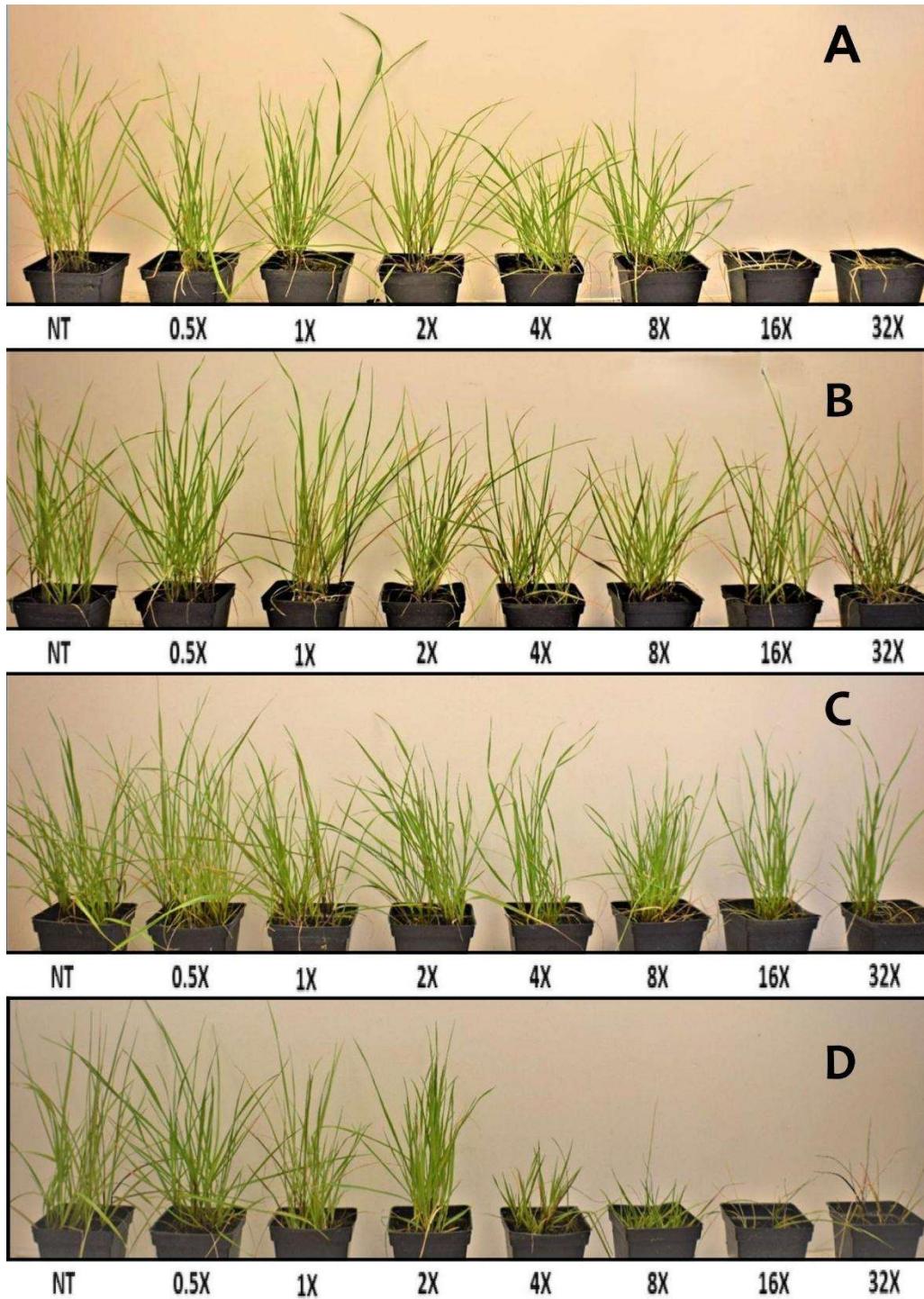


Figure 2.3. Response of highly resistant Italian ryegrass populations IR-14 (A, B), IR-2 (C) and IR-1 (D) to 0.5X-32X rates of pinoxaden (A), diclofop-methyl (B), mesosulfuron (C), and pyroxsulam (D), where NT = non-treated control.

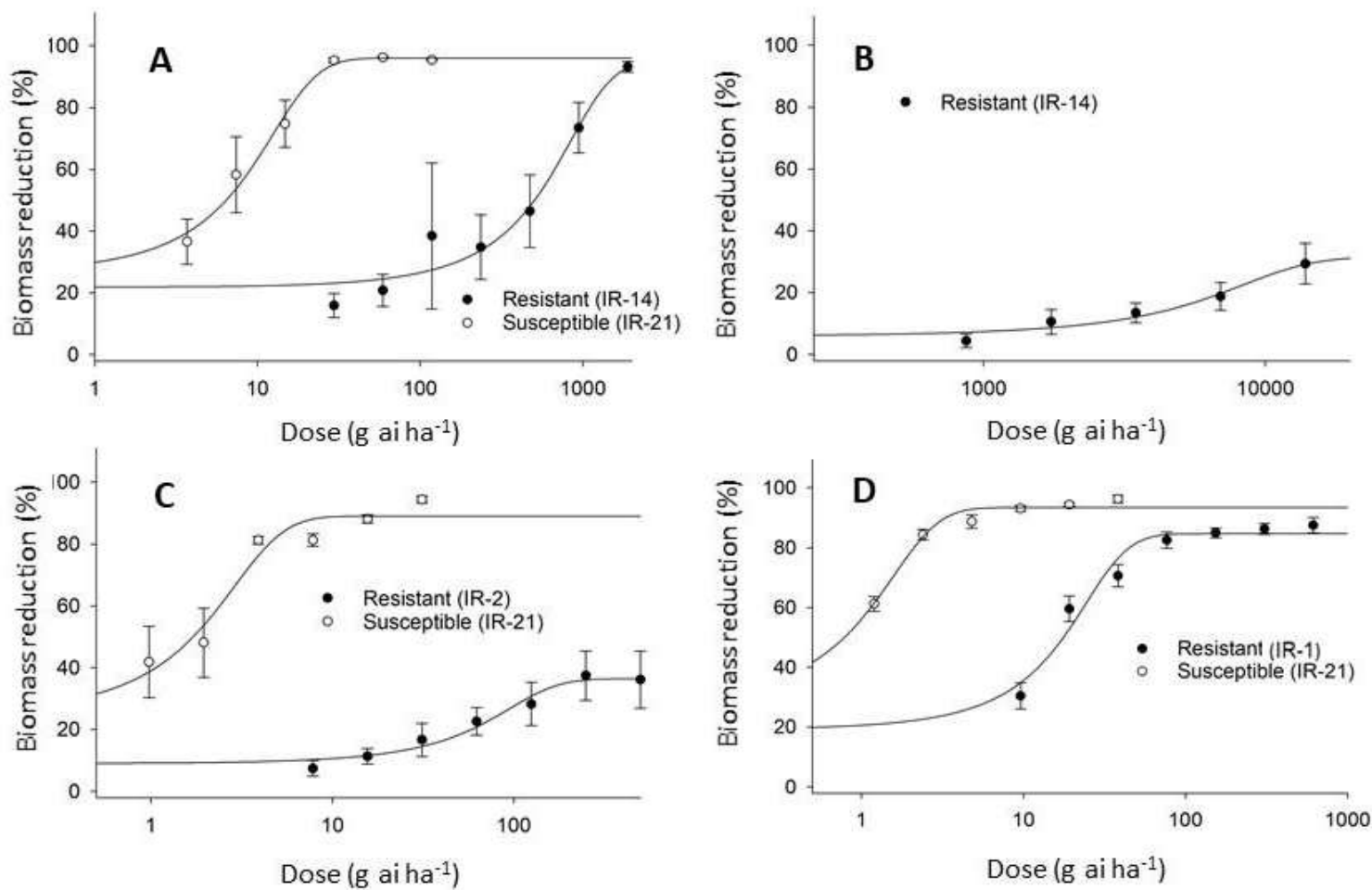


Figure 2.4. Dose response curves of the highly-resistant and susceptible Italian ryegrass populations to (A) pinoxaden, (B) diclofop-methyl, (C) mesosulfuron-methyl and (D) pyroxsulam. Biomass reductions were expressed as dry biomass of treated plants relative to their respective nontreated controls.

Table 2.1. Herbicides and their respective doses used for screening.

S. No.	Common Name	Trade Name	Manufacturer	Mode of action ¹	Dose 1x (g ai or ae/ha)	Application timing ²
1	pinoxaden	Axial XL	Syngenta Crop Protection	ACCCase	59	POST
2	diclofop	Hoelon	Bayer CropScience	ACCCase	545	POST
3	glyphosate	Roundup Powermax	Bayer CropScience	EPSPS	867	POST
4	mesosulfuron	Osprey	Bayer CropScience	ALS	15.6	POST
5	pyroxsulam	Powerflex HL	Corteva Agrosience	ALS	18	POST
6	pyroxasulfone	Zidua	BASF Agricultural Solutions	LCFA	119	PRE

¹ACCCase, acetyl-coenzyme-A-carboxylase inhibitors; ALS, acetolactate synthase inhibitors; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase inhibitors; LCFA, long-chain fatty acid inhibitors

²POST, postemergence; PRE, preemergence

Table 2.2. Categorization of Italian ryegrass populations based on herbicide injury evaluated in greenhouse screening at 1X field herbicide rates during 2023 and 2024.

Herbicide ¹	Resistant ²	Less sensitive ³	Susceptible ⁴
	-----% of total populations ⁵ -----		
pinoxaden	31	19	50
diclofop-methyl	84	16	0
glyphosate	0	0	100
mesosulfuron-methyl	44	9	47
pyroxsulam	22	37	41
pyroxasulfone	0	0	100

¹1X field herbicide rates (g ai or ae ha⁻¹: pinoxaden 59; diclofop-methyl 545; glyphosate 867; mesosulfuron-methyl 15.6; pyroxsulam 18; pyroxasulfone 119)

²Resistant defined as populations exhibiting a response to herbicide with injury levels ranging from 0% - 49%

³Less sensitive defined as populations exhibiting a response to herbicide with injury levels ranging from 50% - 79%

⁴Susceptible defined as populations exhibiting a response to herbicide with injury levels ranging from 80% - 100%

⁵ A total of thirty-two populations of Italian ryegrass were tested

Table 2.3. Dose response summary table with GR₅₀ values and R/S ratios for selected Virginia Italian ryegrass populations.

Herbicide	Population ¹	R ²	RMSE	-----g ai ha ⁻¹ -----		R/S
				GR ₅₀	SEM	
pinoxaden	R (IR-14)	0.57	22.7	505.5	84.5	79
	S (IR-21)	0.61	18.5	6.4	1.13	
diclofop-methyl	R (IR-15)	0.31	25.2	3,748.7	1,248.9	-
	R (IR-14)	0.20	15.4	>17,440	-	-
mesosulfuron-methyl	R (IR-2)	0.31	16.3	>499.2	-	-
	R (IR-14)	0.31	25.3	49.8	16.9	29.3
	S (IR-21)	0.37	22.5	1.7	0.6	
pyroxsulam	R (IR-1)	0.77	14.3	79	8.8	98.8
	R (IR-14)	0.64	9.4	5.8	3.8	7.3
	S (IR-21)	0.85	5.1	0.8	0.11	-

¹R, resistant population; S, susceptible population; RMSE, root square mean error; R/S, ratio of GR₅₀ for resistant and susceptible populations; SEM, standard error of the mean

²GR₅₀ refers to herbicide concentration which reduces plant growth by 50%, based on the above dry biomass 3 weeks after treatment. Values have been rounded to the nearest decimal point

Table 2.4. Cross and multiple resistance to ACCase- and ALS- inhibiting herbicides in 32 Italian ryegrass populations from the Eastern Virginia region based on the results of the initial greenhouse screening at 1X field herbicide rates.

Herbicide mode of action (MOA) combinations ¹	Herbicide active ingredient combinations ²	Populations with cross or multiple resistance ³	
		Count	%
ACCase (den) + ACCase (fop)	pinoxaden + diclofop	9	28
ACCase (den) + ALS	pinoxaden + mesosulfuron	7	22
ACCase (den) + ALS	pinoxaden + pyroxsulam	2	6
ACCase (fop) + ALS	diclofop + mesosulfuron	13	41
ACCase (fop) + ALS	diclofop + pyroxsulam	7	22
ALS + ALS	mesosulfuron + pyroxsulam	7	22
ACCase (den) + ACCase (fop) + ALS + ALS	pinoxaden + diclofop x mesosulfuron + pyroxsulam	2	6

¹ACCase, acetyl-coenzyme-A-carboxylase; ALS, acetolactate synthase; den, phenylpyrazole family; fop; aryloxyphenoxypropionate family.

²1X field herbicide rates (g ai or ae ha⁻¹: pinoxaden 59; diclofop-methyl 545; glyphosate 867; mesosulfuron-methyl 15.6; pyroxsulam 18; pyroxasulfone 119

³Cross-resistance is referred as resistance to different herbicide families within the same herbicide SOA; multiple-resistance is referred as resistance to minimum two unique herbicide

3. Distribution and Characterization of Herbicide-Resistant Palmer amaranth in Virginia

3.1 Abstract

Palmer amaranth poses a significant challenge for management in row-crop production systems in Virginia, making it one of the most problematic weeds due to its rapid growth, prolific seed production and widespread herbicide resistance to multiple modes of action. From 2022 to 2024, field surveys were conducted aimed at understanding the distribution of herbicide-resistant Palmer amaranth. A total of 68 field populations were collected and subsequently screened in greenhouse for sensitivity to trifloxysulfuron, glyphosate, glufosinate, fomesafen, atrazine, mesotrione, 2,4-D, and dicamba at 1X labeled field rate. Palmer amaranth populations with the most resistant populations were further evaluated in dose-response assays using six different rates (0.5, 1, 2, 4, 8 and 16X), while a known susceptible population was tested at five rates (0.125, 0.25, 0.5, 1, and 2X). Survivors were characterized as resistant (0-49% injury), less sensitive (50-79% injury), and susceptible (80-100% injury). Greenhouse screenings revealed resistance to ALS-, EPSPS-, PSII-, PPO-inhibitors and synthetic auxin herbicide, in 70%, 86%, 10%, 4%, and 1% of the total number of populations respectively, with several populations exhibiting multiple resistance. The ratio of I_{50} values indicated that the most resistant population had 14-, 18-, and 47-fold resistance to fomesafen, atrazine, and trifloxysulfuron, respectively. The glyphosate-resistant population had I_{50} value of 1,238 g ae ha⁻¹; however, no susceptible standard was available to compare but this value is approximately 43% higher when compared to 1X field dose (868 g ae ha⁻¹). Overall, these findings confirm the widespread distribution of ALS- and EPSPS-resistant Palmer amaranth across Virginia, and highlight the

severity of resistance evolution in this species. The presence of multi-resistant populations presents a substantial threat to sustainable crop production and underscores the urgency of implementing diversified, integrated weed management strategies.

3.2 Introduction

In the last few decades, Palmer amaranth (*Amaranthus palmeri* S.Watson) has emerged as one of the most problematic, difficult-to-control weeds due to its rapid evolution of herbicide resistance and hence widely studied weed species in the major row-crop production systems. According to a survey conducted by the Weed Science Society of America, Palmer amaranth emerged as the most troublesome weed species in all crops, fruits and vegetables in 2025 in the United States (Van Wychen, 2025). Palmer amaranth is native to the southwestern United States including New Mexico, Texas, and southern California and parts of northwestern Mexico. However, its presence outside its native range was reported in Virginia in 1915 (Bryson & DeFelice, 2009; Sauer, 1957).

Palmer amaranth is an upright, dioecious, branching, summer annual, broadleaf weed species capable of exceeding two meters in height. Female Palmer amaranth plants can produce up to 600,000 seeds, which can remain dormant in the soil for multiple years and can yet be viable for approximately three to four years (Keeley et al., 1987). Its ability to persist, and season long competitiveness is further enhanced by its extended emergence window, which spans from May to August in the Midwest and could extend into September in the Southeastern United States (Jha et al., 2008; Spaunhorst et al., 2014). The competitive ability of Palmer amaranth is also supported by its quick growth rate of 0.18 - 0.21 cm per growing degree days (Horak & Loughin, 2000) and its exceptionally high photosynthetic rate ($81 \mu\text{mol m}^{-2} \text{s}^{-1}$), which is about three to four times higher than that of crops like cotton or soybean (Ehleringer, 1983; Gibson, 1998). This exceptionally high photosynthetic capacity enables Palmer amaranth to fix carbon more rapidly,

resulting in faster biomass accumulation and canopy development, which in turn allows it to outcompete crops for light, water, and nutrients and quickly dominate the crop canopy.

Furthermore, wind-mediated pollination facilitates long-distance dispersal of resistance alleles, promoting the spread of herbicide resistance (Franssen et al., 2001; Ward et al., 2013). Collectively, these biological and ecological traits allow Palmer amaranth to aggressively compete with crops for sunlight, moisture, nutrients, and space, often resulting in substantial yield losses across all major crops. In corn production, Palmer amaranth at a density of eight plants m^{-1} has been associated with yield reduction of up to 91%, with losses driven by simultaneous crop-weed emergence rather than weed density alone (Massinga et al., 2001). In cotton, Palmer amaranth densities of up to 10 plants in a 9.1 m of row length have caused a lint yield reduction of 11% and 59%, respectively (Morgan et al., 2001). Similarly, in another study, interaction between soybean and Palmer amaranth emergence shows reduction in soybean yield to an extent of 15-22% with Palmer amaranth plants emerging simultaneously with soybean or within a period of one week. However, these yield reductions were found to decrease progressively with later weed emergences during the season (Korres et al., 2020). In another study, under high Palmer amaranth infestation levels of eight plants per meter of row, soybean yield reduction up to 79% have been observed (Bensch et al., 2003). Diversified weed management strategies are available for weed control and despite the fact there are various non-chemical options present including cultural, mechanical, genetic and biological methods, herbicides will always have an important place in a integrated weed management strategy (Fernández-Quintanilla et al., 2018; Moss, 2019; Shaner, 2014; Swanton & Weise, 1991).

Repeated reliance on herbicides belonging to the same site of action increases selection pressure on weeds and promotes evolution of resistance. Under sustained selection pressure,

Palmer amaranth has become a prominent example of weeds that quickly develop resistance to multiple herbicide modes of action. One of the earliest confirmed cases involved resistance to Group 3 herbicides, which inhibit microtubule assembly and are mostly used as preemergence herbicides and dicot plants tend to be less sensitive compared to monocots (Hess, 1987; Molin & Khan, 1997; Vaughn, 2000). In 1989, trifluralin-resistant populations were confirmed at eight locations across South Carolina, with varying levels of cross-resistance to four other herbicides of dinitroaniline family (Gossett et al., 1992). To date, Palmer amaranth has evolved resistance to herbicides from nine different modes of action. These include Group 2 (Acetolactate Synthase Inhibitors), Group 3 (Microtubule Assembly Inhibitors), Group 4 (Auxin Mimics), Group 5 (PSII inhibitors), Group 9 (Enolpyruvyl Shikimate Phosphate Synthase Inhibitors), Group 10 (Glutamine Synthetase Inhibitors), Group 14 (Protoporphyrinogen Oxidase Inhibitors), Group 15 (Very Long-Chain Fatty Acid Synthesis Inhibitors) and Group 27 (Hydroxyphenyl Pyruvate Dioxygenase Inhibitors) (Heap 2025).

The introduction and widespread usage of Group 2 acetolactate synthase (ALS) inhibiting herbicides since early 1980s further contributed to selection pressure in Palmer amaranth (Gaeddert et al., 1997). ALS inhibitors work by disrupting the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine which leads to inhibition of cell division and plant growth (Whitcomb, 1999). This herbicide group is characterized by exceptionally high potency at very low application rates (Whitcomb, 1999). Regardless, often use of ALS inhibitors have led to the rapid evolution of resistance in high number of weed species (Mallory-Smith et al., 1990; Primiani et al., 1990). In 1993, Palmer amaranth's resistance to Group 2 herbicides was first reported in Kansas (Horak & Peterson 1995). Palmer amaranth resistant to ALS-inhibiting herbicides has become widely established across the southern parts of the United States (Bond et

al., 2006), with reports from Arkansas in 1994, followed by North Carolina, South Carolina, and Georgia in 1995, 1997 and 2000 respectively, and later in Mississippi and Florida by 2008 (Burgos et al. 2001; Heap 2025; Horak and Peterson 1995; Vencill et al. 2002). Resistance to ALS-inhibiting herbicides in Palmer amaranth is often expressed at high levels. Early studies from Kansas documented Palmer amaranth populations surviving imazethapyr and thifensulfuron applications at up to eight times the labeled field rates (Horak and Peterson 1995). In Arkansas, Burgos et al. (2001) reported even greater resistance levels, with an imazaquin-resistant population exhibiting a 117-fold increase in resistance to pyriithiobac relative to a susceptible biotype, along with cross-resistance to multiple ALS-inhibiting chemistries. Also, cross-resistance to different chemistries of Group 2 herbicides has often been observed in all weed species, specially, Palmer amaranth (Burgos et al., 2001; Gaeddert et al., 1997). This type of resistance means that plant develops resistance to several other herbicides within the same mechanism of action (Hall et al., 2018).

Glyphosate is a non-selective, systemic herbicide that inhibits enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS). A very good mobility in the plant, and capability to reach every meristem, high use rate, affordable price and invention of crops tolerant to it led to high demand for this herbicide (Duke, 2018). The introduction of glyphosate-resistant (GR) crops initially offered farmers an effective substitute for managing Palmer amaranth populations resistant to Group 2 herbicides. However, overuse of glyphosate soon led to the evolution of glyphosate-resistant Palmer amaranth populations. The first confirmed case of GR Palmer amaranth was reported in Georgia in 2005 in cotton fields (Culpepper et al., 2006) followed by a rapid geographic spread of resistance in Palmer amaranth in North Carolina, Tennessee, Illinois, Arkansas, Michigan, South Carolina, and Virginia (Heap 2025; Nandula et al. 2012; Norsworthy

et al., 2008; Steckel et al., 1997; York et al. 2007). To date, about 34 US states have reported resistance to glyphosate (Heap 2025). In 2009, Virginia soybean growers provided early evidence of failures to control Palmer amaranth with glyphosate in Greensville County (Ahmed, 2011), which was later confirmed in 2011 (Heap 2025). Palmer amaranth populations that are resistant to glyphosate have developed in farming systems that have been subjected to frequent glyphosate applications with minimal variation in weed control approaches (Beckie 2011; Culpepper et al. 2006).

The evolution of ALS and glyphosate resistance in Palmer amaranth has increased reliance on protoporphyrinogen oxidase (PPO) inhibiting (Group 14) herbicides as an effective tool for pre-emergence (PRE) and post-emergence (POST) weed management in row crops particularly in soybean and cotton. Most of the Group 14 herbicides function as contact herbicides and are applied postemergence, resulting in restricted movement within the plant from the treated areas. However, a few PPO inhibitors, such as flumioxazin and sulfentrazone are used for pre-emergence control. Fomesafen, another Group 14 herbicide with both pre-emergence and post-emergence activity, experienced a substantial increase in use following the spread of glyphosate-resistant Palmer amaranth (Whitaker et al. 2010). Despite the fact of fomesafen being very potent on Palmer amaranth, resistance was soon reported across multiple US states led by Arkansas in 2011, Missouri and Tennessee in 2015, Indiana and Illinois in 2016, Kansas in 2021, and North Carolina in 2025 (Heap 2025; Salas et al. 2016; Umphres et al. 2018).

Photosystem-II (PSII) inhibitor (Group 5) herbicides act by binding to QB site of Photosystem-II, blocking electron transport and decreasing production of NADPH which results in decreased photosynthetic carbon fixation (Oettmeier, 1999). This group, particularly atrazine, have long served as foundational tool in corn weed management and is supported by corn's natural

ability to detoxify atrazine via glutathione *S*-transferase (GST)-mediated conjugation (Shimabukuro et al. 1970). However, extensive use has driven the evolution of atrazine resistance in Palmer amaranth, which has a similar GST-based metabolic detoxification pathway (Nakka et al., 2017). Resistance to atrazine was first reported in Texas in 1993 followed by cases of resistance in Kansas in 1995 (Heap, 2025; Peterson, 1999). Resistant biotype of Palmer amaranth can detoxify atrazine 24-fold higher than a susceptible biotype (Nakka et al., 2017). Subsequently, resistance to atrazine was reported in Georgia in 2008 (Vencill, 2008). The most recent cases of atrazine resistance were found in North Carolina in 2022 and New York in 2024 whereas atrazine resistance was also reported in Nebraska in 2011 and 2016 separately (Chahal et al., 2019; Heap, 2025; Jhala et al., 2014). From 1995 to 2008, reports of resistance to atrazine slowed as farmers adapted quickly to GR crops and used a ‘one shot tool’ glyphosate to control weeds. However, reliance on atrazine resurged due to escalating glyphosate resistance in Palmer amaranth. Atrazine now ranks as the herbicide with the highest number of resistant weed species globally, with 66 species documented (Heap, 2025).

In addition to PSII inhibitors, herbicides that inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD) have become an essential tool in corn production systems (Bollman et al., 2008; Chahal et al., 2019, 2015; Fleming et al., 1988; Swanton et al., 2007). HPPD inhibitors are considered highly valuable because they exhibit strong synergistic interactions with herbicides from other herbicide groups like atrazine, resulting in broadened weed control spectrum and enhanced efficacy (Singh et al., 2018; Sutton et al., 2002). Mesotrione is a commonly used HPPD inhibiting herbicide that showed effective control of ALS-, glyphosate- and atrazine-resistant weeds (Ganie & Jhala, 2017; Sutton et al., 2002). However, Palmer amaranth biotypes resistant to mesotrione have been reported across Midwest (Kansas, Nebraska, Iowa) and the Southeast (North

Carolina) (Heap, 2025). In Kansas, Palmer amaranth populations have been found to exhibit resistance to multiple HPPD-inhibiting herbicides; however, the precise mechanism and pattern of inheritance of resistance remains unclear (Thompson et al., 2012). Concurrently, efforts to develop and commercialize HPPD-tolerant crops such as tobacco, soybean and cotton are ongoing (Hawkes et al., 2001, 2019; Jhala et al., 2023; Siehl et al., 2014; Singh et al., 2018). Introduction and adoption of these technologies will likely elevate HPPD inhibitor usage which will further intensify the selection pressure and increase the risk of multiple-resistant weed populations and herbicide-tolerant volunteer crops (Jhala et al., 2021; Singh et al., 2018).

Glufosinate-tolerant crops introduced an additional and effective tool for managing Palmer amaranth. Glufosinate is a non-selective, contact herbicide that provides broad-spectrum weed control and rapidly induces plant tissue necrosis. It is used in glufosinate-tolerant crops such as soybean, canola, cotton and corn (Takano et al., 2019). The incorporation of diverse herbicide tank mixtures containing multiple modes of action has been instrumental in slowing the evolution of glufosinate resistance. To date, confirmed cases of glufosinate-resistant Palmer amaranth have been limited to Arkansas in 2020 and North Carolina in 2022 (Heap, 2025; Jones et al., 2022; Preiss et al., 2022). Effective control of Palmer amaranth with glufosinate is strongly influenced by weed growth stage and timing of application. According to the product label, application should be made when plants are less than 7.5 cm tall, as control declines once plants exceed 10 cm (Jones et al., 2022; Steckel et al., 1997). Consequently, delayed applications that target larger plants increase the likelihood of reduced efficacy and may contribute to the selection of resistance.

Auxin mimic herbicides represent another major class of herbicides that has become important for controlling Palmer amaranth resistant to other herbicide groups. They provide selective control of dicot weeds, systemic activity and act by mimicking indole-3-acetic acid which

disrupts hormonal balance and affects the plant vascular tissue (Grossmann, 2010; Sterling & Hal, 1997). Herbicides such as 2,4-D, and dicamba are used globally and rank just behind ALS- and EPSPS-inhibitors (Busi et al., 2018). Some of the most commonly used herbicides from this group are dicamba and 2,4-D (Todd et al., 2020). Although the first case of resistance in weeds to herbicides from this group was reported in 1957, resistance in Palmer amaranth is more recent. Resistance in Palmer amaranth to 2,4-D was confirmed in Kansas in 2015, 2018 and 2021 and there was a recent report from North Carolina as well (Argueta Menendez, 2025; Heap, 2025; Kumar et al., 2019). Regardless the assertions that 2,4-D is a less prone to resistance evolution, the adoption of auxin tolerant crops intensified selection pressure considering the expanded acreage treated with these herbicides (Egan et al., 2011; Moreno-Serrano et al., 2024; Torra et al., 2024). Dicamba-resistant Palmer amaranth populations have been reported in Tennessee and Arkansas between 2020 and 2023 (Foster & Steckel, 2022; Heap, 2025).

The continued reliance on a single mode of action (MOA) herbicides, followed by a shift to a new MOA only after resistance evolves, has exacerbated the evolution of multiple-herbicide resistance in weed populations. Some of the first cases of multiple resistance in Palmer amaranth were reported in Georgia and Mississippi when the populations were found resistant to both glyphosate and ALS-inhibitor pyrithiobac-sodium (Bond et al., 2010; Sosnoskie et al., 2011). Since then, reports of multiple resistance have increased across the United States, posing challenges because such populations greatly restrict effective chemical weed control options. There are many recent cases of multiple resistance in Palmer amaranth (Heap, 2025). One of the most extreme cases is multiple-resistant Palmer amaranth population to ALS, PSII, PPO, HPPD, EPSPS-inhibitors and auxin mimic herbicides reported in Kansas in 2021 (Heap, 2025; Shyam et al., 2021). Multiple herbicide resistance population to four sites of action- ALS, PSII, HPPD and

EPSPS-inhibitors was reported in 2022 in Iowa (Hamberg et al., 2024; Heap, 2025). The most recent case of multiple herbicide resistance was reported in North Carolina where one population was resistant to five herbicides including, 2,4-D, fomesafen, glyphosate, mesotrione, and thifensulfuron (Argueta Menendez, 2025). Over the past decade, herbicide resistance in this species has become a global problem. Besides the United States, there have been reports of resistance from South America (Argentina, Brazil, Uruguay), Africa (South Africa), Asia (Israel) and Europe (Spain, Italy, Turkey) (Heap, 2025).

In Virginia, Palmer amaranth management has traditionally relied on EPSPS- and ALS-inhibiting herbicides, however resistance to both of these herbicide groups has been reported (Scruggs et al., 2020). Growers in multiple regions have recently reported control failures in soybean, corn, and cotton production systems, suggesting that resistance in Palmer amaranth may be more extensive and diverse than currently documented. This highlights the knowledge gap which needs to be addressed. Despite these observations, no coordinated, statewide survey or assessment of herbicide-resistant Palmer amaranth and the level of resistance in the populations has been conducted in Virginia in recent years. Therefore, the objectives of this study were to (1) to survey Palmer amaranth populations across Virginia for herbicide resistance, and (2) to quantify the level of resistance using dose-response assays. The findings of this study will provide an updated understanding of herbicide resistance in Palmer amaranth in Virginia and support the development of more targeted weed control strategies. Providing growers with the latest information on which herbicides remain effective is essential for implementing judicious herbicide use and mitigating the further evolution and spread of resistance.

3.3 Materials and Methods

3.3.1 *Sample collection and preparation*

A field survey was conducted from 2021 and 2024 and 68 Palmer amaranth (PA) populations were collected from soybean (*Glycine max*), corn (*Zea mays*), and cotton (*Gossypium hirsutum*) fields across three main row-crop production regions in Virginia i.e., the Coastal Plain, Piedmont, and Blue Ridge Mountains. These samples were collected later in the season from the plants that had survived herbicide treatments in row-crops. Sampling locations were selected based on reports of control failures from extension agents and growers. Seed heads were harvested from multiple plants within each field and composited to represent a single population. GPS coordinates of sampling location and field cropping history were also recorded at each site. Samples were brought to Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia and were placed in dryers at 55-60° C for 5 days to facilitate threshing and subsequently stored in a cold room at 5° C for four months prior to screening.

3.3.2 *Greenhouse setup and plant material handling*

For each population, seeds were broadcasted in 50-cell trays, followed by transplanting three seedlings at an early one-leaf stage into 10 x10 cm pots. Sunshine® Mix #1 (Sun Gro Horticulture, Agawam, MA, USA) was used as a potting mix for all trays and pots. Greenhouse conditions were maintained at a 16-h photoperiod, with supplemental lighting provided by MHTST-400-U-D grow lights (MHT LIGHTING, New York, NY, USA). Greenhouse temperature conditions were maintained at 35/28 °C day/night regime. Plants received daily overhead irrigation throughout the experiment, except when irrigation was withheld to satisfy rainfast intervals required for specific herbicide applications.

3.3.3 *Herbicide screening*

Greenhouse studies for herbicide screening were conducted from 2023 to 2025 at Virginia Tech's Eastern Shore Agricultural Research and Extension Center, Painter, Virginia. Palmer amaranth seedlings were treated with different herbicides at 1X recommended field rates (Table 3.1) when seedlings attained a height between 5 to 10 cm. Herbicide applications were made using a motorized spray chamber equipped with TeeJet® XR 8002 EVS nozzle (Spraying Systems Co., Wheaton, IL) and calibrated to deliver 187 L ha⁻¹ at 276 kPa. The study was set up with three replications in randomized complete block design and was repeated over time. Three weeks after treatment, plants were evaluated for herbicide injury using a 0-100% scale, where 0% means no visible injury and 100% indicated complete plant necrosis. Based on visible injury ratings, populations were classified into three categories: susceptible (80–100% injury), less sensitive (50–79% injury), and resistant (0–49% injury). Populations that were classified as resistant based on the initial screening results, where each population was tested to each herbicide separately, were used to determine multiple resistance as well.

3.3.4 *Dose-response*

Dose-response assays were conducted for different herbicides based on the results from screening with 1X rate. Of all the populations, one resistant and susceptible population per herbicide was selected for subsequent dose-response evaluation. Resistant populations received seven doses (0, 0.5, 1, 2, 4, 8, 16X the labeled rate), whereas susceptible populations received six doses (0, 0.125, 0.25, 0.5, 1, 2X). Herbicide treatments were arranged in a completely randomized design with three replications and two experimental runs. Data (% visible injury) were collected at 21 days after treatment by using above mentioned injury scale of 0-100%.

3.3.5 Statistical analysis

Spatial distribution of Palmer amaranth responsiveness to the tested herbicides were mapped using ArcGis (ArcGIS Version 10.8.2; Esri, 2020). To demonstrate the distribution of Palmer amaranth over spatial scale the inverse distance technique was applied (Garetson et al., 2019). Visible injury data from dose-response assays were analyzed in JMP (Version 18; SAS Institute Inc., 2024) using a three-parameter log logistic regression equation (Eqn (1)). Dose-response curves were generated in SigmaPlot v16.0 (Systat Software, Inc., 2024). The I₅₀ values of resistant and susceptible population were estimated based on the fitted models and herbicide resistance was expressed as resistant-to-susceptible ratio (R/S).

$$Y = \frac{c}{(1 + (-a \times (Dose - b)))} \quad (1)$$

Y : Injury

a : growth rate

b : infection point

c : asymptote

3.4 Results and Discussion

3.4.1 *Screening for herbicide resistance*

In the initial screening, Palmer amaranth populations were evaluated at the labeled field rate for each herbicide (Table 3.1). Results from this screening (Table 3.2) were used to identify representative susceptible and highly resistant populations for subsequent dose–response assays. Outcomes from the dose-response experiments are summarized in Table 3.3.

3.4.2 *Trifloxysulfuron*

Trifloxysulfuron, the only ALS-inhibiting herbicide evaluated in this study, provided limited control of most Palmer amaranth populations. Prior to this screening, the presence of ALS-resistant Palmer amaranth populations in Virginia was expected due to several reports of control failures from growers. Of the 68 populations collected, 66 were screened, as two accessions (PA-44 and PA-47) were excluded due to insufficient seed availability and poor germination. Across the evaluated accessions, 70% of the populations exhibited resistance, 24% populations were classified as less sensitive, and only 6% were susceptible (Table 3.2). These results indicate a widespread distribution of ALS-resistant Palmer amaranth populations throughout Virginia (Figure 1.2A). Palmer amaranth has a long history of resistance to ALS-inhibiting herbicides. Since the first report from Kansas in 1995, there have been frequent reports from Arkansas, Tennessee, North Carolina, South Carolina, Georgia, Florida, Mississippi, Arizona, Illinois, Delaware, Maryland, Wisconsin, Connecticut and Iowa (Burgos et al., 2001; Heap, 2025). Results from dose-response assays further demonstrated substantial resistance levels in Palmer amaranth populations. The most resistant Palmer amaranth population (PA-16) required 93.1 g ai ha⁻¹ of trifloxysulfuron to achieve 50% growth reduction (I_{50}), representing a

47-fold resistance relative to the susceptible population ($I_{50} = 1.97 \text{ g ai ha}^{-1}$) (Table 3.3; Figure 3.4). The Palmer amaranth resistance to ALS herbicides is widespread in United States with the highest number of cases coming from the Southern part of the country and including states neighboring Virginia like Maryland, Delaware and North Carolina (Heap, 2025). The prevalence of ALS resistance observed in Virginia aligns with nationwide trends, particularly in the southern United States, where the highest number of confirmed cases has been reported including neighboring states such as Maryland, Delaware, and North Carolina (Heap, 2025). Similar challenges have been documented in Arkansas, where glufosinate-resistant Palmer amaranth populations exhibited extreme levels of cross-resistance to ALS herbicides. In a recent case, the most ALS-resistant population required $2,846 \text{ g ai ha}^{-1}$ of imazethapyr to achieve 50% mortality compared with 40 g ai ha^{-1} for the most susceptible population, resulting in a 71-fold resistance ratio. As in our study, I_{90} values could not be calculated because none of the applied doses achieved $\geq 90\%$ mortality, suggesting resistance levels beyond the highest rates tested (Carvalho-Moore et al., 2025).

3.4.3 *Glyphosate*

For glyphosate, of the 68 Palmer amaranth populations collected, 58 were screened for glyphosate response due to insufficient viable seed. None of the populations were susceptible to glyphosate, 14% showed reduced sensitivity, and the rest 86% were resistant (Table 3.2; Figure 3.2). At the labeled field rate of 868 g ae ha^{-1} , only eight populations showed greater than 50% injury, confirming the dominance of resistant populations. Since there was no susceptible population available, dose-response assay was only conducted for the resistant population, and therefore, R/S value could not be estimated. The I_{50} value for the resistant population was 1,237.6

g ae ha⁻¹ (Table 3.3 and Figure 3.4) which is 1.4 times higher than 1X rate used in this experiment, however, complete control could only be achieved by 8X of labeled rate (data not shown). Till date, Palmer amaranth's resistance to glyphosate has been reported in 34 U.S. states with the most recent cases reported in New York and North Carolina in 2024 and 2025, respectively (Heap, 2025). Resistance to glyphosate in Palmer amaranth from Virginia was first documented in 2011 and reported an LD₅₀ 1,470 g ae ha⁻¹ for resistant population, representing a 4.6-fold increase compared with the susceptible population (Ahmed, 2011). More pronounced resistance levels have been documented in Arkansas, where LD₅₀ values ranged from 37 g ae ha⁻¹ in the most susceptible to 4,083 g ae ha⁻¹ in the most resistant populations, resulting in R/S ratios up to 110-fold. In the same study it was estimated that 90% mortality of the most resistant population required 13,178 g ae ha⁻¹, 12 times the recommended 1x field rate (Carvalho-Moore et al., 2025). Similarly, confirmation of glyphosate-resistant populations from New York showed up to six times more glyphosate required to reduce biomass reduction by 90%. The calculated LD₅₀ values for most resistant and most susceptible populations from this experiment were 902 g ae ha⁻¹ and 13.3, respectively which resulted in R/S ratio of approximately 67.7-fold (Butler-Jones et al., 2024). Overall, these findings highlight the severity of glyphosate resistance in Palmer amaranth populations across the surveyed region. The lack of any susceptible accessions, coupled with the high doses required to achieve greater control, reflects the advanced stage of resistance evolution and the diminishing efficacy of glyphosate as a standalone management tool.

3.4.4 *Glufosinate*

Glufosinate provided excellent control of all Palmer amaranth populations screened in this study. Only two accessions showed injury between 95-99%, whereas the remaining populations exhibited 100% control (Table 3.2; Figure 3.2). To date, only two confirmed cases of Palmer amaranth resistance to glufosinate have been reported in the U.S. The first case was reported in Arkansas in 2020, and the second case was reported in North Carolina in 2022 (Heap, 2025). Besides Palmer amaranth, another species from *Amaranthaceae* family, common waterhemp (*Amaranthus tuberculatus* (Moq.) JD Saueris) has also been reported to be resistant to glufosinate in Illinois (Heap, 2025). The absence of resistance in any of the tested Virginia populations reinforces that glufosinate remains a highly effective option for managing Palmer amaranth in the region. These findings indicate that growers can continue to rely on this herbicide, although sustained stewardship and integration of diverse control practices will be critical to delaying future resistance evolution.

3.4.5 *Atrazine*

Atrazine responses varied among the tested Palmer amaranth populations but resistance was not widespread unlike in the case of trifloxysulfuron, or glyphosate. Approximately 10% of the evaluated populations exhibited resistance to atrazine, while 66% were susceptible. Reduced sensitivity was found in 24% of populations (Table 3.2; Figure 3.2). The first case of resistance was reported in 1993 in Texas, and since then frequent cases from multiple, and widely separated U.S. states has been reported. Recent reports of atrazine-resistant Palmer amaranth in North Carolina and New York, the evolution of atrazine resistance in Virginia is not unexpected,

particularly considering the continued and widespread use of atrazine in corn production systems across the state (Doucoure et al., 2020; Heap, 2025). In Virginia, resistance to atrazine has been already reported in smooth pigweed in 1986; in redroot pigweed in 1993 and in common lambsquarters in 1979, however, atrazine-resistant Palmer amaranth has never been reported in this state (Heap, 2025). The dose-response analysis indicated a 17.8-fold resistance level, with an I_{50} value of 2,979 g ai ha⁻¹ for resistant population (PA19) compared with 167.8 g ai ha⁻¹ for the susceptible population (Table 1.3; Figure 3.4). Similar resistance levels have been reported in the northeastern United States. In greenhouse confirmations of resistance in populations from New York and Connecticut, doses required to reduce biomass by 50% were 1,725 g ai ha⁻¹ and 165 g ai ha⁻¹, respectively. When compared with the susceptible reference (159 g ai ha⁻¹), these values corresponded to 11-fold resistance in the New York population and 7-fold resistance in the Connecticut population, with GR_{90} estimates increasing the R/S ratio to 23-fold (Rieley et al., 2025). Collectively, these findings indicate that while atrazine remains effective against many populations, resistance is emerging and may become more prevalent without proactive management strategies.

3.4.6 *Mesotrione*

Out of the total 68 populations collected, 59 were evaluated for mesotrione response. Only 5% of the screened accessions exhibited resistance and 2% were less sensitive while remaining 93% of populations was susceptible. Although, the proportion of resistant population is low (5%) these findings provide an early indication that mesotrione resistance may be emerging in Virginia.

To date, no cases of resistance to mesotrione or other HPPD-inhibiting herbicides have been reported in Virginia. However, mesotrione-resistant Palmer amaranth populations have been documented in Kansas, Nebraska, Iowa, and North Carolina (Heap, 2025). Despite the detection of a small number of resistant populations, mesotrione remains an effective herbicide for Palmer amaranth control in Virginia. However, continued efficacy will depend on integrating mesotrione with other sites of action particularly tank-mixing with atrazine and supplementing with non-chemical practices to ensure robust and sustainable control across diverse field conditions (Singh et al., 2018; Sutton et al., 2002).

3.4.7 *Fomesafen*

The initial screening results showed that resistance to fomesafen is not yet widespread in Virginia. Only 4% of the tested populations were reported to be resistant, and reduced sensitivity was observed in 19% of tested populations. (Table 3.2). Approximately 77% of populations were susceptible, whereas around 80% of susceptible populations had $\geq 90\%$ injury. Palmer amaranth's resistance to fomesafen displays a scattered distribution across the South-Central, Midwestern, and Southeastern United States, with most recent reported case from North Carolina in 2025 (Heap, 2025). Prior to this study, there had been no documented cases of fomesafen-resistant Palmer amaranth in Virginia. The results from our dose-response analysis estimated an I_{50} value of 984 g ai ha^{-1} for the most resistant population (PA-10) compared with 69 g ai ha^{-1} for the susceptible population, thus indicating a 14-fold resistance level (Table 3.3; Figure 3.4). Given that the 1x field rate used in this study was 420 g ai ha^{-1} , even doubling the labeled rate would not achieve 50% control based on the estimated I_{50} . Similar findings were reported in Arkansas, where resistance to this herbicide occurred at relatively low frequency. The

most resistant Arkansas population had an LD₅₀ of 289 g ai ha⁻¹ slightly above the labeled rate used in that study (280 g ai ha⁻¹) corresponding to a 14-fold R/S ratio compared with the susceptible population (LD₅₀ = 20 g ai ha⁻¹). However, in order to achieve LD₉₀ value, fomesafen have to be applied at 894 g ai ha⁻¹, more than three times the labeled rate (Carvalho-Moore et al., 2025).

3.4.8 2,4-D

Out of the total 68 Palmer amaranth populations screened for 2,4-D, only one was resistant. This population was collected in Northampton County. Percentage of populations with reduced sensitivity to 2,4-D was significantly higher (46%) and 53% of total populations were susceptible which means that performance of this herbicide on Palmer amaranth populations in Virginia varies between different localities (Table 3.2). Although Palmer amaranth does not have an extensive history, of resistance to 2,4-D, our results indicate that resistance to this Group 4 is present in only one among 68 surveyed populations in Virginia, suggesting that this marks the first evidence of emerging 2,4-D resistance in the state. This trend aligns with recent confirmations of 2,4-D-resistant Palmer amaranth in North Carolina in 2025; prior to that, resistance had only been reported twice, both in Kansas (2015 and 2018) (Heap, 2025). Before the current study, no cases of resistance to Group 4 herbicides had been documented in Virginia.

3.4.9 Dicamba

Unlike 2,4-D which had about 53% susceptible populations, dicamba (another Group 4 herbicide) provided excellent control of all tested populations, with every population classified as susceptible (Table 3.2). Of the total 68 susceptible populations, more than half (35

populations) had an injury of 90% or higher, whereas about 11 populations were completely controlled (100% injury). Although dicamba resistance has previously been reported in Tennessee in 2020 and Arkansas in 2023 (Heap, 2025). Our results indicate that dicamba remains a highly effective postemergence option for Palmer amaranth management in the region. However, findings from Tennessee demonstrate that repeated and intensive use of dicamba can select for resistance within relatively short time frames, underscoring the importance of proactive stewardship and diversified weed-management programs to preserve dicamba efficacy over the long term (Foster & Steckel, 2022).

3.4.10 Multiple resistance

Multiple resistance to two or more herbicide sites of action was observed in 69% of all tested Palmer amaranth populations. A particularly high proportion (60%) of accessions exhibited resistance to both Group 2 (ALS inhibitors) and Group 9 (EPSPS inhibitors) (Table 3.4), a combination frequently reported in several other states as well (Argueta Menendez, 2025; Bagavathiannan & Norsworthy, 2016; Bond et al., 2010; Kumar et al., 2019; Garetson et al., 2019; Shyam et al., 2021; Sosnoskie et al., 2011). Notably, multiple resistance involving three sites of action i.e. ALS-, PS II- and EPSPS- inhibitors were found in populations, collected in Accomack, Goochland, and King William counties and multiple resistance to ALS-, HPPD- and EPSPS inhibitors were found in Southampton and Hanover counties representing the most complex resistance profiles detected in the current study (Table 3.4). Similar case was reported by Kohrt et al. (2017) where resistance to glyphosate, atrazine and thifensulfuron was confirmed in Palmer amaranth population from Michigan. ALS resistance in *Amaranthus* spp. is commonly conferred by target-site substitutions (e.g., Ala122, Pro197, Asp376, Trp574, Ser653), and sometimes metabolic resistance; glyphosate resistance often involves *EPSPS* gene amplification,

mutation at the target-site or reduced translocation (Beckie & Tardif, 2012; Gaines et al., 2010, 2011; Nandula et al., 2012). PPO resistance is frequently associated with *PPX2* alterations (e.g., Δ Gly210), while PSII resistance (e.g., atrazine) relates to *psbA* Ser264Gly and/or enhanced detoxification (GST-mediated conjugation) (Salas et al., 2016; Giacomini et al., 2017; Devine & Shukla, 2000; Dayan et al., 2014). Synthetic auxin resistance via conjugation, transport, or oxidative metabolism, though mechanisms in Palmer amaranth remain unclear (Mithila et al., 2011). The widespread development of multiple resistance likely reflects long-term reliance on herbicide from the same sites of action, limited use of residual PRE herbicides, and the species' inherent biological advantages such as high fecundity, dioecy, rapid gene flow, and substantial metabolic plasticity (Norsworthy et al. 2012; Délye et al. 2013; Dayan et al. 2014; Gaines et al. 2020). Given the extent of resistance documented in Palmer amaranth, sustainable management will require diversified, program-based strategies emphasizing rotating both the crop and herbicide modes of action, and complementary non-chemical weed-control options such as cover crops, optimized crop competition, and seedbank-reduction practices (Norsworthy et al. 2012; Beckie & Harker 2017; Bagavathiannan & Norsworthy 2016).

3.5 Conclusions

The findings from this research confirm that the Palmer amaranth populations in Virginia have evolved resistance to trifloxysulfuron, fomesafen, atrazine, and glyphosate. Additionally, results from the initial screening suggest resistance to mesotrione and 2,4-D, however, further confirmation of resistance is required. In contrast, dicamba and glufosinate provided complete control of all tested populations at labeled field rates, demonstrating that these herbicides remain dependable options, particularly in fields where multiple resistance limits the number of effective chemistries available. Overall, the results suggest that herbicide resistance in Palmer amaranth

populations in Virginia is established and expanding which emphasizes the need of integrated weed management strategies aimed at slowing down further resistance evolution. The insights from this study will help growers and agricultural stakeholders in Virginia in making informed, strategic and more sustainable decisions for Palmer amaranth management.

3.6 References

- Ahmed, A. M. A. T. (2011). *Control, assessment and glyphosate resistance of Palmer amaranth (Amaranthus palmeri S. Wats.) in Virginia* (Doctoral dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.).
- Argueta Menendez, R. J. (2025). *Investigating a multiple herbicide-resistant Palmer amaranth (Amaranthus palmeri) population from North Carolina*. (Master's thesis, North Carolina State University, Raleigh, NC.).
- Beckie, H. J., & Harker, K. N. (2017). Our top 10 herbicide-resistant weed management practices. *Pest management science*, 73(6), 1045-1052.
- Beckie, H. J., & Tardif, F. J. (2012). Herbicide cross resistance in weeds. *Crop Protection*, 35, 15-28.
- Beckie, H. J., Hall, L. M., Tardif, F. J., & Blackshaw, R. E. (2001). Herbicide resistance in Canada—Where are we today. *Integrated Weed Management: Explore the Potential*, 1–36.
- Bensch, C. N., Horak, M. J., & Peterson, D. (2003). Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Science*, 51(1), 37–43.
- Bond, J. A., & Oliver, L. R. (2006). Comparative growth of Palmer amaranth (*Amaranthus palmeri*) accessions. *Weed Science*, 54(1), 121–126.
- Bond, R. C., Nandula, V. K., & Reddy, K. N. (2010). ALS resistance in glyphosate-resistant Palmer amaranth biotypes from Mississippi. *Proceedings of the Southern Weed Science Society*, 63, 84.
- Bryson, C. T., & DeFelice, M. S. (Eds.). (2009). *Weeds of the South*. University of Georgia Press.
- Burgos, N. R., Kuk, Y. I., & Talbert, R. E. (2001). *Amaranthus palmeri* resistance and differential tolerance of *Amaranthus palmeri* and *Amaranthus hybridus* to ALS-inhibitor herbicides. *Pest Management Science*, 57(5), 449–457.
- Busi, R., Goggin, D. E., Heap, I. M., Horak, M. J., Jugulam, M., Masters, R. A., & Wright, T. R. (2018). Weed resistance to synthetic auxin herbicides. *Pest Management Science*, 74(10), 2265–2276.
- Carvalho-Moore, P., Norsworthy, J. K., Souza, M. C., Barber, L. T., Piveta, L. B., Meiners, I., & Porri, A. (2025). Resistance profile of glufosinate-resistant Palmer amaranth accessions and herbicide options. *Weed Technology*, 39, e31.
- Chahal, P. S., Jugulam, M., & Jhala, A. J. (2019). Mechanism of atrazine resistance in atrazine- and HPPD inhibitor-resistant Palmer amaranth. *Canadian Journal of Plant Science*, 99(6), 815–823.
- Culpepper, A. S., Grey, T. L., Vencill, W. K., Kichler, J. M., Webster, T. M., Brown, S. M., & Hanna, W. W. (2006). Glyphosate-resistant Palmer amaranth confirmed in Georgia. *Weed Science*, 54(4), 620–626.
- Dayan, F. E., Owens, D. K., Tranel, P. J., Preston, C., & Duke, S. O. (2014). Evolution of resistance to phytoene desaturase and protoporphyrinogen oxidase inhibitors—state of knowledge. *Pest management science*, 70(9), 1358-1366.

- Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*, 29(11), 649-658.
- Devine, M. D., & Shukla, A. (2000). Altered target sites as a mechanism of herbicide resistance. *Crop protection*, 19(8-10), 881-889.
- Duke, S. O. (2018). The history and current status of glyphosate. *Pest Management Science*, 74(5), 1027–1034.
- Egan, J. F., Maxwell, B. D., Mortensen, D. A., Ryan, M. R., & Smith, R. G. (2011). 2,4-D-resistant crops and the potential for evolution of resistant weeds. *Proceedings of the National Academy of Sciences*, 108(11), E37–E37.
- Esri. (2020). *ArcGIS (Version 10.8.2)* [Computer software]. Esri.
- Fernández-Quintanilla, C., Peña, J. M., Andújar, D., Dorado, J., Ribeiro, A., & López-Granados, F. (2018). Is the current state of the art of weed monitoring suitable for site-specific weed management in arable crops? *Weed Research*, 58(4), 259–272.
- Franssen, A. S., Skinner, D. Z., Al-Khatib, K., Horak, M. J., & Kulakow, P. A. (2001). Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. *Weed Science*, 49(5), 598–606.
- Gaeddert, J. W., Peterson, D. E., & Horak, M. J. (1997). Control and cross-resistance of an acetolactate synthase inhibitor-resistant Palmer amaranth biotype. *Weed Technology*, 11(1), 132–137.
- Gaines, T. A., Zhang, W., Wang, D., Bukun, B., Chisholm, S. T., Shaner, D. L., ... & Westra, P. (2010). Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of the National Academy of Sciences*, 107(3), 1029-1034.
- Gaines, T. A., Shaner, D. L., Ward, S. M., Leach, J. E., Preston, C., & Westra, P. (2011). Mechanism of resistance of evolved glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). *Journal of Agricultural and Food Chemistry*, 59(11), 5886-5889.
- Gaines, T. A., Duke, S. O., Morran, S., Rigon, C. A., Tranel, P. J., Küpper, A., & Dayan, F. E. (2020). Mechanisms of evolved herbicide resistance. *Journal of Biological Chemistry*, 295(30), 10307-10330.
- Giacomini, D. A., Umphres, A. M., Nie, H., Mueller, T. C., Steckel, L. E., Young, B. G., ... & Tranel, P. J. (2017). Two new PPX2 mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. *Pest management science*, 73(8), 1559-1563.
- Gossett, B. J., Murdock, E. C., & Toler, J. E. (1992). Resistance of Palmer amaranth to the dinitroaniline herbicides. *Weed Technology*, 6(3), 587–591.
- Grossmann, K. (2010). Auxin herbicides: Current status of mechanism and mode of action. *Pest Management Science*, 66(2), 113–120.
- Heap, I., & Duke, S. O. (2018). Overview of glyphosate-resistant weeds worldwide. *Pest Management Science*, 74(5), 1040–1049.
- Horak, M. J., & Loughin, T. M. (2000). Growth analysis of four *Amaranthus* species. *Weed Science*, 48(3), 347–355.
- Jha, P., Norsworthy, J. K., Riley, M. B., Bielenberg, D. G., & Bridges, W. (2008). Acclimation of Palmer amaranth to shading. *Weed Science*, 56(5), 729–734.

- Jhala, A. J., Beckie, H. J., Peters, T. J., Culpepper, A. S., & Norsworthy, J. K. (2021). Interference and management of herbicide-resistant crop volunteers. *Weed Science*, 69(3), 257–273.
- Jones, E., Leon, R. G., & Everman, W. J. (2022). Biological effects on Palmer amaranth surviving glufosinate. *Agrosystems, Geosciences & Environment*, 5(4), e20315.
- Kumar, V., Liu, R., Boyer, G., & Stahlman, P. W. (2019). Confirmation of 2,4-D resistance in a Kansas Palmer amaranth population. *Pest Management Science*, 75(11), 2925–2933.
- Kohrt, J. R., Sprague, C. L., Swathi Nadakuduti, S., & Douches, D. (2017). Confirmation of a Three-Way (Glyphosate, ALS, and Atrazine) Herbicide-Resistant Population of Palmer Amaranth (*Amaranthus palmeri*) in Michigan. *Weed Science*, 65(3), 327–338. doi:10.1017/wsc.2017.2
- Mithila, J., Hall, J. C., Johnson, W. G., Kelley, K. B., & Riechers, D. E. (2011). Evolution of Resistance to Auxinic Herbicides: Historical Perspectives, Mechanisms of Resistance, and Implications for Broadleaf Weed Management in Agronomic Crops. *Weed science*, 59(4), 445-457.
- Nakka, S., Godar, A. S., Wani, P. S., Thompson, C. R., Peterson, D. E., Roelofs, J., & Jugulam, M. (2017). Physiological and molecular characterization of HPPD-inhibitor resistance in Palmer amaranth. *Frontiers in Plant Science*, 8, 555.
- Nandula, V. K., Reddy, K. N., Koger, C. H., Poston, D. H., Rimando, A. M., Duke, S. O., & Ribeiro, D. N. (2012). Multiple resistance to glyphosate and pyriithiobac in Palmer amaranth from Mississippi. *Weed Science*, 60(2), 179–188.
- Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., ... & Barrett, M. (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed science*, 60(SP1), 31-62.
- Norsworthy, J. K., Griffith, G. M., Scott, R. C., Smith, K. L., & Oliver, L. R. (2008). Confirmation and control of glyphosate-resistant Palmer amaranth in Arkansas. *Weed Technology*, 22(1), 108–113.
- Salas, R. A., Burgos, N. R., Tranel, P. J., Singh, S., Glasgow, L., Scott, R. C., & Nichols, R. L. (2016). Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. *Pest management science*, 72(5), 864-869.
- SAS Institute Inc. (2024). *JMP® (Version 18)* [Computer software]. SAS Institute Inc.
- Scruggs, E. B. (2020). Control and fecundity of Palmer amaranth and common ragweed [Doctoral dissertation, Virginia Tech].
- Systat Software, Inc. (2024). *SigmaPlot (Version 16.0)* [Computer software]. Systat Software, Inc.
- Takano, H. K., Beffa, R., Preston, C., Westra, P., & Dayan, F. E. (2019). Reactive oxygen species trigger the fast action of glufosinate. *Planta*, 249(6), 1837–1849.
- Todd, O. E., Figueiredo, M. R., Morran, S., Soni, N., Preston, C., Kubeš, M. F., & Gaines, T. A. (2020). Synthetic auxin herbicides: Finding the lock and key to weed resistance. *Plant Science*, 300, 110631.
- Van Wychen, L. (2025). 2025 survey of the most common and troublesome weeds in broadleaf crops, fruits & vegetables, and hemp in the United States and Canada [Data set]. *Weed Science Society of America*.
- Whitcomb, C. E. (1999). An introduction to ALS-inhibiting herbicides. *Toxicology and Industrial Health*, 15(1–2), 232–240.

3.7 Figures & Tables

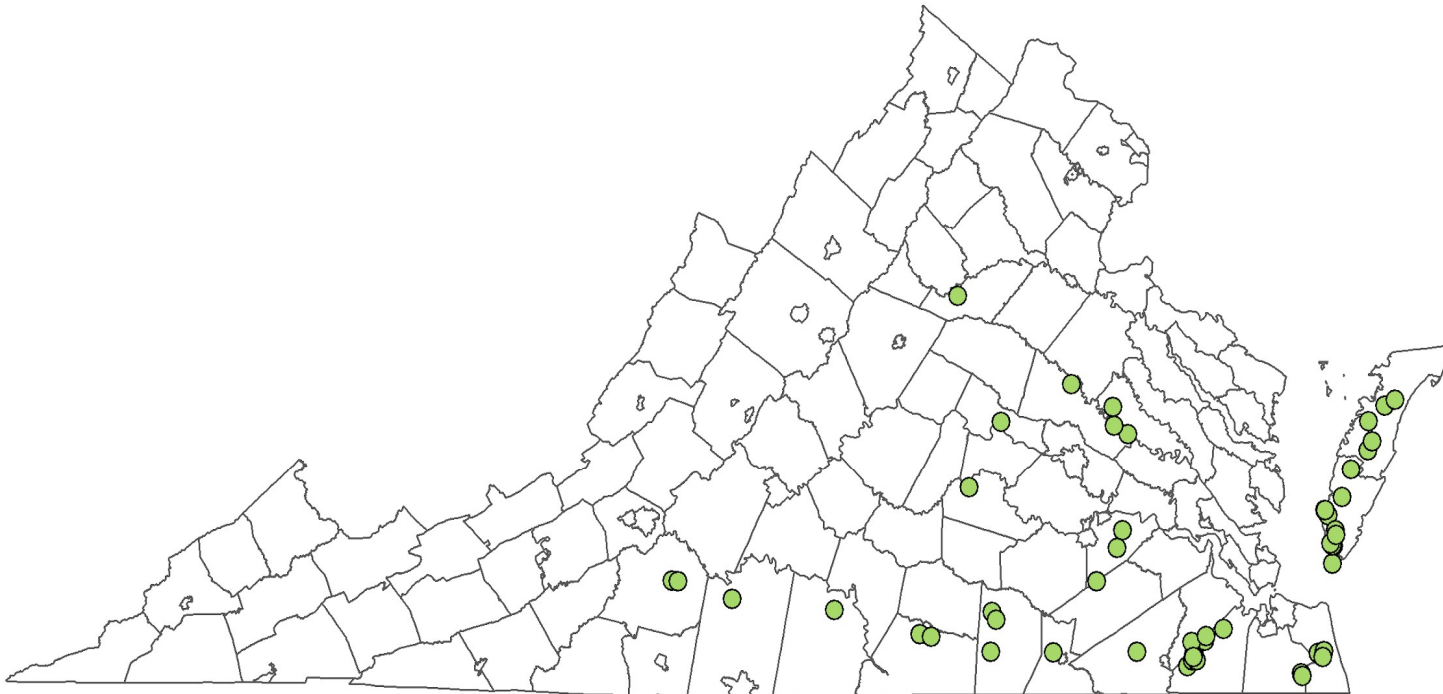


Figure 3.1. Geographical locations of the fields where Palmer amaranth was sampled in Virginia from 2022 to 2024.

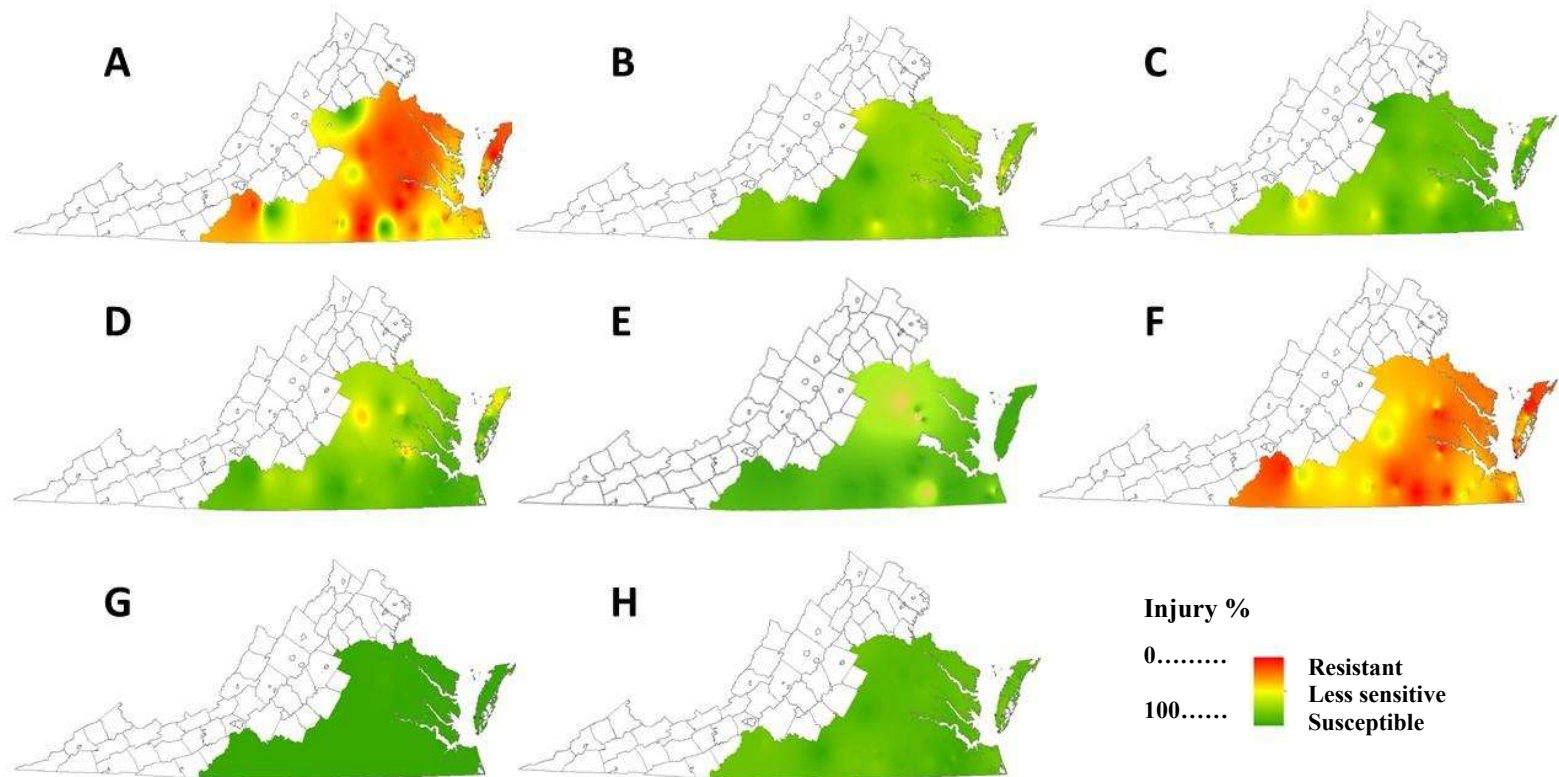


Figure 3.2. Spatial distribution of responsiveness of Palmer amaranth populations based on inverse distance weighted (IDW) interpolation of injury to 8 herbicides: (A) trifloxysulfuron-sodium, (B) 2,4-D, (C) fomesafen, (D) atrazine, (E) mesotrione, (F) glyphosate, (G) glufosinate, (H) dicamba.

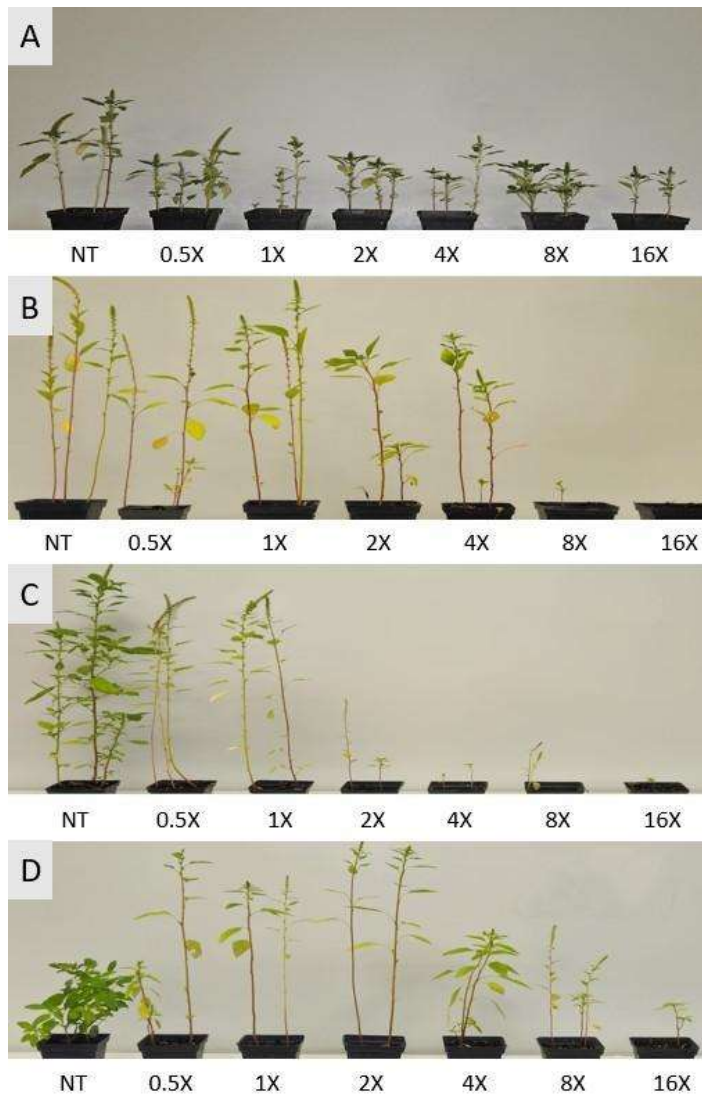


Figure 3.3. Response of highly resistant Palmer amaranth populations: PA-16 (A), PA-10 (B), PA-16 (C) and PA-10 (D) to progressively higher rates of (A) trifloxysulfuron-sodium, (B) fomesafen, (C) atrazine and (D) glyphosate compared with non-treated (NT) control.

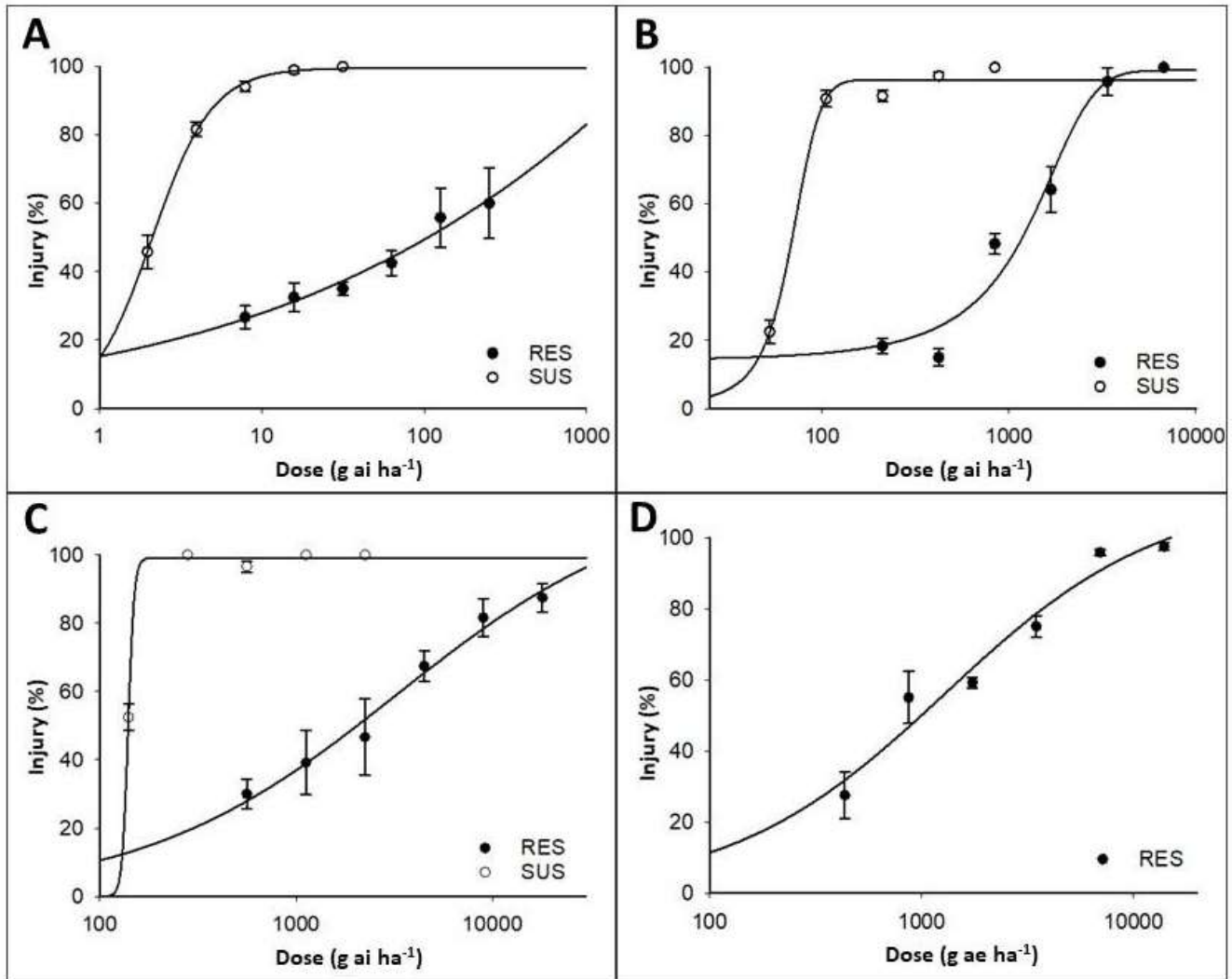


Figure 3.4. Nonlinear logistic dose-response curves of resistant and susceptible populations to (A) trifloxysulfuron-sodium, (B) fomesafen, (C) atrazine and (D) glyphosate. Visible injury was rated on a scale of 0 (no injury) to 100 (plant death) at 21 days after treatment. RES, resistant population; SUS, susceptible population..

Table 3.1. List of herbicides with different modes of action applied postemergence at 1X field rate for greenhouse screening of Palmer amaranth populations.

S. No.	Common Name	Trade Name	Manufacturer ²	Mode of action ¹	Dose 1x (g ai or ae/ha)
1	trifloxysulfuron	Envoke	Syngenta	ALS	15.8
2	glyphosate	Roundup Powermax	Bayer	EPSPS	868
3	glufosinate	Liberty	BASF	GS	595
4	atrazine	Aatrex 4L	Syngenta	PS II	1,345
5	mesotrione	Callisto	Syngenta	HPPD	105.4
6	fomesafen	Reflex	Syngenta	PPO	420
7	2,4-D	Low Vol 4	Loveland	Synthetic auxins	532
8	dicamba	Xtendi Max	Bayer	Synthetic auxins	560

¹ALS, acetolactate synthase inhibitors; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase inhibitors; HPPD, 4-hydroxyphenylpyruvate dioxygenase inhibitors; PPO, protoporphyrinogen oxidase inhibitors; GS, glutamine synthetase inhibitors; PS II, photosystem II inhibitors

²Manufacturer: Syngenta Crop Protection, Bayer CropScience, BASF Agricultural Solutions, Loveland products

Table 3.2. Resistance profiling of Palmer amaranth populations to herbicides with different modes of action in Virginia.

Herbicide	Resistant ¹	Less sensitive ²	Susceptible ³
	-----% of total populations ⁴ -----		
trifloxysulfuron-sodium	70	24	6
glyphosate	86	14	0
glufosinate	0	0	100
atrazine	10	24	66
mesotrione	5	2	93
fomesafen	4	19	77
2,4-D	1	46	53
dicamba	0	0	100

¹Highly resistant defined as populations exhibiting a response to herbicide with injury levels ranging from 0% - 49%

²Moderately resistant defined as populations exhibiting a response to herbicide with injury levels ranging from 50% - 79%

³Susceptible defined as populations exhibiting a response to herbicide with injury levels ranging from 80% - 100%

⁴Total of sixty-eight populations of Palmer amaranth were tested

Table 3.3. Dose response analysis of Palmer amaranth resistant and susceptible populations indicating I₅₀ values and level of resistance to different herbicides.

Herbicide	Population ¹	I ₅₀ ²	R/S ³
trifloxysulfuron-sodium	R (PA-16)	93.1	47
	S (PA-43)	2.0	
fomesafen	R (PA-10)	983.6	14
	S (PA-50)	68.7	
atrazine	R (PA-19)	2,979	18
	S (PA-41)	167.8	
glyphosate	R (PA-40)	1,237.6	-
	S (NA)	-	

¹R, resistant population; S, susceptible population

²I₅₀ refers to herbicide concentration which reduces plant growth by 50%, based on the above biomass visual injury assessment 3 weeks after treatment. Values have been rounded to the nearest decimal point

³R/S, ratio of I₅₀ for resistant and susceptible population

Table 3.4. Multiple resistance among tested populations of Palmer amaranth. Multiple resistance was determined based on initial greenhouse screening at 1X rates where each population was tested to each herbicide separately.

Mode of action	Herbicide combinations	Number of populations tested	Populations with multiple resistance	
			No.	%
ALS + Synthetic auxins	trifloxysulfuron + 2,4-D	66	1	2
ALS + PPO	trifloxysulfuron + fomesafen	66	2	3
ALS + PSII	trifloxysulfuron + atrazine	66	6	9
ALS + HPPD	trifloxysulfuron + mesotrione	59	2	3
ALS + EPSPS	trifloxysulfuron + glyphosate	58	35	60
Synthetic auxins + PPO	2,4-D + fomesafen	68	0	0
Synthetic auxins + PSII	2,4-D + atrazine	68	0	0
Synthetic auxins + HPPD	2,4-D + mesotrione	59	0	0
Synthetic auxins + EPSPS	2,4-D + glyphosate	58	0	0
PPO + PSII	fomesafen + atrazine	68	0	0
PPO + HPPD	fomesafen + mesotrione	59	0	0
PPO + EPSPS	fomesafen + glyphosate	58	0	0
PSII + HPPD	atrazine + mesotrione	59	0	0
PSII + EPSPS	atrazine + glyphosate	58	5	9
HPPD + EPSPS	mesotrione + glyphosate	58	3	5
ALS + PSII + EPSPS	trifloxysulfuron + atrazine + glyphosate	58	2	3
ALS + HPPD + EPSPS	trifloxysulfuron + mesotrione + glyphosate	58	2	3

¹1X herbicide rates (g ai or ae ha⁻¹): trifloxysulfuron 15.8; glyphosate 868; glufosinate 595; atrazine 1,345; mesotrione 105.4; fomesafen 420; 2,4-D 532; dicamba 560.