CONFIRMATION OF GLYPHOSATE-RESISTANT PALMER AMARANTH IN NEW YORK AND RESPONSES TO ALTERNATIVE CHEMISTRIES AND ELEVATED TEMPERATURES

A Thesis

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ABSTRACT

Palmer amaranth (*Amaranthus palmeri* S. Watson) is one of the most troublesome weeds in North America due to its rapid growth rate, substantial seed production, competitiveness, and the evolution of herbicide resistant populations. Though frequently encountered in the South, Midwest, and mid-Atlantic regions of the US, Palmer amaranth (AMAPA) was recently identified in soybean fields in Genesee, Orange, and Steuben counties, NY, where glyphosate was the primary herbicide for in-crop weed control. This research, conducted in 2022 and 2023, aimed to 1) describe the dose response of three putative resistant NY AMAPA populations to glyphosate, 2) determine their mechanisms of resistance, and 3) assess their sensitivity to other postemergence (POST) herbicides commonly used in NY crop production systems. Based on the effective dose necessary to reduce aboveground biomass by 50% (ED_{50}), the NY AMAPA populations were 42- to 67-times more resistant to glyphosate compared to a glyphosate-susceptible (GS) population. Additionally, the NY populations had elevated 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene copy numbers located within extrachromosomal circular DNA (eccDNA), ranging from 25 to 135. NY populations were also poorly controlled by labeled rates of the Weed Science Society of America (WSSA) Group 2 herbicides. Some variability was observed among the populations in response to WSSA Group 5 and 27 herbicides. All populations were effectively controlled by labelled rates of herbicides belonging to WSSA Groups 4, 7, 10, 14, and 22. Additionally, a preliminary study was conducted to assess the impact of temperature on AMAPA's early growth and sensitivity to glyphosate based on glyphosate-resistance (GR) status. Temperature did not affect the early growth of the GR NY population; however, elevated temperatures reduced the biomass of the GS Nebraska population. The dose of glyphosate required to reduce the aboveground biomass of the

GR population by 50% ranged from 451 to 1,170 g ae ha^{-1} depending on chamber temperature. The ED_{50} value of the medium-temperature treatment was significantly lower than that of the high-temperature treatment; however, no other contrasts were statistically different. This result is suspected to be spurious due to the lack of a biological explanation. Additional research is warranted to confirm whether NY AMAPA populations have evolved resistance to the tested herbicide SOAs, to further investigate the impact of temperature on glyphosate sensitivity, and to develop effective AMAPA management strategies suitable for NY crop production.

BIOGRAPHICAL SKETCH

Aleah Louise Butler-Jones was raised in South Orange and Orange, New Jersey. She is the youngest child of Dr. Sheila Louise Butler and Anthony Everton Jones. She graduated from Benedictine Academy, an all-girls catholic high school, in 2015 and attended Cornell University. In 2019, she graduated with a bachelor's in science in agricultural sciences with a minor in horticulture and concentration in sustainable cropping systems. During her time as an undergraduate student, she worked as a research assistant for Cornell Small Farms Program and in the lab of Dr. Thomas Björkman. In addition, she regularly competed as part of the Cornell University Weed Science Team under the guidance of Dr. Antonio 'Toni' DiTommaso. Between her undergraduate and graduate studies, she worked in the lab of Dr. Jenny Kao-Kniffin at Cornell University. She pursued her master's degree in horticultural biology in the lab of Dr. Lynn Sosnoskie at Cornell AgriTech, formerly New York State Agricultural Experiment Station.

DEDICATION

I dedicate this to all who have loved and supported me. There are too many names to list everyone who has been essential to my academic journey. Mum, Dad, Zach, I owe so much to you, far more than can be described in this limited format. Without you all, my journey would have ceased before ever beginning. For that, I am indebted to you and eternally thankful. I love you to the moon and back and everything in between!

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CHAPTER 1: Literature Review

Weed Management in the United States

Due to their resilient and dynamic nature, weeds present a constant challenge for crop production. In the United States, weeds are estimated to be responsible for $33 billion USD in crop production losses annually (Pimentel et al. 2005). Weeds and their management in production systems have been consistently ranked as of high importance for growers (Jerkins and Ory 2016). Herbicides are the most utilized form of weed management due to their high efficacy and relatively low cost compared to other weed management strategies. Globally, herbicides consistently account for the largest portion of pesticide expenditure. In 2012, 45% of pesticide costs, worldwide, went towards herbicides. Further, the United States' (US) herbicide spending comprised over 12% of global pesticide expenditure. At greater than $5.1 billion USD, herbicides accounted for approximately 58% of the US pesticide market in 2012; over 85% of this went towards agricultural production. In 2012, the three most popular active ingredients in the US agricultural sector (glyphosate, atrazine, and S-metolachlor, respectively) were herbicidal compounds (US EPA 2017). Herbicide use in New York (NY) is similar to that of the rest of the nation. In 2022, herbicides were applied to over 740,000 hectares across 9,254 farms in NY, the most of any pesticide category (USDA NASS 2024).

Physical weed control, often in the form of tillage and cultivation, is a frequently used strategy of weed management; however, heavy reliance on soil disturbance-based methods of weed control has several drawbacks. Though tillage can be effective for the suppression of many weed species, repeated and excessive physical disturbance can harm soil structure, reduce aggregate stability, increase compaction, and reduce soil organic matter, among other factors

(Smith et al. 2011, Tebrügge and Düring 1999). While physical control strategies are deployed by both agronomic and specialty crop producers across the conventional and organic operation spectrum, organic and specialty crop growers can be particularly reliant upon tillage and cultivation for weed management. Synthetic pesticides are not allowed to be used in certified organic production (National Organic Program 2000). While herbicides approved for use in organic production exist, they have not been widely adopted due to their high cost and low efficacy when compared to synthetic herbicides. Generally, organic herbicides are ineffective against broadleaf weeds above 10 cm, monocotyledonous weeds, and perennials (Ferguson 2004). In addition, they lack systemic and residual activity, instead only damaging tissue the product has directly contacted. High application rates are usually needed for effective levels of weed reduction. For example, Brainard et al. (2013) found that applications of at least 21.4 L acetic acid ha^{-1} or 5.4 L clove oil ha^{-1} were required for 90% control of mustard (*Brassica juncea* (L.) Czern.). In general, organic herbicides must be used in conjunction with other weed control methods for adequate weed control on-farm.

While conventional operations may use synthetic herbicides, fewer products are labeled for use in specialty crops, compared to agronomic commodities, for several reasons. Specialty crops are planted on fewer hectares than agronomic crops leading to fewer potential herbicide sales. Additionally, there is greater liability potential owing to the high crop value and sensitivity of specialty crops to many herbicides. This leads to little incentive to register herbicides in these crops. As such, specialty crop producers generally rely upon a comparatively small range of herbicides with restricted weed control spectra, mechanical cultivation, and hand weeding (Fennimore and Doohan 2008). Hand weeding is the most expensive weed control tactic due to

the physical labor it requires. Furthermore, the cost of hand weeding has become significantly more expensive due to an increase in wages and reduction in the size of the agricultural workforce. Between October 1998 and October 2023, the national average hourly wage paid to field workers rose from $7.15 to $18.24, an increase of approximately 155% (USDA NASS 1998, USDA NASS 2023). The rise in hourly wages can be partially attributed to the decline in the number of agricultural workers. Between 2012 and 2022, the number of hired farm laborers dropped by approximately 20%, from 2,736,417 workers to 2,184,493 workers (USDA NASS 2012; USDA NASS 2024). Guest worker programs, such as the H2-A temporary agricultural program, can help growers source workers in light of labor shortages; however, the documentation associated with this program can be hard to manage (Bampasidou and Salassi 2019). In addition, to qualify for the H2-A temporary agricultural program, farm operators must (i) offer a job that is temporary or seasonal in nature, (2) prove that there are insufficient numbers of qualified US workers that are able, willing, and available to do the work, and (iii) show that employing H2-A workers will not negatively impact the wages and working conditions of comparably employed US workers (USCIS n.d.).

Glyphosate Use Around the World

N-(phosphonomethyl) glycine, or glyphosate, was first synthesized in 1950 by Dr. Henri Martin while working for a small pharmaceutical company. Due to its lack of medicinal properties, glyphosate was excluded from the literature and the rights to this compound were sold to Aldrich Chemical. While there were cursory explorations of its biological activity by other companies, glyphosate was generally disregarded until it was resynthesized and examined for herbicidal properties in 1970 by Dr. John Franz, a chemist working for the Monsanto

Company. Greenhouse and field testing showed that glyphosate was a highly effective systemic herbicide (Dill et al. 2010). In 1974, the Monsanto Company first released glyphosate to the market as the foliar-applied/post-emergence, non-selective herbicide RoundUp® (Benbrook 2016, Duke and Powles 2008).

Glyphosate works by inhibiting 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway. This leads to a significant reduction in feedback inhibition and, in turn, the accumulation of shikimate. Though the exact manner in which this accumulation kill plants is not completely understood, two mechanisms have been proposed. One hypothesized mechanism is that the crucial production of aromatic amino acids is hindered to the point of insufficient protein synthesis. Others have suggested that the lack of feedback inhibition causes increased carbon flow to the shikimate pathway, thus resulting in a dearth of carbon available for other essential processes. EPSPS is a ubiquitous enzyme found across all higher plants. Because of this, glyphosate is effective against an extremely wide range of plant species. In addition, to being non-selective, glyphosate is relatively quickly absorbed by plant surfaces and translocated throughout the plant via the phloem. As such, the compound is transported to meristematic tissues and other metabolic carbon sinks (Duke and Powles 2008). For these reasons, glyphosate became one of the most popular post-emergence herbicides in the world. In 1995, US farmers and ranchers applied 12.5 million kg of glyphosate, making it the seventh most purchased pesticide that year (Benbrook 2016). Prior to the introduction of glyphosate-tolerant crops (GTCs), glyphosate was primarily used for broad spectrum weed control in non-cropped areas including along roadsides, before or between the planting of fields, and in the weed-free strip along the understory of tree crops.

Herbicide Tolerant Crops

RoundUp Ready® Technology

While glyphosate was a popular herbicide prior to the introduction of glyphosate-resistant (GR) traits, its use expanded exponentially after the release of GR soybean [*Glycine max* (L.) Merr.], first commercialized as RoundUp Ready® soybean, in 1996. For example, in 2005, about ten years after the release of RoundUp Ready® technology, over 44.6 million kg of glyphosate was applied in the US. That year, agricultural applications accounted for 88% of the total use (Benbrook 2016). In total, over 90% of soybean and 60% of cotton acres were planted to GR varieties in 2005 (Duke 2018). In 2014, approximately 747 million kg of glyphosate were applied for agricultural production worldwide, accounting for 90% of glyphosate use globally. In the United States alone, over 113 million kg of glyphosate was applied in agriculture, making up 15% of total agricultural glyphosate use worldwide. Eighty percent of global agricultural glyphosate use went towards herbicide tolerant (HT) soybean, cotton, and corn (Benbrook 2016). In 2016, glyphosate accounted for 26% of the global market value of herbicides (Peters and Strek 2018). Between 1974, the year glyphosate was released to the market, and 2014, 1.37 billion kg of glyphosate was applied by the agricultural sector. In this same period, agricultural use of glyphosate increased 300-fold while non-agricultural use increased 43-fold (Benbrook 2016). Soybeans grown in NY are dominated by HT traits including those for glyphosate, glufosinate, dicamba, and 2,4-D. RoundUp Ready® stacked with LibertyLink® (glufosinate) traits in soybeans, as well as corn, are the most commonly planted in NY. While growers may spray glufosinate, glyphosate is primarily used (EA Smith, personal communication). RoundUp

Ready® alfalfa varieties are also commercially grown in NY; however, they are not widely grown across the state (MJ Stanyard and ME Hunter, personal communication).

A Dearth in the Release of New Herbicides

Synthetic herbicides were first introduced in the 1940s with 2,4-D. A golden age of herbicide discovery and commercialization occurred over the next forty years. Between the 1950s and 1980s, new herbicide active ingredients across several herbicide sites of action (SOAs) were being developed about every two to three years (Dayan and Duke 2020); however, this pace has slowed in the last three decades. There are several reasons for the plateau. First, the introduction of GR crops in 1996 revolutionized weed management in agronomic production systems. Grower adoption of GR corn, cotton, and soybean exceeded 90% by 2016. As a result, weed management in these systems became more simple, cheaper, and highly dependent upon glyphosate (Duke 2018). This led to the lower use and subsequent devaluing of other herbicide SOAs in agronomic cropping systems.

Another contributing factor to the slowdown of herbicide development may be the consolidation of agrochemical companies. Compared to the 1970s, fewer research groups are focusing on herbicide discovery. In addition, the associated research and development (R&D) costs are significantly higher (Duke 2012). Between 2000 and 2016, the cost of producing a new herbicide SOA grew from $184 million to almost $286 million, an approximately 55% increase (Dayan 2019). Finally, newly released herbicides must clear stricter environmental and toxicological regulatory hurdles, only increasing the cost to bring new products to market (Westwood et al. 2018). Further, the registration of many currently approved herbicides may be in jeopardy due to lawsuits waged against the US Environmental Protection Agency (EPA)

regarding the Endangered Species Act (ESA). It was found that the EPA had failed to adequately consult with the U.S. Fish and Wildlife Services (FWS) or the National Marine Fisheries Service (NMFS) about ESA compliance for pesticide registrations, leaving several herbicides vulnerable to lawsuits. In 2023, the federal district court ruled that the EPA must fully comply with the ESA (Anonymous 2024).

Biology and Ecology of Palmer amaranth

Distribution

Palmer amaranth (*Amaranthus palmeri* S. Watson) is a C_4 summer annual native to the Sonoran Desert, which extends from the southwestern US into northern Mexico (Ehleringer 1983, Sauer 1957). Palmer amaranth is one of the ten diecious *Amaranthus* spp. native to North America (Sauer 1972, Steckel 2007). Prior to the 1880s, records indicate that Palmer amaranth was only found in Arizona, California, New Mexico, and Texas. It began spreading into the southeastern and midwestern US in the early twentieth century (Sauer 1957) primarily through human-mediated seed dispersal and habitat creation through agricultural growth (Ward et al. 2013). For example, *A. palmeri* was first detected in Oklahoma in 1926 (Sauer 1957), in Arkansas in 1962, in Nebraska and Ontario, Canada in 1963, in South Carolina in 1966, in Florida in 1967, and in West Virginia in 1970 (Sauer 1972). Since then, Palmer amaranth has spread throughout the southeastern, midwestern, and mid-Atlantic US across agronomic and specialty crop production systems. Though less common in the northeastern US, a GR population was discovered in Connecticut in 2019 (Heap 2024) and in NY starting in 2019 (LM Sosnoskie, personal communication).

Physical Description

A. palmeri has lanceolate, alternate leaves when young which become diamond-shaped as the plant matures. Older leaves have characteristically long petioles that are as long or longer than the leaf blades. While leaves sometimes have a chevron watermark on their adaxial surface, it is not always present and should not be solely depended upon as an identifying feature. The stems are smooth and can range from green to red or vertically red-green striped in color. Palmer amaranth has an upright growth habit and can grow up to 3 m tall (Sellers et al. 2003, Ward et al. 2013). Male and female flowers occur on separate plants. Flowers are produced on tightly clustered, terminal inflorescences that can be highly branched and reach 1 m in length. Females have stiff bracts which make the female inflorescences feel rougher or more prickly than male inflorescences (Ward et al. 2013).

Reproductive Biology

Flowering can begin as soon as three weeks after germination depending on the environmental conditions (Keeley et al. 1987). Being a diecious species, Palmer amaranth is an obligate outcrosser (Franssen et al. 2001, Tranel et al. 2002). It is wind-pollinated and male flowers produce an ample amount of pollen which can travel relatively far distances. Sosnoskie et al. (2012) demonstrated that the glyphosate resistance trait can be transferred through the pollen of male GR to glyphosate-susceptible (GS) female Palmer amaranth plants located up to 300 m away under field conditions. Palmer amaranth is a precocious seed producer. Keeley et al. (1987) observed that viable seed could be produced two to three weeks after the beginning of flowering. *A. palmeri* seeds are smooth, 1 to 2 mm in diameter, round to disc-shaped, brown to black, and often shiny in appearance (Ward et al. 2013).

Female Palmer amaranth plants are highly fecund. In California, plants that germinated between March and June produced 200,000 to 600,00 seeds per plant; however, plants that germinated between July and September produced fewer than 115,000 seeds per plant (Keeley et al. 1987). Sellers et al. (2003) found that Palmer amaranth plants in late May to early June in Missouri produced over 250,000 seeds per plant. Female Palmer amaranth plants produce high amounts of seed even when in competition with crops. Bensch et al. (2003) reported that Palmer amaranth produced a maximum of 32,300 seed m^{-2} at a density of eight plants m^{-1} when grown in competition with soybean in Kansas. Similar results were observed in Palmer amaranth grown in competition with corn in Kansas. In this experiment, Palmer amaranth seed yield was 1,800 to 91,000 seeds m^{-2} as weed density increased from 0.5 to 8 plants m^{-1} of crop row when it emerged when corn (*Zea mays.* L.) was at the four-to seven-leaf stages. At these same densities, weed seed production increased significantly, ranging from 140,000 to 514,000 seeds m^{-2}, when Palmer amaranth emerged at the same time as corn (Massinga et al. 2001).

Seed Dispersal Mechanisms

The seed of Palmer amaranth can be dispersed through several means including waterways, irrigation, mammalian and avian movement, contaminated seed mixes, agricultural equipment (i.e., on plows, combines, spreaders, etc.), and agricultural practices such as harvesting and spreading manure, improperly prepared compost, and crop waste materials (DeVlaming and Proctor 1968, Farmer et al. 2017, Norsworthy et al. 2009, Proctor 1968, Yu et al. 2021). Because of their small size and generalist dispersal mechanisms, Palmer amaranth is easily introduced to new and previously non-effected agricultural habitats. For example, populations of GR Palmer amaranth were first detected in Orange and Genesee counties, New

York between 2020 and 2022. While the exact means of introduction are not known, it is strongly suspected that seed was introduced by human-mediated means. The Orange County population may have been introduced by infested custom harvest equipment from the mid-Atlantic. The Genesee population likely originated from contaminated, spoiled sorghum seed purchased from the US Great Plains that was spread onto a grower's field (LM Sosnoskie, personal communication). Regardless of the means of introduction, Palmer amaranth can easily spread across a field and into other fields if proper preventative measures are not taken. In South Carolina, Norsworthy et al. (2014) deposited 20,000 Palmer amaranth seeds in a 1 m^{-2} area to simulate the seed deposition of a single GR Palmer amaranth female plant. Though the fields had no history of Palmer amaranth's occurrence, Palmer amaranth covered 95 to 100% of all the fields within three years of its introduction. In addition, the authors observed total crop loss in the test fields due to the inability to harvest the crop (Norsworthy et al. 2014).

Seedbank Biology and Germination Conditions

Managing the soil seedbank and minimizing additions to it are key to long-term weed management. To create an effective weed management plan for *A. palmeri*, one must understand how this species' seeds behave in the seedbank, their longevity, and the factors influencing germination. Palmer amaranth is a ruderal species that, in response to accessible moisture, quickly germinates and completes its lifecycle (Ehleringer 1985). In a study comparing the germination speed of nine *Amaranthus* spp. under temperature regimes ranging from 5 to 35 C, all viable Palmer amaranth and smooth pigweed (*Amaranthus hybridus* L.) seed germinated within one day when subjected to a mean temperature of 30 C. All other species took three to eight days to reach 50% germination. Further, Palmer amaranth was the only species that

germinated at every temperature tested (Steckel et al. 2004). This is unsurprising considering that Palmer amaranth originates from the xeric environment of the Sonoran desert and has evolved to quickly germinate in response to available moisture (Ehleringer 1985) .

While Palmer amaranth can germinate under a wide range of conditions, it germinates best under warmer soil conditions. While Palmer amaranth can start to emerge when soil temperatures reach 18 C at a depth of 5 cm (Keeley et al. 1987), Wright et al. (1999) found that only 40% of the seeds germinated at 14 C. Further, Wright et al. (1999) observed peak germination at 26 C. In a study comparing the germination and growth of Palmer amaranth, redroot pigweed (*Amaranthus retroflexus* L.), and common waterhemp (*Amaranthus rudis* Sauer) under different temperatures, Palmer amaranth did not germinate when exposed to a 15/10 C day night temperature; however, germination peaked at 35/30 C and, like all other species tested, significantly declined above this temperature (Guo and Al-Khatib 2003). The environmental conditions experienced by the mother plant also affect the seed characteristics of the progeny. In testing the maternal effects on the drought tolerance of progeny seed, Matzrafi et al. (2021) found that Palmer amaranth plants grown under drought conditions produced seeds that were 18% larger and 20 to 32% less dormant than those of non-stressed plants. Further, these seeds were able to germinate in drier conditions compared to seeds from non-stressed plants (Matzrafi et al. 2021).

Being a small seeded species, Palmer amaranth cannot germinate and successfully become established when buried below a certain depth. Keeley et al. (1987) found that, when buried less than 1.3 cm in the soil, Palmer amaranth had a germination and establishment efficiency of 40% whereas seed located deeper than 5.1 cm, only 7% of individuals successfully

established. The inverse relationship between successful germination and seed depth might be attributed to the decrease in light quantity and quality and more stable temperatures deeper in the soil (Jha et al. 2010). Though they did not test Palmer amaranth specifically, Gallagher and Cardina (1998) observed that the seed of redroot pigweed and smooth pigweed had greater requirements for red light at lower temperatures, suggesting that light may only be needed for the germination of the most dormant seeds in the soil seedbank. While Palmer amaranth seed germination is most successful at shallow depths, seed viability is greater deeper in the soil profile (Sosnoskie et al. 2013). In seed burial experiments, the viability of Palmer amaranth seed buried 10 cm deep or shallower was reduced by at least 47% and 85% one and three years after burial, respectively. The viability of seeds buried at 40 cm, however, was significantly higher over this same time period. Mean reduction in percent viability was 39% after one year and 78% after three years. In addition, there were no differences in seed viability between GR and GS individuals. Seed recovery varied over the year with lower recovery in the summer through early fall and higher recovery in the late winter through early spring, possibly reflecting fluctuations in herbivory due to changes in the accessibility of other food sources (Sosnoskie et al. 2013). In a separate study examining the impact of large arthropod and rodent exclusion on Palmer amaranth seed herbivory, Sosnoskie et al. (2013) found that 44% and 34% of the seeds were recovered, respectively, from traps that excluded these groups compared to 25% seed recovery observed in the non-exclusion traps.

Growth Rate, Competitive Ability, and Economic Impact

In addition to influencing germination and emergence, temperature impacts Palmer amaranth's photosynthetic and growth rate. Palmer amaranth photosynthetic rate peaks when leaf

temperatures reach 42 C. To take advantage of its high photosynthetic capacity, Palmer amaranth's leaves move in a diaheliotropic manner (Ehleringer 1983). Palmer amaranth produced significantly more biomass than redroot pigweed and common waterhemp when grown at 25/20 C and 35/30 C (Guo and Al-Khatib 2003), which might be attributed to its very high photosynthetic capacity at high temperatures (Ehleringer 1983). Sellers et al. (2003) observed similar results in the growth rate of Palmer amaranth compared to five other pigweed species: redroot pigweed, common waterhemp, smooth pigweed, tumble pigweed (*Amaranthus albus* L.), and spiny amaranth (*A. spinosus* L.). Palmer amaranth amassed up to 65% greater biomass and was at least 37% taller than the other species two weeks after planting (WAP). At 14 WAP, Palmer had the highest mean biomass out of the six species tested (Sellers et al. 2003). In a field study, Horak and Loughin (2000) compared the growth of Palmer amaranth, common waterhemp, redroot pigweed, and tumble pigweed planted in June and July in Kansas. At harvest, Palmer amaranth was the tallest and had the highest plant volume, dry weight, and leaf area. In addition, Palmer amaranth generated 32 to 83% more dry biomass than common waterhemp, redroot pigweed, and tumble pigweed (Horak and Loughin 2000). While Palmer amaranth is more competitive than other *Amaranthus* species in general, its rate of growth and development varies across different populations suggesting the existence of different ecospecies of Palmer amaranth across its range (Bond and Oliver 2006). Compared to soybean, Palmer amaranth is less tolerant of cooler rootzone temperatures. When grown at 16 C, Palmer amaranth experienced an 80% reduction in dry biomass compared to those grown at 24 C (control); however, soybean's dry biomass was only reduced 26% compared to the control. Additionally, an eight degree increase in aerial day/night temperatures from 26/22 C to 34/30 C increased

Palmer amaranth dry weight by 1.5- to 16-times compared to the controls. In comparison, soybean suffered a 10 to 20% reduction in dry weight compared to the controls with this rise in temperature (Wright et al. 1999).

In addition to exhibiting altered growth under different temperature regimes, Palmer amaranth displays photosynthetic and morphological plasticity when grown in shaded conditions. For example, Jha et al. (2008) observed that Palmer amaranth increased its photosynthetic rates in response to increasing photosynthetic active radiation (PAR) when grown under 47% shade compared to levels like those experienced by unshaded plants. Furthermore, specific leaf area increased 43% when shading was increased to 87% (Jha et al. 2008).

Palmer amaranth is one of the most troublesome and economically damaging weeds in the United States (Ward et al. 2013, Van Wychen 2022). Though soybean is a relatively competitive plant, Palmer amaranth easily outcompetes it and significantly reduces soybean yield. In an experiment investigating the competitiveness of these pigweed species in a soybean production system, Palmer amaranth planted at a density of eight plants m^{-1} of row at soybean planting reduced soybean yield by 79% compared to a weed-free check. In comparison, common waterhemp and redroot pigweed reduced yields by to 56% and 38%, respectively (Bensch et al. 2003). When grown in competition with corn, Palmer amaranth that emerged with corn reduced corn yield by 11% to 91% as weed density increased from 0.5 to 8 plant m^{-1} of row. Similar trends were observed in the response of the corn leaf area index to increasing weed density. The timing of emergence; however, had a greater impact on corn yield loss than weed density (Massinga et al. 2001). Palmer amaranth commonly outcompetes agronomic crops such as corn, soybean, and cotton (*Gossypium* spp.) when crop and weed emergence occur concurrently

(Chandi et al. 2013). In addition to causing major yield loss, Palmer amaranth can decrease harvest efficiency (Ward et al. 2013) and force growers, custom applicators and harvesters, and agricultural processors and distributors to take extra time to thoroughly clean their farm equipment to prevent the spread of Palmer amaranth into new areas (Roberts and Florentine 2022).

Herbicide Resistance and Palmer amaranth

An Overview of Herbicide Resistance

Herbicides are the primary tool deployed to manage weeds in the United States. Herbicides work by interfering with the biochemical machinery that supports plant growth. The general means by which they do this can be grouped into three categories: light activation of reactive oxygen species (ROS), inhibition of cellular metabolism, or interference in cell division and growth. Herbicides are classified by the physiological mechanism by which they kill plants. As of 2024, there are 29 herbicide SOAs recognized by the Herbicide Resistance Action Committee (HRAC) (HRAC 2024).

Herbicide resistance is the inherited ability of a plant to survive and reproduce after being exposed to a typically lethal dose of herbicide. Weeds can be resistant to more than one herbicide with the same SOA (cross resistance) and/or resistant to herbicides across two or more SOAs (multiple resistance) (WSSA 1998). While herbicide resistance traits existed within plants prior to the discovery of synthetic herbicides (Délye et al. 2013), the widespread and repeated use of synthetic herbicides since the mid-twentieth century has imposed a strong selection pressure leading to the evolution and proliferation of herbicide resistance in hundreds of species (Gaines

et al. 2020). Since the release of genetically engineered HT crops, the diversity of herbicides and SOAs used in many agronomic production systems have decreased. Between 1994 and 2006, herbicide SOA diversity used in soybean production decreased from approximately eight SOAs applied per unit area to approximately two SOAs applied per unit area. For cotton production, herbicide SOA diversity peaked in 1999 at 7.6 SOAs applied per unit area but fell to 5 SOAs applied per unit area by 2006 (Kniss 2018). The decline in the diversity of herbicides used in crop production coincided with the release and widespread integration of GR crops. For example, over 90% of soybean, 60% of cotton, and 50% of corn acres in the US were planted with GR varieties in 2007. Glyphosate became the dominant herbicide used in these crops (Duke 2018). As of February 2024, there are 530 unique cases (species x site of action) of HR weeds worldwide according to the International Database for Herbicide Resistant Weeds. This spans 272 individual plant species and 168 herbicides belonging to 21 of the 31 known herbicide SOAs. 132 unique cases occur in the US spanning across more than 39 crops including corn, soybean, cotton, perennial fruits, and vegetables (Heap 2024).

Herbicide resistance mechanisms can be generally grouped into two categories: target-site resistance (TSR) mechanisms and non-target site resistance (NTSR) mechanisms. Target-site resistance is caused by genetic mutations which lead to (i) a change in target protein structure, (ii) amplification of the gene containing the target site, or (iii) an increase in expression of the target protein. In contrast, mechanisms behind non-target site resistance encompass all mechanisms that either decrease the concentration of herbicide available to interact with the target site protein or enable the plant to contend with target site inhibition. These can include reduced herbicide absorption, sequestration of the active ingredient into vacuoles, decreased

translocation, and enhanced metabolism. The mechanisms behind NTSR are intricate and often involve many genes across large gene families (Gaines et al. 2020).

Incidence of Herbicide Resistance in Palmer amaranth

In addition to its high competitive ability, Palmer amaranth poses a serious threat to US crop production due to its proclivity for evolving herbicide resistance. Palmer amaranth is involved in 14% of unique cases of herbicide resistance globally and accounts for about 53% of the unique cases in the United States. According to the International Herbicide-Resistant Weed Database, populations of *A. palmeri* have evolved resistance to herbicides across nine SOAs: acetolactate synthase (ALS)- (WSSA Group 2), microtubule- (WSSA Group 3), auxin-mimicking herbicides (WSSA Group 4), photosystem II (PSII)- (WSSA Group 5), 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)- (WSSA Group 9), glutamine synthetase- (WSSA Group 10), protoporphyrinogen oxidase (PPO) - (WSSA Group 14), very long-chain fatty acid synthesis (VLCFA)- (WSSA Group 15), and hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting (WSSA Group 27) herbicides (Heap 2024). Additionally, several populations resistant to multiple SOAs have been identified. For example, a population resistant to herbicides from six Weed Science Society of America (WSSA) Groups, specifically those within Groups 2, 4, 5, 9, 14, and 27, was reported in Kansas (Shyam et al. 2021).

Resistance to Microtubule-Inhibitors in Palmer amaranth

Resistance to the dinitroaniline herbicides, a family of microtubule-inhibitors, was one of the first documented in Palmer amaranth. Microtubule inhibitors are primarily soil incorporated, pre-planting applied herbicides that work by disrupting microtubule function during mitosis preventing the alignment an separation of chromosomes during meiosis (Chen et al. 2021). In

1989, trifluralin resistance was discovered in eight Palmer amaranth populations in South Carolina. While the label recommended rate of trifluralin (560 g ha^{-1}) effectively controlled the susceptible population, at least six-times the recommended rate was required to effectively control the resistant population (Gossett et al. 1992). Resistance to trifluralin and pendimethalin were reported in Tennessee in 1998 and Arkansas in 2016, respectively (Heap 2024). The mechanism of resistance is currently unknown.

Resistance to ALS-Inhibitors in Palmer amaranth

Acetolactate synthase (ALS)-inhibitors impede ALS, a key enzyme in the biosynthesis of branched chained amino acids (Tranel and Wright 2002, Ward et al. 2013). ALS is also referred to as acetohydroxyacid synthase (AHAS); ALS- and AHAS-inhibitor may be used to reference the same herbicide active ingredients, depending on the source. ALS-inhibiting herbicides have been widely used to manage Palmer amaranth since the 1980s. As a result of this strong selection pressure, resistance of Palmer amaranth to this class of herbicides is widespread (Tranel and Wright 2002). The first incidence of ALS- resistant Palmer amaranth was reported in Kansas in 1993 (Horak and Peterson 1995, Mayo et al. 1995) and has since spread and/or evolved throughout the midwestern, mid-Atlantic, and southeastern US (Heap 2024). Cross resistance to several ALS-inhibitors is common in this species. For example, Mayo et al. (1995) found that label recommended rates of acifluorfen, lactofen, chlorimuron, and imazaquin provided less than 80% control of the Kansan Palmer amaranth population tested. Similarly, Horak and Peterson (1995) found that the resistant Palmer amaranth population from Kansas survived exposure to imazethapyr and thifensulfuron applied at 560 g ha^{-1} and 36 g ha^{-1}, respectively, eight-times the labeled rate.

Most ALS resistance is caused by target site mutations which reduces herbicide sensitivity; however, non-target site resistance to these herbicides occurs and is less well understood (Gaines et al. 2020, Tranel and Wright 2002, Yu and Powles 2014). Increased herbicide metabolism is one mechanism underlying NTSR. Target-site resistance to ALS-inhibitors can vary in strength depending on the site of the mutation (Gaines et al. 2020, Yu and Powles 2014). ALS-resistance traits can be shared between *Amaranthus* spp. For example, Wetzel et al. (1999) found that ALS-resistance could be passed from Palmer amaranth to common waterhemp through hybridization. Additionally, Tranel et al. (2002) reported that ALS-resistance could be transferred from smooth pigweed, a monoecious species, to common waterhemp, a diecious species. Diverse mutations of the ALS gene occur naturally within several species including several *Amaranthus* spp. (Franssen et al. 2001, Ward et al. 2013) and common ragweed (*Ambrosia artemisiifolia* L.) (Tranel et al. 2004). This, along with widespread and repeated use of ALS-inhibiting herbicides within the same field, likely contributed to most ALS-resistance in Palmer amaranth (Franssen et al. 2001, Ward et al. 2013). To date, numerous populations of Palmer amaranth have evolved resistance to ALS-inhibiting herbicide active ingredients such as chlorimuron-ethyl, cloransulam-methyl, foramsulfuron, imazethpyr, iodosulfuron-methyl-sodium, mesosulfuron-methyl, pyrithiobac-sodium, rimsulfuron, and trifloxysulfuron-sodium (Heap 2024).

Resistance to PSII-Inhibitors in Palmer amaranth

Photosystem II (PSII)-inhibitors are some of the oldest and most frequently used herbicides in crop production. This group of herbicides inhibits photosynthesis by binding to the D1 protein within the photosystem II complex, thus interfering with electron transport (Dayan et

al. 2019). In 1993, atrazine resistance was first reported in Texas. It was subsequently reported in Kansas, Georgia, Nebraska, and North Carolina in 1995, 2008, 2011, and 2022, respectively (Heap 2024). An atrazine-resistant population in Nebraska required a dose of 11,136 g ha^{-1}, almost ten-times the 1X rate, to cause a 90% reduction in dry biomass (Jhala et al. 2014). Atrazine resistance in Palmer amaranth is conferred by a NTSR mechanism, more specifically through rapid detoxification of the compound (Nakka et al. 2017a). To date, several populations of Palmer amaranth have evolved resistance to PSII-inhibitors such as atrazine, bromoxynil, and metribuzin (Heap 2024).

Resistance to EPSPS-Inhibitors in Palmer amaranth

Glyphosate is the only EPSPS-inhibiting herbicide. It works by inhibiting EPSPS enzyme synthesis, a key component in the shikimic acid pathway, resulting in plant death (Duke and Powles 2008). Contrary to the previously-held belief that the evolution of GR weeds was highly improbable (Bradshaw et al. 1997), if not impossible, the first GR population of Palmer amaranth was detected in Georgia in 2004 (Culpepper et al. 2006). Glyphosate-resistant populations were subsequently reported in North Carolina in 2005 (Heap 2024), Arkansas in 2006 (Norsworthy et al. 2008), and Tennessee in 2006 (Steckel et al. 2008). Between 2005 and 2023, GR Palmer amaranth was reported in 31 states in the United States (Heap 2024). In addition, it has been detected in Mexico, South Africa, Argentina, Brazil, and Uruguay (Heap 2024, Gaines et al. 2021). Many of these populations occur in production systems with repeated glyphosate use and little reliance on other weed management tactics (Ward et al. 2013).

For US-based populations of *Amaranthus palmeri*, the mechanism of resistance is the amplification of extrachromosomal circular DNA (eccDNA) containing the EPSPS gene thus

significantly increasing the expression of the enzyme targeted by glyphosate (Gaines et al. 2010, Molin et al. 2018, 2020a). Even without the selection pressure of glyphosate, Vila-Aiub et al. (2014) found that EPSPS gene amplification in *Amaranthus palmeri* did not negatively affect fitness in GR biotypes. Research suggests that US glyphosate-resistant Palmer amaranth originated from a single population in Mississippi which spread across the nation and into South America across more than one invasion event (Gaines et al. 2021, Molin et al. 2020a). Molin et al. (2018) hypothesized that glyphosate resistance evolved once in the United States and spread across several populations through the spread of pollen and/or seed by humans or natural modes of dispersal. Their findings, along with those of Molin et al. (2020), confirm that GR Palmer amaranth found in the US originated from a single population despite its widespread prevalence. Gaines et al. (2021) found that the presence of GR Palmer amaranth in South America is the result of two disparate events: first, the introduction of Palmer amaranth and in-situ evolution resistance to glyphosate in Argentina and then a subsequent importation of GR Palmer amaranth into Brazil and Uruguay from the US in the 21st century based on the presence of the eccDNA replicon.

Resistance to HPPD-Inhibitors in Palmer amaranth

Introduced in the 1990s, hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides belong to one of the most recently discovered herbicide sites of action. HPPD-inhibiting herbicides prevent carotenoid biosynthesis eventually leading to membrane disruption and chlorophyll degradation (Mitchell et al. 2001). These herbicides have been, and continue to be, commonly used products for the management of glyphosate-, atrazine- and ALS-inhibitor resistant Palmer amaranth. HPPD-inhibitor-resistant Palmer amaranth was first detected in

Kansas in 2009. By 2021, resistant populations were discovered in Nebraska, Wisconsin, and North Carolina (Heap 2024). Jhala et al. (2014) found that a population within Nebraska was four- to 23-times more resistant to HPPD-inhibiting herbicides than the susceptible population tested depending on the herbicide used. HPPD-inhibitor-resistance in Palmer amaranth is primarily caused by the rapid detoxification of the herbicide (NTSR), but also increased expression of HPPD gene expression (TSR) (Nakka et al. 2017b). Multiple populations of Palmer amaranth have evolved resistance to HPPD-inhibiting herbicide active ingredients such as mesotrione, tembotrione, and topramezone (Heap 2024).

Resistance to PPO-Inhibitors in Palmer amaranth

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides block the PPO enzyme, an essential catalyst for the conversion of protoporphyrinogen IX (PPGIX) to protoporphyrin IX (PPIX). PPGIX and PPIX are required for the formation of chlorophyl and heme. PPO inhibition causes the generation of ROS which damages protein and lipid membranes, thus fatally harming the plant (Salas et al. 2016). PPO-resistant Palmer amaranth was first detected in Arkansas in 2011 (Heap 2024). Schwartz-Lazaro et al. (2017) found 100% mortality was only achieved in a Palmer amaranth population from Arkansas when plants were treated with 32- to 256-times the label recommended rate of saflufenacil, flumioxazin, and carfentrazone. In Palmer amaranth, PPO-resistance is conferred by the deletion of glycine at the 210th amino acid within the PPO enzyme (Salas et al. 2016). A number of Palmer amaranth populations have evolved resistance to PPO-inhibiting herbicides such as acifluorfen, carfentrazone-ethyl, fomesafen, lactofen and pyraflufen-ethyl (Heap 2024).

Resistance to Synthetic Auxins in Palmer amaranth

Auxin-mimicking herbicides are the oldest synthetic herbicides used in crop production. For example, 2,4-D was first synthesized in 1940 and released to the market in 1946 (Troyer 2001). While the exact mechanism behind their herbicidal properties are not known, synthetic auxins can initiate uncontrolled cell division, essentially causing plants to grow themselves to death (Dayan et al. 2019). In 2015, resistance to synthetic auxins in Palmer amaranth was first detected in Kansas (Kumar et al. 2019). Kumar et al. (2019) reported that the resistant population was over three-times more resistant to 2,4-D than the susceptible population tested. A dicamba-resistant population was discovered in Tennessee in 2020. Vieira et al. (2020) found that repeated exposure to drift reduced the sensitivity of Palmer amaranth to dicamba within two generations. The mechanism of resistance for the auxinic herbicides has not yet been described; however, Palmer amaranth populations resistant to 2,4-D or dicamba have been reported (Heap 2024).

Resistance to VLCFA-Inhibitors in Palmer amaranth

Very long-chain fatty acid-inhibiting (VLCFA) herbicides prevent the formation of VLFCAs, thus inhibiting shoot and root growth. They are commonly used to control monocotyledonous weeds in corn and cotton production (Brabham et al. 2019). Resistance to VLCFA-inhibitors in Palmer amaranth was first recorded in Arkansas in 2016 (Heap 2024). While the exact mechanism of VLCFA-resistance in Palmer amaranth is not known, it is possibly caused by increased gene expression leading to increased metabolism of the herbicide (Rangani et al. 2021). To date, only two Palmer amaranth populations resistant to a VLCFA-inhibitor, specifically S-metolachlor, have been reported (Heap 2024).

Resistance to Glutamine Synthetase Inhibitors in Palmer amaranth

Glufosinate is the only commercialized glutamine synthetase-inhibiting herbicide. This class of herbicides inhibits glutamine synthetase, a key enzyme in nitrogen metabolism in plants, which leads to the inhibition of photosynthesis and destruction of cells (Dayan et al. 2019). In 2020, resistance to glutamine synthetase-inhibitors was first reported in Palmer amaranth. To date, resistance populations have only been reported in cotton fields within Arkansas (2020) and North Carolina (2021) (Heap 2024). Three Palmer amaranth populations from Arkansas were found to be five- to 27-times more resistant to glufosinate than the susceptible populations tested (Priess et al. 2022). Glufosinate-resistance in Palmer amaranth is likely conferred by gene amplification and increased glutamine synthetase expression; however, ongoing investigations of additional mechanisms are being conducted (Carvalho-Moore et al. 2022).

Multiple Resistance in Palmer amaranth

In addition to evolving resistance to a diversity of individual herbicides, several populations of Palmer amaranth are resistant to herbicides with multiple SOAs. Increasing numbers of these populations are being found in the United States. For example, Bagavathiannan and Norsworthy (2016) evaluated the sensitivity of 215 roadside Palmer amaranth populations from across the Mississippi Delta-region of Arkansas to pyrithiobac (WSSA Group 2) and glyphosate (WSSA Group 9). 73% and 89% of the populations had < 10% mortality after exposure to twice the label rates of pyrithiobac (73 g ha^{-1}) and of glyphosate (870 g ha^{-1}), respectively. Further, 97% of the resistant populations were resistant to both herbicides. In addition to being resistant to PPO-inhibiting herbicides, Schwartz-Lazaro et al. (2017) observed that Arkansan Palmer amaranth populations were resistant to microtubule-, ALS-, and EPSPS-

inhibitors. EPSPS- and PPO-inhibitor resistant Palmer amaranth populations were also detected in southwestern Nebraska (Oliveira et al. 2021). In Kansas, an individual population resistant to chlorsulfuron (WSSA Group 2), 2,4-D (WSSA Group 4), atrazine (WSSA Group 5), glyphosate (WSSA Group 9), and mesotrione (WSSA Group 27) was identified (Kumar et al. 2019). Within the last five years, the first six-way resistant population of Palmer amaranth was discovered in Kansas (Shyam et al. 2021). This population evolved resistance to herbicides within WSSA Groups 2, 4, 5, 9, 14, and 27. Though these incidences have been mostly limited to the United States' southeast and Great Plains regions, populations with multiple resistance are spreading northward. For example, a newly introduced population of Palmer amaranth in Wisconsin was found to be resistant to imazethapyr, atrazine, and glyphosate (Faleco et al. 2022). Palmer amaranth's proclivity for evolving herbicide resistance only makes this troublesome weed even more concerning. With climate change, it is expected that Palmer amaranth's range will expand northward in regions of Canada and Europe. Considering this, other tactics for managing this noxious weed must continue to be explored.

Alternative Weed Management Strategies and Palmer amaranth

Given the proliferation of herbicide resistance in Palmer amaranth populations across the country, other weed control techniques should be utilized to manage this troublesome species. Preventative control is the first step in key to preventing the introduction of Palmer amaranth to previously non-infested areas and its spread afterwards. Palmer amaranth can easily be brought into new areas on agricultural equipment so proper cleaning and sanitation of equipment should be executed to prevent further spread (Yu et al. 2021).

Once Palmer amaranth is present, physical control tactics can be effective at reducing its negative impacts on crop yield and depositions to the soil seedbank. Norsworthy (2008) found that conventional tillage in a soybean production system reduced Palmer amaranth's density in the top 5 cm of the seedbank by at least 80% within one year. DeVore et al. (2013) observed similar results with deep tillage causing an 81% reduction in Palmer amaranth emergence in soybean over a two-year period. When used in combination with double cropping of soybean and wheat, emergence was reduced by 95% over two years (DeVore et al. 2013). Similar results were observed in cotton with the combination of deep tillage and high residue cover crops reducing Palmer amaranth emergence by 85% in the first year (DeVore et al. 2012). Jha and Norsworthy (2009) observed that spring cultivation has little impact on the emergence of Palmer amaranth; however, emergence was reduced by at least 73% after soybean canopy closure.

While cover crops can be a useful tool within a weed management regimen, enough biomass must be accumulated for significant reductions in Palmer amaranth. In examining the impact of winter cover crops on Palmer amaranth competition in cotton production, Webster et al. (2013) found that 8,600 kg ha^{-1} of cover crop biomass was needed for 90% Palmer amaranth control; however, 100% yield loss was observed in non-herbicide treated plots. The presence of GR Palmer amaranth has driven growers to increase their reliance on more expensive weed management tactics such as hand-weeding (Sosnoskie and Culpepper 2014). Heavy reliance on soil disturbance based methods of weed control has several drawbacks including increased fuel and labor costs and negative impacts on soil structure, stability, and health (Jerkins and Ory 2016, Smith et al. 2011). Because of growing concerns regarding soil degradation, production

costs, and herbicide resistance, there is increased interest in novel weed management technologies such as harvest weed seed control and electrical weeding.

Harvest weed seed control (HWSC) is a group of tools and strategies aimed at preventing additions to the weed seedbank. It includes collecting weed seed during harvest and either concentrating them in chaff rows or destroying the seeds using impact mills. Though widely used in Australia, this technology has not been adopted to the same degree within the United States for several reasons. Despite this, HWSC could be a very effective tool in minimizing Palmer amaranth's presence in the weed seedbank. For example, models produced by Shergill et al. (2020) predicted that a 50% effective use of HWSC could reduce the size of the Palmer amaranth seedbank by 73% within five years.

Another potentially useful technology is electrical weeding, also called electrical weed control (EWC). Though the exact mechanism is not known, it is theorized that EWC works by passing an electrical current through a weed, which generates heat through Ohmic heating causing rapid heating, cell membrane rupture, and plant death (Brodie 2018). Though the impacts of EWC on Palmer amaranth were not test explicitly, Schreier et al. (2022), using a Weed Zapper™ that manages weeds exceeding annual crop canopies, found that electrocution was more effective at increasing visual control ratings when applied at later growth stages several annual weed species including common ragweed, giant ragweed (*Ambrosia trifida* L.), horseweed (*Conyza canadensis* L.), barnyardgrass (*Echinochloa crus-galli* L.), yellow foxtail (*Setaria pumila* Roem. & Schult.), and giant foxtail (*Setaria faberi* Herrm.). Additionally, they reported that the numbers of viable weed seeds could also be reduced using EWC relative to untreated checks.

Palmer amaranth is an incredibly troublesome weed in cropping systems. In addition to its high competitive ability, its propensity to evolve herbicide resistance and the expected spread of its range makes this weed particularly concerning for stakeholders across the country and world. Diversifying weed management strategies are needed for the successful management of herbicide-resistant Palmer amaranth. Early detection, vigilant management, and prevention of its spread are integral to limiting the extent to which Palmer amaranth is a detriment to crop production.

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CHAPTER 2: Confirmation of Glyphosate-Resistant Palmer amaranth (*Amaranthus palmeri*) Populations in New York and Responses to Alternative Chemistries

Introduction

Across US agricultural production, herbicides have become an indispensable tool for weed control due to their high efficacy and relatively low cost compared to other control options (Varanasi et al. 2016). However, herbicide resistance (HR) and the proliferation of HR weeds pose a major threat to various cropping systems across the world (Norsworthy et al. 2012, Westwood et al. 2018). The number of unique cases of HR weeds in the US has more than quadrupled in the last thirty years (Heap 2024). Furthermore, very few new herbicide sites of action (SOAs) have been released in approximately thirty years (Dayan and Duke 2020, Duke 2012, Shaner and Beckie 2014). While various weed species have evolved resistance to a single herbicide and herbicide SOA, some populations exhibiting resistance to multiple herbicide SOAs have been identified.

One such weed is Palmer amaranth (*Amaranthus palmeri* S. Watson; AMAPA), a C_4 summer annual native to the southwestern United States and northern Mexico. It is consistently ranked as one of the most troublesome and economically significant weeds for US corn and soybean production (Ehleringer 1983, Ward et al. 2013, Van Wychen 2022). This diecious species poses a serious threat to cropping systems because of its rapid growth rate (Ward et al. 2013), early and prolific seed production (Keeley et al. 1987), abundant pollen production and vast dispersal (Sosnoskie et al. 2012), and morphological and phenological plasticity to environmental conditions (Ehleringer 1983, Keeley et al. 1987, Spaunhorst et al. 2018). AMAPA

seed dispersal results from various natural and anthropogenic factors including the movement of agricultural equipment required by mowing, tillage, and harvesting operations (Norsworthy et al. 2008), wildlife (Farmer et al. 2017), wind and water movement (Norsworthy et al. 2014, Sosnoskie et al. 2012), and soil application of manure contaminated by ingested seeds (Yu et al. 2021). AMAPA is also becoming an increasingly troublesome weed for several vegetable crops such as sweet potato [*Ipomea batatas* (L.) Lam.], pumpkin (*Cucurbita pepo* L.) and asparagus (*Asparagus officinalis* L.) (Boyd et al. 2022).

AMAPA's presence in New York (NY) was first confirmed in 2019 when three populations were identified in soybean fields in Steuben, Orange, and Genesee counties (LM Sosnoskie, personal communication). In 2021, NY soybean production covered over 131,000 ha and was valued at over $205 million USD. In the northeastern US, diversified cropping systems are commonplace, often including both agronomic and specialty crops. Soybeans are commonly included in the crop rotation of vegetable production systems. Vegetable crops are important commodities in NY, which is a top ten state for the production of annual specialty crops. NY ranks second for cabbage and snap beans in the US and is one of the top-ten producing states for onions, pumpkins, squash, and sweet corn (USDA NASS 2024). Given that the same field preparation equipment is used for both agronomic and specialty crops, it is important to evaluate the efficacy of different herbicide options for managing AMAPA once it has spread into vegetable production systems. Controlling AMAPA in vegetable production systems may pose greater challenges due to the restricted availability of herbicides approved for use on these crops, hindering the rotation of herbicides from sites of action, and increasing the risk of selecting herbicide-resistant populations.

Palmer amaranth, like several other weed species, is not always resistant to single herbicide chemistries; rather, several populations of Palmer amaranth have evolved resistance herbicides across to two or more SOAs. Sixty-nine populations of Palmer amaranth in the US have evolved resistance to multiple sites of action (SOAs) including acetolactate synthase (ALS)-, microtubule-, auxin-mimicking, photosystem II (PSII)-, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)-, glutamine synthetase-, protoporphyrinogen oxidase- (PPO), very long-chain fatty acid synthesis (VLCFA)-, and hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides corresponding to WSSA Groups 2, 3, 4, 5, 9, 10, 14, 15, and 27, respectively (Heap 2024). For example, a population resistant to chlorsulfuron (WSSA Group 2), 2,4-D (WSSA Group 4), atrazine (WSSA Group 5), glyphosate (WSSA Group 9), and mesotrione (WSSA Group 27) was identified in Kansas (Kumar et al. 2019).

Between 1987 and 2007, US glyphosate usage increased from less than 5,000 metric tons yr^{-1} to over 80,000 metric tons yr^{-1} due to the widespread adoption of glyphosate-resistant (GR) crops and the expansion of reduced and no-tillage agriculture which often relies heavily on herbicides for weed control (Battaglin et al. 2014; Benbrook 2016). In the US, AMAPA's mechanism of resistance to glyphosate relies on the amplification of the EPSPS coding gene contained within extrachromosomal circular DNA (eccDNA), thus increasing enzyme copy numbers (Gaines et al. 2010, Molin et al. 2018, 2020a). Glyphosate-resistant populations of AMAPA were first identified in Georgia in 2006 (Culpepper et al. 2006) and are now found in over 28 US states as well as in Argentina, Brazil, Mexico, South Africa, and Uruguay (Heap 2024, Küpper et al. 2018, Molin et al. 2020). In the northeastern US, GR Palmer amaranth has been reported in Connecticut (Heap 2024), Pennsylvania (DD Lingenfelter, personal

communication), and New Jersey (TE Besançon, personal communication). Given its competitiveness, confirmed resistance to various herbicide SOAs, and expected range expansion due to climate change (Eceiza et al. 2022), AMAPA poses a major threat to cropping systems in the northeastern US.

The purpose of this study was to 1) confirm the presence of GR Palmer amaranth populations in NY through dose-response studies, 2) determine the mechanism of resistance of these populations, and 3) assess their sensitivity to alternative herbicide SOAs commonly used in corn, soybean, and vegetable production. We hypothesize that the suspected GR accessions are resistant through the amplification of eccDNA containing the gene coding for EPSPS, thus significantly increasing enzyme copy numbers. Further, we hypothesize that the NY Palmer amaranth population will exhibit reduced sensitivity to field use rates of at least one herbicide from WSSA Groups 2, 5, and 27.

Materials and Methods

Plant Material and Research Sites

In 2020, 2021, and 2022, three suspected GR AMAPA accessions were identified and collected from soybean fields in Steuben, Orange, and Genesee counties, NY respectively (Figure 2.1). Seedhead samples were collected from 20 female AMAPA plants detected within soybean fields that were sprayed with glyphosate during the growing season. Field edges were avoided during sampling. Seedheads were then threshed to separate the seeds which were subsequently cleaned and stored at 4 C until the start of the experiments. The Steuben County population (NY-STE) was discovered and collected from a farm near Howard, NY (42.36°N,

77.51°W). Most fields within this operation were in a corn-soybean-alfalfa rotation. The Orange County population (NY-ORA) was collected from a soybean and diversified vegetable farm near Florida, NY (41.33°N, 74.37°W). The Genesee County population (NY-GEN) was collected from a soybean farm located near Pavilion, NY (42.88°N, 78.02°W). In addition to these three populations, a glyphosate-susceptible (GS) AMAPA population collected from Keith County, Nebraska (NE-S) in 2017 was used in the experiments (Oliveira et al. 2021). Seeds from this population were provided by Dr. Rodrigo Werle at University of Wisconsin-Madison, WI.

Glyphosate Dose Response

In February through April 2023, the study was conducted as a completely randomized design (CRD) with ten replications per treatment and was repeated twice in sequence. Prior to planting, seeds were stratified at 4 C for at least 6 months. Five to ten AMAPA seeds were planted in 7.6 cm diameter pots filled with Lambert LM-111 growing media (Lambert, 106 Chemin Lambert, Rivière-Ouelle, Québec, Canada) and hand-thinned to one plant per pot after emergence. Growing media moisture was maintained for the duration of the experiment through daily irrigation. Plants were grown in greenhouses located at Cornell AgriTech in Geneva, NY set to a constant temperature of 25 C with a 16 h day length. Natural lighting was supplemented with 400 W high pressure sodium lamps.

All seedlings were grown to the two- to four-leaf stage at which time glyphosate (RoundUp PowerMax®, Bayer Crop Science, 800 N Lindbergh Boulevard, St. Louis, MO, USA) was applied at 27, 54, 109, 218, 435, 870 (label application rate), 1,740, 3,480, and 6,960 g ae ha^{-1}. A non-treated control (NTC) was also included for comparison. Applications were made using a single-nozzle cabinet sprayer (DeVries Manufacturing, 86956 MN-251, Hollandale, MN,

USA) equipped with a TeeJet 8002VS nozzle (TeeJet Technologies, Spraying Systems Co., 200 W. North Avenue, Glendale Heights, IL, USA). The cabinet sprayer was calibrated to deliver a volume of 187 L ha^{-1} at 276 kPa. At 21 days after treatment (DAT), plant survival was evaluated visually as dead (no green tissue = 0) or alive (green tissue and evidence of regrowth = 1). Aboveground biomass was also collected and recorded at 21 DAT, dried at 60 C for seven days, and then the dry weights were recorded. Biomass data was converted to percent biomass relative to the NTC using Equation 1:

$$\text{Percent biomass} = \frac{\text{DB}_{EU}}{\overline{\text{DB}}_{NTC}} \text{x } 100 \quad [1]$$

with DB_{EU} representing the dry biomass of the experimental unit and $\overline{DB}_{NTC}$ corresponding to the mean dry biomass of the NTC for each population.

EPSPS Gene Copy Number Assay

Seeds from the three NY putative resistant populations and from the NE known susceptible accession were grown to the six- to eight-leaf stage under the conditions described above using six replicates. One fully expanded leaf was harvested from the newest growth of each plant, inserted into 1.5 ml microcentrifuge tubes containing metal beads, and placed in a TissueLyser II (Qiagen Sciences, 19300 Germantown Road, Germantown, MD, USA) for one minute at 1,200 rpm. Upon sampling, tissue was stored at -80 C until further processing. Genomic DNA was extracted using a modified cetyltrimethylammonium bromide protocol as described by Xin and Chen (2012). Following DNA extraction, the concentration of nucleic acid was quantified using a NanoDrop 2000 (Thermo Fisher Scientific, 168 3rd Avenue, Waltham, MA, USA). DNA concentrations were standardized to 1 ng μL^{-1} using sterile high-performance

liquid chromatography (HPLC) grade water and used for quantitative polymerase chain reaction (qPCR) as described below.

Quantitative real-time PCR was used to measure gene copy number of EPSPS relative to ALS. Extracted DNA from the suspected GR NY and susceptible NE samples was used for this assay. Additionally, DNA extracted from a confirmed GR AMAPA population (AZ-R) and a confirmed GS population (AZ-S) originating from Pima County, AZ, was included as positive and negative controls (Molin et al. 2018); however, only three replicates were used. Primers EPSF1 (5′-ATGTTGGACGCTCTCAGAACTCTTGGT-3′) x EPSR8 (5′-TGAATTTCCTCCAGCAACGGCAA-3’) and ALSF2 (5′-GCTGCTGAAGGCTACGCT-3′) x ALSR2 (5′- GCG GGACTGAGTCAAGAAGTG-3′) were used as the EPSPS and ALS primers, respectively (Gaines et al. 2010, Tranel et al. 2004). ALS primers were included due to low variability in ALS gene sequences in *Amaranthus* spp. as demonstrated by Tranel et al. (2004).

Quantitative PCR was performed following the procedures described by Gaines et al. (2010). Each individual sample was run in duplicate. Each 20 µL reaction was composed of 2 µL of DNA template containing 10 ng of sample DNA, 1 µL forward primers, 1 µL reverse primers, 10 µL Syber-Green master mix (Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA, USA), and 6 µL sterile HPLC grade water. The 20 µL reactions were amplified by the following thermoprofile on a MyiQ real-time PCR detection system (Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA, USA): 95 C for 15 min, then 30 cycles of 95 C for 30 s and 60 C for 1 min. Real-time fluorescence data were captured during the amplification cycles. Negative controls containing primers without sample DNA or lacking both primers and sample DNA were included. No amplification products were observed in the negative controls.

Relative quantification of EPSPS copy number following the method developed by Gaines et al. (2010) was used to assess data from the qPCR experiment. Relative quantification of EPSPS copy number (ΔC_t) was calculated using Equation 2:

$$\Delta C_t = C_t^{ALS} - C_t^{EPSPS} \quad [2]$$

with C_t^{ALS} and C_t^{EPSPS} representing the number of cycles required for the fluorescent signal to exceed that of the background level (threshold cycle) for samples amplified using the ALS and EPSPS primers, respectively. Change in EPSPS copy number was reported as $2^{\Delta Ct}$ which is the multiplicative increase in EPSPS copy number relative to ALS copy number, the latter of which has proven monogenic inheritance in *Amaranthus* spp. (Trucco et al. 2005). Resistance to ALS-inhibiting herbicides does not result from ALS gene amplification; rather, it is caused by target site mutation at amino acid sites, thus interfering with the binding of ALS herbicides (Gaines et al. 2020).

EPSPS Cassette Marker Assay

AMAPA DNA isolated for the EPSPS gene copy number assay was also used to confirm the presence of eccDNA in the suspected GR Palmer amaranth population through PCR. Each reaction contained 10 ng of DNA template, EconoTaq DNA Polymerase (Lucigen Corporation, 2905 Parmenter Street, Middleton, WI, USA), forward and reverse primers, and sterile HPLC grade water thus producing a 20 µL reaction. Two sets of primer pairs were used in this experiment. Primer pairs AW293 (5′-GTTATAGCAGCAATTCACCAG-3’) x AW275 (5’-CTAGTTGTTTCACTTGTTTGTGTG-3’) and AW216 (5’-GACCTGGGTTGTCTTCATTC-3’) x AW541 (5’-CGATGATCCAACCGTCCA-3’), henceforth referred to as eccDNA markers A [1757 base pairs (bp)] and C (1554 bp) respectively, were used to amplify regions of the

eccDNA genome containing the amplified EPSPS gene (Molin et al. 2018). Reactions containing DNA from resistant and susceptible populations as described for the EPSPS gene copy number assay were used as positive and negative controls, respectively. In addition, separate negative control reactions containing replicon-specific markers, but no sample DNA were included in each amplification run. The PCR thermocycler settings were as follows: 4 min of denaturing at 94 C, followed by thirty 30 s cycles at 94 C, 30 s of annealing at 55 C, a 90 s extension period at 72 C, and a final 5 min extension period at 72 C. The presence or absence of the two eccDNA markers was detected using 1% agarose gel electrophoresis. No amplification products were observed in the negative controls.

Response to Alternative Chemistries

In addition to the glyphosate dose-response study, the sensitivity of the NE-S and suspected GR NY populations to alternative chemistries was also evaluated. Treatments consisted of registered postemergence (POST) herbicides commonly used in field corn, soybean, and vegetable production (Table 2.1) applied at the lowest rate recommended by the label. Plants were grown under the same greenhouse conditions as previously described for the glyphosate dose-response study. The experiment was conducted as a CRD with ten replicates of each population x herbicide combination except for chlorimuron-ethyl, chloransulam-methyl, and glufosinate for which six replicates were used. The study was conducted twice in time. Herbicides were applied when AMAPA reached the two- to four- leaf stage. The same cabinet sprayer and application settings as those described for the dose-response study were used. At 21 DAT, plant survival was evaluated visually as dead (no green tissue = 0) or alive (green tissue and evidence of regrowth = 1). This was used to calculate percent mortality for each combination

of herbicide and population. Aboveground biomass was subsequently collected, dried at 60 C for seven days, and weighed. Relative biomass was calculated using Equation 1.

Statistical Analysis

All statistical analyses were performed using RStudio version 2023.06.1 (R Core Team 2023). For the dose-response experiment, analysis of variance (ANOVA) was performed on the raw data to test for differences in the relative dry biomass (dependent variable) between experimental runs (independent variable). ANOVA confirmed that there were no significant differences between the experimental runs and data were pooled. The assumption of normality was not violated; thus, no transformation of the data was required. Three-parameter and four-parameter log-logistic functions were created using the *drc* package version 3.0-1 (Ritz et al. 2015). Lack-of-fit tested were run on each model using the *modelFit* function within the *drc* package (Ritz et al. 2015). We failed to reject the null hypothesis and concluded that the models fit the data well. ANOVA was performed to assess whether the three- and four-parameters log-logistic models significantly differ. ANOVA confirmed no significant differences in fit between the models, thus the simpler model was chosen. The three-parameter model was used to ascertain the dose of glyphosate required to reduce the biomass of each AMAPA population relative to the NTC by 50% (ED_{50}) using Equation 3 (Knezevic et al. 2007):

$$Y = \frac{d}{1+\exp\left[b(\log x - \log e)\right]} \quad [3]$$

where *Y* is the aboveground dry biomass expressed as a percentage of the mean biomass of the NTC and *x* is the herbicide application rate. Parameters *d* is the upper limit of the log-logistic curve. The ED_{50} is represented by *e* while *b* is the relative slope around parameter *e*. Using the *ED* function within the *drc* package, the dose of glyphosate required to reduce the biomass of

each AMAPA population relative to the NTC by 90% (ED_{90}) was also calculated. Given AMAPA's abundant seed production, high levels of control are required to reduce its persistence in-field and spread to new areas. The resistance level, expressed as the R/S ratio, was calculated by dividing the ED_{50} of each suspected GR population by the ED_{50} of the susceptible population. Statistical analysis was not conducted on the mortality data as the data was reported as percent dead for each herbicide treatment.

For the EPSPS gene copy number assay and the alternative chemistries experiment, a linear mixed model was fit to determine the effect of population on relative EPSPS gene copy number or the effect of herbicide and population on biomass accumulation, respectively, using the lme4 package version 1.1-32 (Bates et al. 2015). To account for the imbalance in the number of replicates, the Satterthwaite approximation for degrees of freedom was used (Kuznetsova et al. 2017). Herbicides, populations, and their interaction were treated as fixed effects while experimental run was considered a random effect. ANOVA was performed followed by Tukey's HSD pairwise comparisons ($\alpha = 0.05$) using the *emmeans* package version 1.8.8 (Lenth 2023). The normality and homoskedasticity assumptions were not violated, thus no transformations of the data were required.

Results and Discussion

Dose-Response Experiment

Resistance to glyphosate of suspected NY-GR populations was confirmed while the NE-S population demonstrated high sensitivity to glyphosate (Figure 2.2) as evidenced by the 100% reduction in biomass following glyphosate applied at 870 g ae ha^{-1}. Conversely, plants from the

NY-GEN, NY-ORA, and NY-STE populations only experienced 15%, 25%, and 10% mortality, respectively, when exposed to this dose. None of the NY populations were completely controlled by the highest dose (6,960 g ae ha^{-1}) of glyphosate; however, they experienced 85 to 95% mortality, and the biomass of the surviving plants was reduced by 69 to 96% (Table 2.3).

The dry biomass of suspected GR populations was reduced 48 to 70% as compared to the biomass of the nontreated control (Figure 2.2). The dose of glyphosate required to reduce the aboveground biomass of the suspected GR populations by 50% ranged from 565 to 902 g ae ha^{-1} (Table 2.3). The ED_{50} value for NY-ORA (565 g ae ha^{-1}) significantly differed from that of NY-STE (902 g ae ha^{-1}), but not NY-GEN (849 g ae ha^{-1}). The ED_{50} values of the NY populations were in within the lower part of the range of the reported ED_{50} values for GR Palmer amaranth populations in the US. For example, similar ED_{50} values were reported for GR AMAPA populations from Georgia and New Mexico with 560 and 458 g ae ha^{-1}, respectively (Culpepper et al. 2006; Mohseni-Moghadam et al. 2013b). Conversely, glyphosate applied at 1,320 g ae ha^{-1} caused a 50% reduction in the biomass of a GR Palmer amaranth population in Nebraska (Chahal et al. 2017), over double the dose required to observe similar results from the NY-ORA population. Similarly, glyphosate applied at 1,538 to 2,603 g ae ha^{-1} led to a 50% reduction in the shoot dry weight of two populations resistant to multiple SOAs in Nebraska (Kumar et al. 2018, 2019). Based on these ED_{50} values, the R/S ratios of the suspected GR resistant populations NY-GEN, NY-ORA, and NY-STE were 68, 42, and 64 respectively (Table 2.2). These R/S ratios were within the range of the reported values for GR Palmer amaranth populations in the US. On the lower end of the range, Culpepper et al. (2006) found that a GR AMAPA population from Georgia was 6.2-times more resistant than the susceptible population tested. Similarly, Kumar et

al. (2018) reported that a multiple herbicide-resistant AMAPA population from Kansas had R/S ratio values ranging from seven to fourteen. Similar to the results reported here, Chahal et al. (2017) observed that the GR populations from Nebraska were 37- to 40-times more resistant to glyphosate than the Nebraskan GS population. On the upper end of the range of R/S values, Norsworthy et al. (2008) reported that the Arkansan GR population's resistance levels were 79- to 115-times greater than that of the susceptible population. Based on the ED_{50}, resistance to glyphosate of all three suspected GR populations from NY has been confirmed.

EPSPS Gene Copy Number and Confirmation of EPSPS Cassette Presence

The NY populations had higher EPSPS copy numbers than the known susceptible populations from NE and AZ. The relative EPSPS gene copy numbers of NY-GEN, NY-ORA, and NY-STE averaged 55, 47, and 91, respectively (Figure 2.3). These results strongly suggest that amplification of the EPSPS gene confers glyphosate resistance in the NY AMAPA populations as described by Gaines et al. (2010) for GR AMAPA plants collected from Georgia. Chahal et al. (2017) found that AMAPA with at least 30 copies of the EPSPS gene survived exposure to glyphosate applied at 870 g ae ha^{-1}. Molin et al. (2018) reported that the EPSPS gene copy numbers of GR populations from Maricopa, Kent, Macon, Wicomico, and Washington counties in AZ, Delaware (DE), Georgia (GA), Kansas (KS), Maryland (MD), and Mississippi (MS), respectively, ranged from 6 to 61 copies. Other members of Amaranthaceae have evolved resistance to glyphosate through the same mechanism. For example, Gaines et al. (2016) confirmed that increased EPSPS gene copy number was the resistance mechanism of several GR kochia (*Kochia scoparia* L.*)* populations from sugar beet and wheat-chemical fallow fields across the Great Plains region of the US. Significantly higher gene copy number was observed

from the NY-STE plants than the NY-ORA plants. Though not explicitly tested in this experiment, the results appear to indicate that higher gene copy numbers of NY-STE may be responsible for the higher level of resistance (R/S = 64) observed in this population as compared to NY-ORA (R/S = 42). Vila-Aiub et al. (2014) found that plants with higher amplification of the EPSPS gene displayed higher levels of resistance compared to those with lower amplification of the EPSPS gene.

In addition, eccDNA markers A and C were amplified in the GR population from AZ and all NY suspected GR populations (Figure 2.4). The bands were similar in size, pattern, and position relative to the DNA ladder. However, sensitive populations from AZ and NE failed to amplify and produce PCR products, implying the absence of eccDNA. All three NY populations contain the EPSPS replicon. Results from this study support previous findings by Molin et al. (2020b) who reported that the mechanism of resistance is the amplification of the EPSPS replicon, or the approximately 400-kb eccDNA containing the EPSPS gene and 58 other genes that encode other competitive functions. Further, this mechanism is unique to GR plants (Molin et al. 2020a, 2020b). Koo et al. (2018) demonstrated that eccDNA is transmitted both mitotically and meiotically during cell division, leading to the rapid development of glyphosate resistance. Glyphosate resistance in Palmer amaranth has been found to have limited genetic diversity. Molin et al. (2018) hypothesized that glyphosate resistance evolved once in the US and spread across several populations. The EPSPS replicon is reported to be highly conserved across GR Palmer amaranth population from AZ, DE, GA, KS, MD, and MS, strongly suggesting that GR Palmer amaranth found in the US originated from a single population despite its widespread prevalence (Molin et al. 2018, 2020a).

Response to Alternative Herbicide Chemistries

ANOVA confirmed that there was no significant difference of AMAPA relative biomass between experimental runs ($P = 0.126$); therefore, data were pooled across runs. All AMAPA populations were fully controlled by applications of linuron (WSSA Group 7), glufosinate (WSSA Group 10), and paraquat (WSSA Group 22) at labeled rates (Table 2.3). While less than 50% mortality was observed in all populations treated with Group 14 herbicides, responses varied based on population. 85 to 100% and 95 to 100% mortality were observed for plants treated with fomesafen and flumioxazin respectively while oxyfluorfen caused 60 to 75% mortality. Mortality 21 DAT following application of ALS-inhibiting herbicides ranged from 0 to 42% across the three NY populations, indicating that none of the suspected GR populations were effectively controlled by the ALS-inhibitors tested in this study. The sensitivity of the NE-S population to Group 2 herbicides varied more than those of the NY populations, ranging from 20% to 70% mortality in response to halosulfuron-methyl and rimsulfuron application, respectively. NY-GEN plants were not effectively controlled by atrazine with 90% of them surviving as compared to only 15 and 40% for NY-ORA and NY-STE, respectively. Based on the percent mortality, mesotrione effectively controlled AMAPA populations NE-S (90%) and NY-ORA (75%); however, only 35 and 30% of NY-GEN and NY-STE plants were killed by the tested application rate.

While mortality data showed reduced sensitivity to Group 2 herbicides for all NY suspected GR populations, biomass data indicated variable responses to chlorimuron-ethyl ($P < 0.025$), chloransulam-methyl ($P < 0.001$), and halosulfuron-methyl ($P < 0.001$) (Figure 2.5). For the chlorimuron-ethyl treatment, the NY-STE population had significantly lower mean percent

biomass compared to the other populations; however, there was no significant difference between those of NE-S, NY-GEN, and NY-ORA. Chlorimuron-ethyl reduced the biomass of the NY-STE, NE-S, NY-GEN, and NY-ORA by 75, 52, 56, and 42% respectively. While all tested populations were ineffectively controlled by chloransulam-methyl, the NY-ORA population had significantly higher relative biomass compared to the other populations followed by NY-GEN, NY-STE, and finally NE-S. The tested populations exhibited a more varied response to halosulfuron-methyl. Halosulfuron-methyl reduced the biomass of NY-GEN, NY-ORA, NY-STE, and NE-S by 11, 43, 57, and 71%, respectively. The NY-GEN population had significantly higher relative biomass than the other populations followed by NY-ORA, NY-STE, and NE-S. For rimsulfuron, the NE-S population had significantly lower mean percent biomass compared to the NY populations; however, there was no significant difference between those of NY-GEN, NY-ORA, and NY-STE. In plants treated with atrazine, relative biomass was higher in NY-GEN (74%) but did not significantly differ between NY-ORA (7%) or NY-STE (14%). Mesotrione reduced the mean percent biomass of NY-GEN and NY-STE by 75% and 80% respectively. In addition to being resistant to glyphosate, NY-GEN may be resistant to at least one herbicide from WSSA Groups 2, 5, and 27. NY-ORA and NY-STE are suspected to be resistant to chlorimuron-ethyl, chloransulam-methyl, halosulfuron-methyl, and rimsulfuron. The variability in responses amongst the populations to these and other ALS-inhibiting herbicides should be further explored. In addition, NY-STE and NY-GEN may be resistant to mesotrione. Further research is required to confirm these suspicions.

The reduced efficacy of ALS-, PSII-, and HPPD-inhibitors at controlling AMAPA populations assessed in this study is consistent with previous reports. The poor control exhibited

by the alternative herbicide chemistries tested in this experiment may be due to application. The application of these herbicides at the lowest label recommended rate may account for responses observed. Several AMAPA populations developed resistance to single herbicides belonging to WSSA Groups 2, 3, 5, 9, 10, 14, 15, and 27. In addition, several populations resistant to more than one herbicide SOA have been identified (Heap 2024). For example, Faleco et al. (2022) found a newly introduced AMAPA population in Wisconsin that showed resistance to atrazine, glyphosate, and imazethapyr. While Chahal et al. (2017) found that glufosinate effectively controlled the multiple-resistance populations from Nebraska, Priess et al. (2022) confirmed the existence of a population resistant to glufosinate and suspected resistance to imazethapyr, pendimethalin, 2,4-D, glyphosate, fomesafen, S-metolachlor, mesotrione, and tembotrione in Arkansas. Finally, a population resistant to WSSA Groups 2, 4, 5, 9, 14, and 27, was reported in Kansas (Shyam et al. 2021).

The adoption of herbicide-tolerant crops over the last few decades has significantly reduced the diversity of SOAs in several cropping systems (Kniss 2018). The excessive dependance on a single SOA, such as glyphosate, has hastened the evolution and proliferation of herbicide resistance across several weed species and production systems (Boyd et al. 2022, Culpepper et al. 2006, Norsworthy et al. 2012). While the newly introduced NY populations are still sensitive to several of the herbicides SOAs tested, specifically auxin mimics, PPO-inhibitors, and photosystem I electron diverters, this sensitivity might be temporary. To maintain the efficacy of these herbicides, efforts must be taken to reduce selection pressure through the deployment of diversified weed management techniques including prevention, cultural practices, and mechanical options for control (Norsworthy et al. 2012). While practicing integrated weed

management offers several benefits, the short-term costs may not favor implementation of best management practices that provide delayed economic benefit. Efforts must continue to be made to facilitate the adoption of multifaceted approaches to weed management.

Conclusions and Broader Implications

This work confirms the presence of GR AMAPA in NY. The suspected GR populations have resistance levels ranging from 42- to 67-times that of the susceptible NE population. In addition, these populations exhibit reduced sensitivity to ALS-, PSII-, and/or HPPD-inhibitors. Future research should assess the sensitivity of these populations to different SOAs and confirm whether they harbor multiple resistance traits. While other herbicide SOAs are still effective in these introduced populations, their effectiveness is unlikely to last forever especially if herbicides are not used judiciously. The reduced sensitivity of AMAPA to multiple herbicide SOAs is particularly concerning, especially considering the very few number of new herbicide sites of action coming to market in the last three decades. The number of herbicides available to NY corn, soybean, and vegetable growers is diminishing and unlikely to rebound because of the rapidly changing regulatory environment due to increased compliance with the Endangered Species Act. Furthermore, it is highly possible that resistance would develop relatively quickly to new herbicides released to market due to increased selection pressure in response to the lack of other chemical control options. Considering all these factors, growers should look to integrated weed management for guidance and incorporate non-chemical-based control options into their production systems. These include mechanical methods such as tillage and cultivation,

preventative measures like harvest weed seed control, and novel technology including electrical weed control.

The presence of GR AMAPA in NY only increases the challenges facing NY growers as other HR species are present in the state including common lambsquarters (*Chenopodium album* L.), smooth pigweed (*Amaranthus hybridus* L.), waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], common ragweed (*Ambrosia artemisiifolia* L.), common groundsel (*Senecio vulgaris* L.), and horseweed [*Conyza canadensis* (L.) Cronquist]. Further, at least two of these species are resistant to multiple herbicide SOAs. Though less studied, herbicide resistant weeds are an issue in vegetable production (Boyd et al. 2022). Because of the diversified nature of northeastern cropping systems, there is an overlap in vegetable and agronomic crop acreage. Growers and land managers must be cautious of practices that can introduce AMAPA and further its spread. AMAPA can easily be introduced to new areas on agricultural equipment, through irrigation, and by birds (Boyd et al. 2022, Norsworthy et al. 2014, Proctor 1968). Equipment, especially those purchased from areas where AMAPA is common, should be scrutinized and thoroughly cleaned. Field applications of manure and spent plant materials present another avenue of introduction for this noxious weed (Yu et al. 2021). With climate change, AMAPA is expected to spread further north (Eceiza et al. 2022). New York finds itself in a similar position to Minnesota regarding AMAPA's presence in the state (Yu et al. 2021). Successful eradication is possible but unlikely. Regardless, management efforts will require rapid institution of a regulatory framework, access to funds, collaboration among various partners, concerted efforts towards educating the public, and the active addressing of new infestations. As such, agricultural professionals and the public

must remain vigilant and take steps to prevent future introductions, eradicate infestations early, and limit their spread.

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Tables and Figures

Table 2.1. Postemergence herbicides used to assess the response of Palmer amaranth populations to herbicide SOAs commonly used in NY agriculture.[a]

Active ingredient	WSSA group number	Herbicide family	Product formulation	Herbicide manufacturer	Application rate
					g ai ha^{-1} or g ae ha^{-1} [d]
Chlorimuron-ethyl	2	Sulfonylureas	Classic®	AMVAC, Newport Beach, CA 92660	13.1
Cloransulam-methyl	2	Triazolopyrimidine	FirstRate®	AMVAC, Newport Beach, CA 92660	35.3
Halosulfuron-methyl[b]	2	Sulfonylureas	Sandea®	Gowan, Yuma, AZ 85366	58.5
Rimsulfuron[b]	2	Sulfonylureas	Matrix® SG	Corteva, Indianapolis, IN 46268	70.1
2,4-D	4	Phenoxy-carboxylates	Embed® Extra	Corteva, Indianapolis, IN 46268	733*
Dicamba	4	Benzoic acid	XtendiMax® With VaporGrip® Technology	Bayer CropScience, St. Louis, MO 63167	559*
Atrazine	5	Triazine	Aatrex® 4L	Syngenta Crop Protection, Greensboro, NC 27419	1,244
Prometryn[b]	5	Triazine	Caparol® 4L	Syngenta Crop Protection, Greensboro, NC 27419	1,122

Linuron	7	Substituted ureas	Lorox® DF	Tessenderlo Kerley, Phoenix, AZ 85008	841
Glufosinate	10	Phosphinic acids	Rely® 280	Bayer CropScience, St. Louis, MO 63167	595
Flumioxazin	14	N-Phenyl-imides	Chateau® SW	Valent, Walnut Creek CA 94596	70.1
Fomesafen[b]	14	Diphenyl ethers	Reflex®	Syngenta Crop Protection, Greensboro, NC 27419	421
Oxyfluorfen	14	Diphenyl ethers	Goaltender®	NuFarm, Alsip, IL 60803	210
Paraquat[b]	22	Bipyridilium	Gramoxone® SL 3.0	Syngenta Crop Protection, Greensboro, NC 27419	754
Mesotrione[b]	27	Triketone	Callisto®	Syngenta Crop Protection, Greensboro, NC 27419	105
Topramezone[c]	27	Pyrazoles	Impact®	AMVAC, Newport Beach, CA 92660	24.5

[a]Abbreviations: ai, active ingredient; ae, acid equivalent; SOA, site of action; WSSA, Weed Science Society of America

[b] Nonionic surfactant at 0.25% (v/v) was added.

[c] Methylated seed oil at 1% (v/v) was added.

[d]Rates displayed with an asterisk (*) are acid equivalent (ae) while those without an asterisk are active ingredient (ai).

Table 2.2. Estimation of regression parameters and glyphosate dose required for 50% (ED_{50}) and 90% (ED_{90}) reduction in biomass of Palmer amaranth populations 21 days after treatment.

Palmer amaranth biotype[a]	Glyphosate				
	Regression parameters (±SE)[b]		ED_{50} (±SE)[a]	ED_{90} (±SE)[a]	R/S[c]
	b	d	g ae ha^{-1}		
NE-S	1.07 (±0.33)	99.98 (±5.74)	13.33 (±5.36)	103.24 (±35.62)	-
NY-GEN	1.24 (±0.16)	101.31 (±3.60)	901.99 (±124.13)	5311.44 (±1165.05)	67.67
NY-ORA	1.62 (±0.34)	88.20 (±3.78)	565.34 (±76.01)	2195.91 (±577.81)	42.42
NY-STE	1.97 (±0.30)	100.53 (±2.94)	848.84 (±81.61)	2579.42 (±447.60)	63.68

[a]Abbreviations: NE-S, glyphosate-susceptible biotype from Keith County, NE; NY-GEN, NY-ORA, NY-STE, glyphosate-resistant populations from Genesee County, Orange County, and Steuben County, NY respectively; ED_{50}, the effective dose of glyphosate required to reduce the biomass of the population relative to the non-treated control (NTC) by 50%; ED_{90}, the effective dose of glyphosate required to reduce the biomass of the population relative to the NTC by 90%; ae, acid equivalent; SE, standard error.

[b]Regression parameters were estimated using a three-parameter log-logistic model, $Y = \frac{d}{1+\exp[b(\log x - \log e)]}$, where b represents the slope of the curve at the inflection point, d represents the upper limit, and e represents the dose of glyphosate needed to cause 50% biomass reduction (ED_{50}) compared to the NTC.

[c]R/S represents the resistant:susceptible ratio between the known-susceptible (NE-S) and the suspected resistant NY populations (NY-GEN, NY-ORA, NY-STE).

Table 2.3. Percent mortality of Palmer amaranth populations (NE-S, NY-GEN, NY-ORA, and NY-STE) following application of several postemergence herbicides. Biotypes with mortality ≤50% were considered ineffectively controlled by an herbicide.[a]

			Palmer amaranth mortality 21 DAT (%)			
Active ingredient	WSSA group number	Herbicide family	NE-S	NY-GEN	NY-ORA	NY-STE
Chlorimuron-ethyl	2	Sulfonylureas	42	25	25	42
Cloransulam-methyl	2	Triazolopyrimidine	33	8	0	8
Halosulfuron-methyl[b]	2	Sulfonylureas	20	15	10	15
Rimsulfuron[b]	2	Sulfonylureas	70	30	10	10
2,4-D	4	Phenoxy-carboxylates	100	80	100	75
Dicamba	4	Benzoic acid	85	95	100	90
Atrazine	5	Triazine	65	10	85	60
Prometryn[b]	5	Triazine	100	90	100	100
Linuron	7	Substituted ureas	100	100	100	100
Glufosinate	10	Phosphinic acids	100	100	100	100
Flumioxazin	14	N-Phenyl-imides	100	95	100	95
Fomesafen[b]	14	Diphenyl ethers	100	85	100	100
Oxyflurofen	14	Diphenyl ethers	75	60	65	70
Paraquat[b]	22	Bipyridilium	100	100	100	100
Mesotrione[b]	27	Triketone	90	35	75	30
Topramezone[c]	27	Pyrazoles	100	65	100	85

[a]Abbreviations: DAT, days after treatment; NE-S, glyphosate-susceptible biotype from Keith County, NE; NY-GEN, NY-ORA, NY-STE, glyphosate-resistant populations from Genesee County, Orange County, and Steuben County, NY respectively; WSSA, Weed Science Society of America

[b] Nonionic surfactant at 0.25% (v/v) was added.

[c] Methylated seed oil at 1% (v/v) was added.

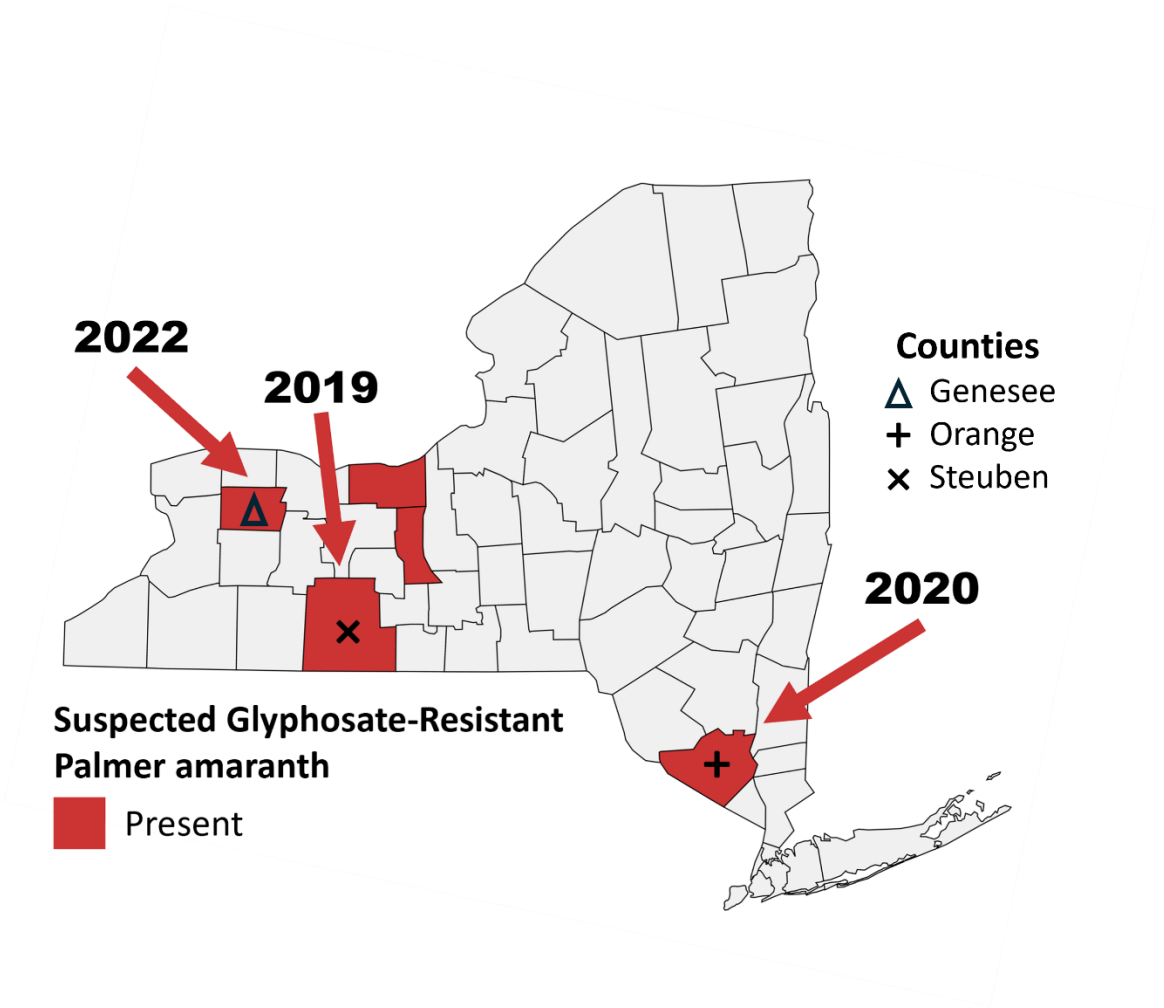

Figure 2.1. Map of New York showing counties (Genesee, Orange, Seneca, Steuben, and Wayne) in which populations of Palmer amaranth have been found. Insufficient seed was obtained from populations in Wayne and Seneca Counties to include in the research trials.

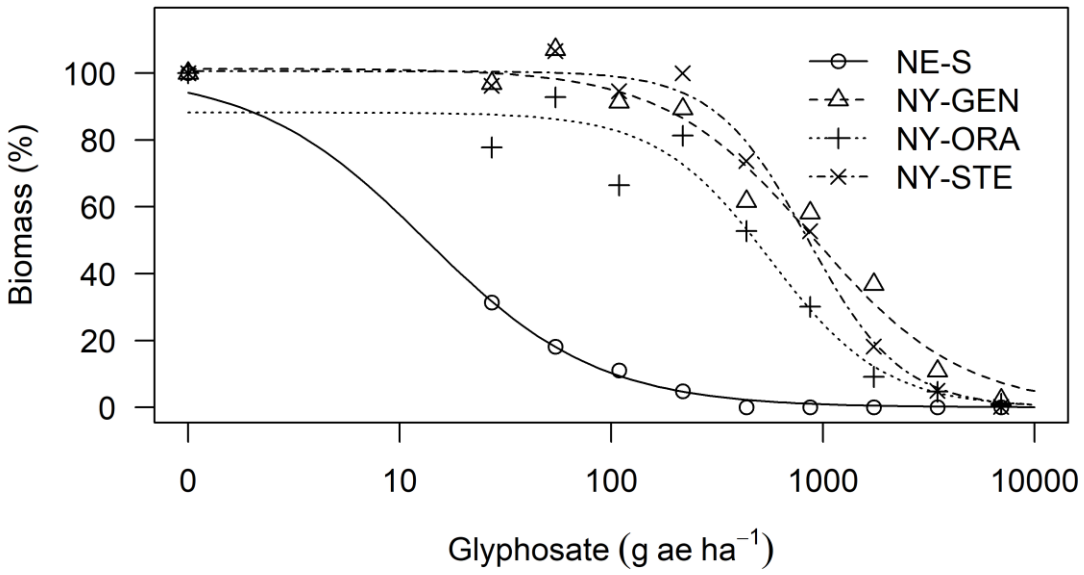

Figure 2.2. Dose-response curves of glyphosate-resistant (NY-GEN, NY-ORA, and NY-STE) and glyphosate-susceptible (NE-S) populations showing percent biomass 21 days after treatment. Percent biomass was calculated with the following equation: $\text{Percent biomass} = \frac{\text{DB}_{EU}}{\overline{\text{DB}}_{NTC}} \text{ x } 100$ with DB_{EU} representing the dry biomass of the experimental unit and $\overline{DB}_{NTC}$ representing the mean biomass of ten untreated control replicates for the appropriate population.

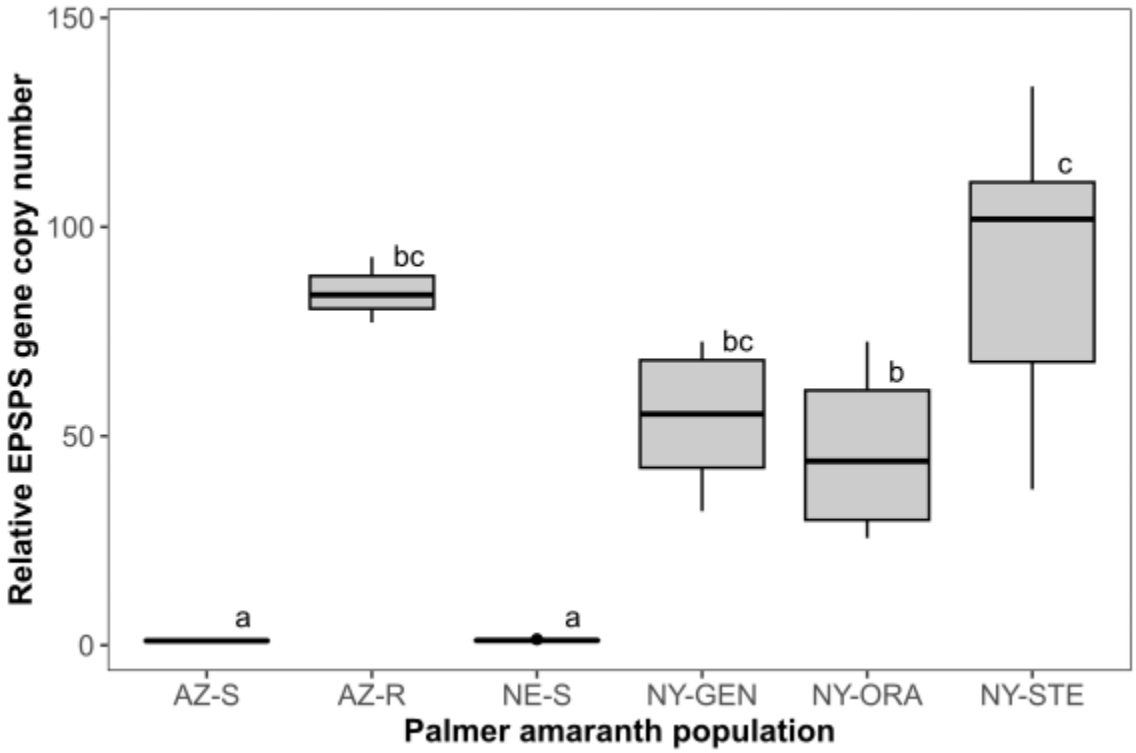

Figure 2.3. The relative *EPSPS* gene copy numbers of glyphosate-resistant and glyphosate-susceptible Palmer amaranth populations (n = 6 biological replicates). NY-GEN, NY-ORA, and NY-STE were suspected-resistant populations. Biotypes NE-S and AZ-S were known glyphosate-susceptible controls while AZ-R was a known glyphosate-resistant control.

Abbreviations: *EPSPS*, 5-enol-pyruvylshikimate-3-phosphate synthase; AZ-R and AZ-S, glyphosate-resistant and -susceptible populations collected in Pima County, AZ, respectively; NE-S, glyphosate-susceptible biotype collected in Keith County, NE; NY-GEN, NY-ORA, NY-STE, glyphosate-resistant populations collected in Genesee County, Orange County, and Steuben County, NY respectively.

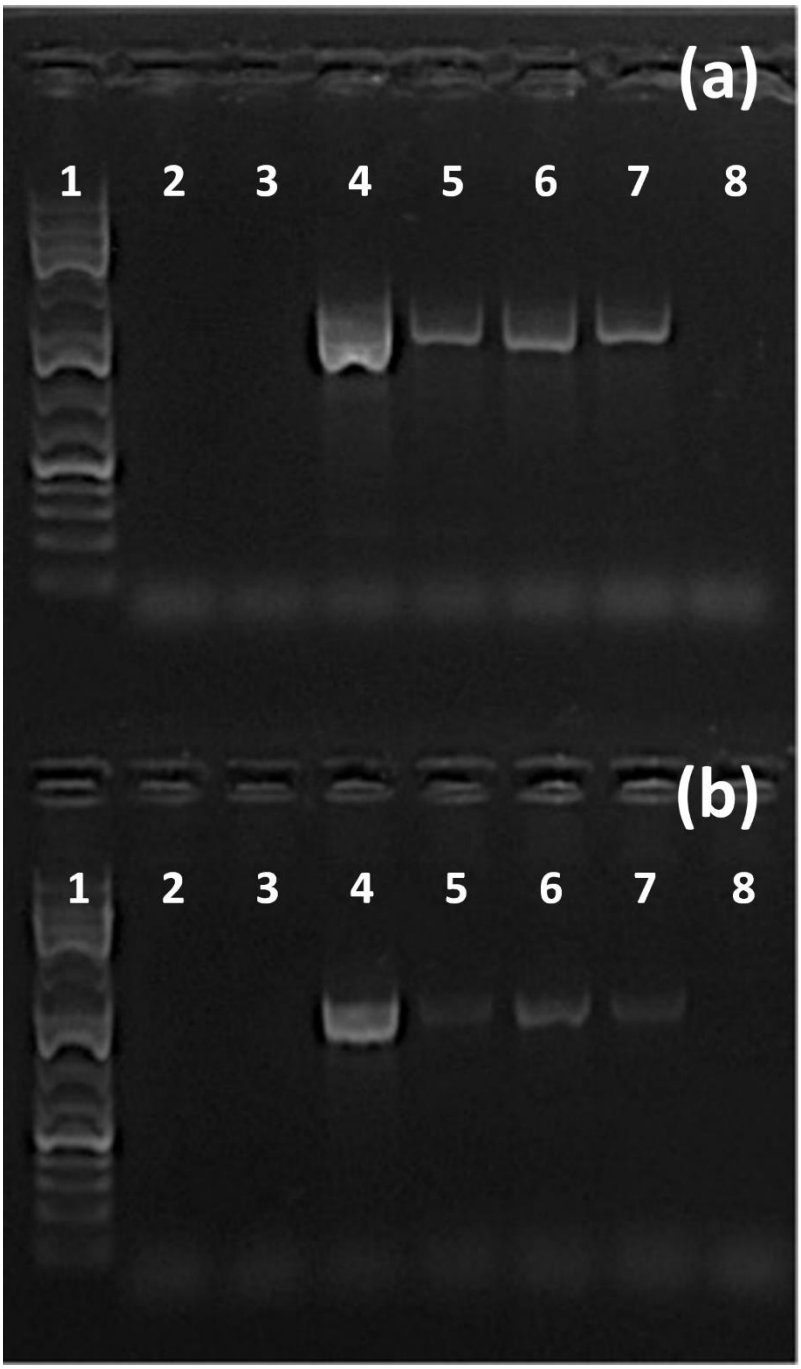

Figure 2.4. Gel image illustrating PCR analysis of the EPSPS eccDNA cassette markers (a) A (1757 bp) and (b) C (1554 bp) in glyphosate-resistant and -susceptible Palmer amaranth populations from Arizona, Nebraska, and New York, USA. Lanes include (1) 1-kilobase (kb)

ladder, (2) no template negative control, (3) AZ-S, (4) AZ-R, (5) NY-GEN, (6) NY-ORA, (7) NY-STE, and (8) NE-S. Individuals from all New York populations amplified both EPSPS cassette primers similar to the AZ-R positive control. Each sample tested displayed results similar to the figure presented above. **Abbreviations:** EPSPS, 5-enol-pyruvylshikimate-3-phosphate synthase; eccDNA, extrachromosomal circular DNA; AZ-R and AZ-S, glyphosate-resistant and -susceptible populations collected in Pima County, AZ; NE-S, glyphosate-susceptible population collected in Keith County, NE; NY-GEN, NY-ORA, NY-STE, glyphosate-resistant populations collected in Genesee County, Orange County, and Steuben County, NY respectively.

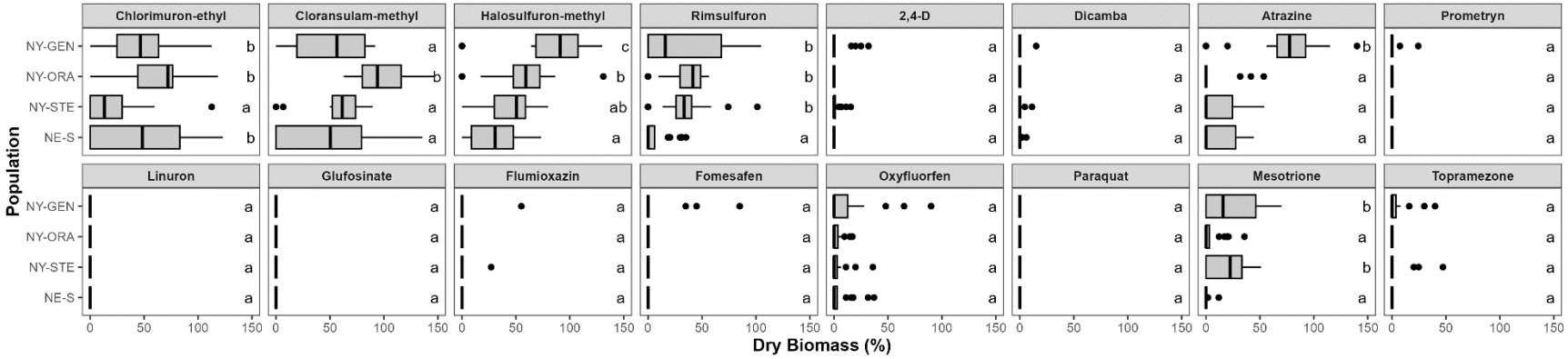

Figure 2.5. Percent biomass 21 days after treatment of Palmer amaranth populations from Nebraska (NE-S) and New York (NY-GEN, NY-ORA, and NY-STE) in response to herbicide. Percent biomass is expressed as a percent of the mean NTC and calculated using the following equation: $\text{Percent biomass} = \frac{\text{DB}_{EU}}{\overline{\text{DB}}_{NTC}} \text{x } 100$ with DB_{EU} representing the dry biomass of the experimental unit and $\overline{DB}_{NTC}$ representing the mean biomass of the appropriate population. Treatments with the same letters did not significantly differ according to Tukey's honestly significant difference ($\alpha = 0.05$).

CHAPTER 3: Preliminary Evaluation of the Influence of Temperature on Glyphosate Sensitivity in Palmer amaranth (*Amaranthus palmeri* S. Watson)

Introduction

In the United States, weeds are estimated to be responsible for $33 billion in crop production losses annually (Pimentel et al. 2005). The increasing global temperatures associated with climate change are expanding the distributions of several agricultural weeds with the potential for resulting impacts on crop production (Clements and Jones 2021). For many weedy species, elevated temperature hastens growth and development (Korres et al. 2016, Ramesh et al. 2017). In addition, higher temperature can decrease the herbicide efficacy depending on the mechanism of resistance. Non-target site resistance (NTSR) mechanisms are particularly susceptible to the effect of temperature with respect to herbicide performance (Gaines et al. 2020). These findings are especially concerning considering that herbicides are the primary method of weed management employed in the US (Varanasi et al. 2016, Ziska 2020). The effects of air temperature on weed growth and glyphosate sensitivity can vary among species and, at times, populations (Matzrafi 2019).

One weed of particular concern is Palmer amaranth (*Amaranthus palmeri* S. Watson; AMAPA). Palmer amaranth is consistently ranked as one of the most difficult to manage and economically important weeds in the US (Van Wychen 2022). This dioecious, C_4 weed is highly competitive in a number of cropping systems for several reasons including its rapid growth rate (Ward et al. 2013), precocious flowering, copious seed production (Keeley et al. 1987), and ability to thrive across a range of growing conditions (Guo and Al-Khatib 2003, Jha et al. 2008, Spaunhorst et al. 2018, Wright et al. 1999). Additionally, several populations of Palmer amaranth

have evolved resistance to herbicides within Weed Science Society of America (WSSA) Groups 2, 3, 4, 5, 9, 10, 14, 15, and 27 individually (Heap 2024). Furthermore, several populations have developed resistance to more than one herbicide with the same mode of action (SOA) or herbicides across two or more SOAs (Heap and Duke 2018, Heap 2024).

Glyphosate, an 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)-inhibiting herbicide (WSSA Group 9), is the most widely used herbicide in the world (Duke 2018). Glyphosate targets EPSPS, an enzyme essential to the proper functioning of the shikimate pathway thus preventing the biosynthesis of aromatic amino acids (Dayan et al. 2019). While it was doubted whether plants could naturally become resistance to glyphosate (Bradshaw et al. 1997), glyphosate-resistant (GR) populations of Palmer amaranth were first detected in Georgia in 2006 (Culpepper et al. 2006). As of 2024, GR Palmer amaranth is found in the majority of states in the United States in addition to Argentina, Brazil, Mexico, South Africa, and Uruguay (Heap 2024, Küpper et al. 2018, Molin et al. 2020a). In addition, Palmer amaranth is present in five counties in New York (NY) despite being rare in the northeast US (LM Sosnoskie, personal communications). Glyphosate-resistance in Palmer amaranth is conferred by the amplification of the EPSPS coding gene (Gaines et al. 2010) housed within extrachromosomal circular DNA (eccDNA) which increases the number of enzyme copies produced (Molin et al. 2020b). While gene amplification sometimes has an associated fitness cost, there is mixed evidence that amplification of the EPSPS gene negatively affects GR Palmer amaranth populations in the absence of glyphosate (Cahoon et al. 2022, Giacomini et al. 2014, Vila-Aiub et al. 2014).

While temperature has been shown to impact the growth and development of Palmer amaranth (Ehleringer 1983, Guo and Al-Khatib 2003, Wright et al. 1999), its impact on

glyphosate sensitivity is less understood. In a recent study, de Souza Rodrigues et al. (2023) evaluated the impact of temperature, carbon dioxide (CO_2) concentrations, and glyphosate resistance on early growth and development of GS and GR Palmer amaranth populations from Georgia. Carbon dioxide concentration, as opposed to temperature, was the main driver with plants grown at 750 ppm CO_2 being significantly taller, more voluminous, and accumulating more biomass than those grown at 410 ppm CO_2. In addition, the GS population exhibited significantly greater height, number of leaves, and plant volume than the GR populations tested. In contrast, temperature did not significantly affect the growth and development of Palmer amaranth with respect to population (de Souza Rodrigues et al. 2023). The study did not include a glyphosate treatment; thus, it is unknown whether the selection pressure posed by glyphosate exposure would alter the experiment's results. We conducted a preliminary study to better understand the influence of elevated temperature on the growth of Palmer amaranth and how that may differ based on glyphosate-resistance. Given that Palmer amaranth is relatively new to NY, the NY populations may have evolved and thus be better adapted to a warmer climate. In addition, glyphosate-resistance conferred by EPSPS gene amplification does not negatively affect the fitness in GR biotypes (Vila-Aiub et al. 2014). We hypothesized that elevated temperatures would increase early plant growth and decrease the sensitivity of the GR populations to glyphosate but would have no impact on that of the GS populations.

Materials and Methods

Plant Material and Research Sites

In 2020, a suspected GR Palmer amaranth population was identified from a soybean field near Howard, NY in Steuben County (42.36°N, 77.51°W), hereafter referred to as GR-NY. Most fields within this operation were in a corn-soy-alfalfa rotation. Seedhead samples were collected from 20 female Palmer amaranth plants found within the main production field that received in-crop glyphosate applications during the growing season. Field edges were avoided during sampling. After in-situ collection, the seedheads were threshed. The resulting seeds were cleaned and stored at 4 C until the start of the experiment. This population was confirmed to be glyphosate-resistant through dose-response studies and molecular EPSPS gene copy number and cassette marker assays as described in Chapter 2. In addition, a glyphosate-susceptible (GS) population was sampled from Keith County, Nebraska (GS-NE) in 2017 was used in this experiment (Oliveira et al. 2021). Oliveira et al. (2021) confirmed the glyphosate sensitivity of the GS-NE population. Seed from this population was donated by Dr. Rodrigo Werle at University of Wisconsin in Madison, WI. Complete herbicide records were not available for both populations.

Glyphosate Dose Response

In 2023 and 2024, growth chamber dose-response studies were conducted at Cornell AgriTech in Geneva, NY (42.86°N, 76.98°W) to evaluate the response of GR and GS Palmer amaranth populations to temperature and glyphosate exposure. Plants were grown in Conviron PGR15 growth chambers (Controlled Environments Limited, 590 Berry Street, Winnipeg, Manitoba, Canada). The study was arranged as a completely randomized design (CRD) with ten

replications per treatment and repeated twice. Palmer amaranth populations GR-NY and GS-NE were used as the resistant and susceptible populations, respectively. The GS-NE population was used as the susceptible population because a GS population has not been discovered in NY thus far. Prior to planting, seeds were stratified at 4 C for at least six months. Five to ten Palmer amaranth seeds were planted in 7.6 cm diameter pots filled with Lamber LM-111. Plants were grown in growth chambers set to a 16 h day length, 60% relative humidity, and one of the following temperature regimes: low (25/15 C, day/night), consistent with current summer average temperatures in NY (Kunkel 2022); intermediate (30/20 C, day/night); and optimum for Palmer amaranth (35/25 C, day/night) (Godar et al. 2015, Guo and Al-Khatib 2003). Growing media moisture was maintained for the duration of the experiment through daily irrigation. Seedlings were thinned to one plant per pot and treated with glyphosate at the two- to four-leaf stage.

Glyphosate was applied at 27.2, 54.4, 108.8, 217.5, 435, 870 (label recommended rate), 1740, and 3480 g ae ha^{-1} (RoundUp PowerMax®, Bayer Crop Science, 800 N Lindbergh Boulevard, St. Louis, MO, USA). Additionally, plants were left unsprayed to serve as a nontreated control (NTC). Applications were made using a single-nozzle cabinet sprayer (DeVries Manufacturing, 86956 MN-251, Hollandale, MN, USA) equipped with a TeeJet 8002VS nozzle (TeeJet Technologies, Spraying Systems Co., 200 W. North Avenue, Glendale Heights, IL, USA). The sprayer was calibrated to deliver a spray volume of 187 L ha^{-1} at 276 kPa. Fourteen days after treatment (DAT), aboveground biomass was collected, dried at 60 C for 7 days, and weighed. Dry biomass was converted to percent biomass relative to the NTC using Equation 1:

$$\text{Percent biomass} = \frac{\text{DBEU}}{\overline{\text{DBNTC}}} \text{x } 100 \quad [1]$$

with DBEU representing the biomass of the experimental unit and $\overline{\text{DBNTC}}$ corresponding to the mean biomass of the NTC for each population.

Statistical Analysis

Statistical analyses were executed in RStudio version 2023.06.1 (R Core Team 2023). For the dose-response experiment, analysis of variance (ANOVA) confirmed no significant differences in percent biomass between the experimental runs and data were pooled. Three-parameter and four-parameter symmetrical log-logistic, four-parameter Weibull, and five-parameter Brain-Cousens (hormesis) models were fit using the **drc** package version 3.0-1 (Ritz et al. 2015). Because models were unable to be fit to the GS-NE population due to its high sensitivity to glyphosate, only data from the GR-NY population was used for this analysis. ANOVA was performed followed by Tukey's pairwise comparisons ($\alpha = 0.05$) using the **emmeans** package version 1.8.8 (Lenth 2023) to determine if there were significant differences between the models. These confirmed no difference between the four models, so the simplest model was used. The three-parameter model was used to determine the dose of glyphosate needed to reduce the biomass of the GR-NY Palmer amaranth populations by 50% (ED_{50}) relative to the NTC using Equation 2 (Knezevic et al. 2007):

$$Y = \frac{d}{1+\exp\left[b(\log x - \log e)\right]} \quad [2]$$

Where *Y* is the aboveground dry biomass described as a percentage of the mean biomass of the NTC and *x* is the dose of glyphosate. Parameters *d* and *e* are the upper limit of the log-logistic curve and ED_{50}, respectively. Parameter *b* is the relative slope around parameter *e*.

Using the complete dataset, a linear mixed model was fit to determine the effect of temperature and population on the untreated Palmer amaranth biomass using the **lme4** package version 1.1-32 (Bates et al. 2015) with the Satterthwaite approximation for degrees of freedom (Kuznetsova et al. 2017). Temperature and population were treated as fixed effects while growth chamber was treated as a random effect. Square root transformations were performed on the NTC Palmer amaranth biomass to meet the normality assumption. ANOVA was performed followed by Tukey's pairwise comparisons ($\alpha = 0.05$) using the **emmeans** package version 1.8.8 (Lenth 2023) to determine if there were significant differences in NY-GR biomass based on temperature.

Results and Discussion

The GS-NE and GR-NY populations reached the two- to four-leaf stage 16 to 21 days after planting (Table 3.1). The biomass of the nontreated GS-NE was affected by temperature (Figure 3.1). The nontreated GS-NE Palmer amaranth biomass was highest in the low-temperature (25/15 C) chamber followed by the medium- (30/20 C) and high-temperature chambers (35/25 C), respectively. Fourteen days after treatment, the nontreated GS-NE Palmer amaranth in the high-temperature chamber accumulated 58% less biomass than those in the low-temperature chamber (Table 3.1). The nontreated GR-NY was not similarly affected by temperature, although there was a trend towards reduced biomass with increasing temperatures. Based on these findings, our hypothesis that increased temperatures would increase early Palmer amaranth plant growth was not supported. Rather, biomass of the nontreated Palmer amaranth

was highest in the low-temperature chamber (GS-NE) or did not differ between temperatures (GR-NY).

These results contrast with those of earlier studies which reported increased Palmer amaranth growth and better performance under warmer conditions. Ehleringer (1983) found that Palmer amaranth reached peak photosynthetic capacity when leaf temperature reached 42 C. Wright et al. (1999) reported that increasing temperatures from 26/22 C to 34/30 C increased Palmer amaranth biomass by over 400% 14 DAT. Similar results were observed by Guo and Al-Khatib (2003). They found that Palmer amaranth accumulated significantly more biomass at when grown 35/30 C and 25/20 C than 15/10 C (Guo and Al-Khatib 2003). In contrast, de Souza Rodrigues et al. (2023) reported that temperature did not significantly affect the early growth and development of Palmer amaranth regardless of its glyphosate-resistance status. It is possible that plants in the high-temperature chamber were negatively impacted by drought stress; however, this is unlikely due to frequent watering (once to twice per day), although soil moisture was not explicitly monitored. Additionally, Palmer amaranth is well adapted to drought conditions (Ehleringer 1983, 1985, Ward et al. 2013). Another possibility is that, given its origins in Nebraska, the GS-NE population may have a lower optimal temperature than populations from the southern US where Palmer amaranth is most problematic and studied. In the aforementioned studies, Palmer amaranth seeds were collected from Utah, North Carolina, Kansas, and Georgia.

Glyphosate-resistance status did not affect the biomass of nontreated Palmer amaranth 14 DAT ($P = 0.744$). This suggests there may not be a fitness cost associated with glyphosate resistance, specifically EPSPS gene amplification, in Palmer amaranth. Other studies reported similar results that EPSPS amplification does not incur a fitness cost in GR Palmer amaranth

(Giacomini et al. 2014, Vila-Aiub et al. 2014). In contrast, a field based study found that Palmer amaranth plants with EPSPS amplification produced significantly lower biomass and fewer seeds compared to GS plants (Cahoon et al. 2022). The environmental conditions experienced by the mother plant also affect competitiveness and herbicide sensitivity of the progeny. In testing the maternal effects on the drought tolerance of progeny seed, Matzrafi et al. (2021) found that Palmer amaranth plants from California and Kansas grown under drought conditions produced seeds that were 18% larger and 20 to 32% less dormant than those of non-stressed plants. Further, these seeds were able to germinate in drier conditions compared to seeds from non-stressed plants (Matzrafi et al. 2021). Osipitan et al. (2021) examined the effect of maternal drought-stress on the herbicide sensitivity of the progeny. They found that maternal Palmer amaranth plants grown under drought conditions produced progeny that was significantly less sensitive to S-metolachlor, rimsulfuron, saflufenacil, and simazine (Osipitan et al. 2021). Controlling for genetic background is important for fitness cost studies (Giacomini et al. 2014, Vila-Aiub et al. 2009, 2011). Unlike the above-mentioned studies, our experiment did not control for genetic background because GS populations of Palmer amaranth were not detected in New York at the time of the of study. Differences in biomass might be observed between GS and GR populations with similar genetic backgrounds.

Dose-response curves could not be fit to the GS-NE data due to this population's high sensitivity to glyphosate. Following exposure to glyphosate applied at 27.2 g ae ha^{-1}, 1/32-times the field recommended rate (870 g ae ha^{-1}), 100% biomass reduction was observed 14 DAT (data not shown). At the 870 g ae ha^{-1} rate, plant biomass from the GR-NY population was reduced by 61 to 73% compared to the nontreated control, depending on the chamber temperature (Figure

3.2). The dose of glyphosate required to reduce the aboveground biomass of the GR population by 50% (ED_{50}) ranged from 451 to 1,170 g ae ha^{-1} (Table 3.2). The ED_{50} value of the medium-temperature treatment (451 g ae ha^{-1}) was significantly lower than that of the high-temperature treatment (1,170 g ae ha^{-1}); however, no other contrasts were significantly different. This result is suspected to be spurious due to the lack of a biological explanation.

Though temperature has been shown to affect glyphosate sensitivity in other weed species, their resistance is due to NTSR mechanisms. For example, Nguyen et al. (2016) reported that the glyphosate sensitivity of a GR jungle rice population [*Echinochloa colona* (L.) Link] increased when grown at 30 C compared to 20 C; however, temperature had no impact on the glyphosate sensitivity of the GS population tested. The altered response to glyphosate in these species was due to reduced glyphosate uptake (NTSR) at higher temperatures. Neither population exhibited increased EPSPS gene copy number (Nguyen et al. 2016). Guo et al. (2023) reported similar results from an experiment evaluating the influence of temperature on glyphosate efficacy in goosegrass [*Eleusine indica* (L.) Gaertn.]. Greater shikimic acid accumulation was observed under the lower temperature regime compared to the high temperature regime. GR goosegrass grown at 30/20 C were 8.9-times more resistant to glyphosate than the GS population; however, the resistance level decreased to 3.1 when temperatures were lowered to 20/15 C. For both GR and GS populations, lower translocation was observed at 20/15 C than 30/20 C at 3 DAT, again a NTSR mechanism (Guo et al. 2023).

In testing the effect of temperature on sensitivity of *Amaranthus patulus* Bertol. to several herbicides, Park et al. (2021) reported that glyphosate was more effective at controlling *A. patulus* when grown at 35 C compared to 30 C; however, there were no differences in percent

control between plants grown at 25 C and 35 C. The authors did not discuss the mechanism of resistance for this species. Kaundun et al. (2019) found that a larger proportion of GR Palmer amaranth plants from Argentina survived exposure to glyphosate when grown at 30/26 C compared to those grown at 20/16 C; however, resistance in these populations was not due to EPSPS amplification. Rather, the resistance was largely conferred by a proline 106 to serine (P106S) target-site mutation in addition to EPSPS over-expression. This P106S mutation was not detected in any of the plants assessed belonging to US populations (Kaundun et al. 2019). Both TSR, specifically P106S mutation, and NTSR glyphosate resistance mechanisms were identified in Argentinian Palmer amaranth populations; however, EPSPS amplification was not detected (Larran et al. 2022).

Temperature does seem to impact herbicide efficacy in US Palmer amaranth populations; however, the effects seem limited to NTSR mechanisms. For example, Godar et al. (2015) reported that Palmer amaranth was less sensitive to mesotrione (WSSA Group 27) when grown at 40/30 C compared to 32.5/22.5 C due to an increase in the rate of mesotrione metabolism. Additionally, metabolism-based resistance to 2,4-D (WSSA Group 4) was reported in a six-way-resistant population of Palmer amaranth in Kansas. This resistance mechanism is suspected to be moderated by cytochrome P450 activity (Rudell et al. 2023). Rudell et al. (2023) found that the resistant Palmer amaranth population were 23-times more resistant than the susceptible population when grown at 34/24 C; however, the resistance level decreased to two when temperatures were lowered 24/14 C. When plants were treated with malathion, a P450 inhibitor , and followed by 2,4-D, resistant plants grown at 34/24 C became significantly more sensitive to

2,4-D (Rudell et al. 2023). Given that EPSPS amplification is a TSR mechanism, it may be unlikely for temperature to influence glyphosate efficacy in the GR-NY population.

It is expected that climate change will reduce herbicide efficacy and select for NTSR in many weed species (Matzrafi et al. 2016); however, elevated temperatures are only one of the environmental changes associated with climate change. Increased atmospheric CO_2 concentrations can also decrease herbicide efficacy by modifying the rate of detoxification, metabolism, and/or translocation, all factors in non-target site herbicide resistance (Matzrafi 2019, Ziska 2020). Though glyphosate resistance in Palmer amaranth may not be affected by increased temperatures, it is still highly competitive under current and future climates (Kistner and Hatfield 2018, Massinga et al. 2001, Ward et al. 2013). Further, projected increases in temperature due to climate change is likely to expand Palmer amaranth's range further north into regions of Canada and Europe (Kistner and Hatfield 2018). The selection pressure imposed by and range expansion expected with climate change must be considered when developing long-term weed management strategies and evaluating the sustainability of these approaches.

Next Steps and Future Considerations

This preliminary study is a first step in understanding the role of temperature in Palmer amaranth's glyphosate sensitivity. Additional research is needed to determine whether temperature influences glyphosate's efficacy in GR and GS Palmer amaranth. In future experiments, the lower range of glyphosate doses will be expanded to increase the chance of observing gradual differences in the GS population. In addition, plants will be grown longer prior to harvest. This additional time may allow plants to segregate more clearly according to

treatment effects. Increasing the number of replicates is also advisable to reduce variability and increase the study's statistical power. Finally, it may be sensible to acquire a susceptible population from South Dakota. While a GS population has not yet been identified in New York, we suspect that the GR-NY population was brought into New York in Palmer amaranth contaminated, spoiled sorghum seed sourced from South Dakota (LM Sosnoskie, personal communication). Assuming this is accurate, a GS population of Palmer amaranth from South Dakota may have a similar genetic background as the GY-NY population which would better control difference due to genetics.

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Tables and Figures

Table 3.1. Mean biomass of untreated glyphosate-susceptible (GS-NE) and glyphosate-resistant (GR-NY) Palmer amaranth populations 14 days after treatment under different temperature regimes.

Temperature (C day/night)	Biomass (g)		Days from planting to treatment[b]
	GS-NE[a] (±sd)	GR-NY[a] (±sd)	
Low (25/15)	0.119 (±0.050)	0.114 (±0.056)	21
Medium (30/20)	0.092 (±0.028)	0.083 (±0.038)	16
High (35/25)	0.069 (±0.040)	0.084 (±0.068)	17

[a]Abbreviations: GS-NE, glyphosate-susceptible population from Keith County, NE; GR-NY, glyphosate-resistant populations from Steuben County, NY; ED_{50}, sd, standard deviation.

[b]Treatment occurred when plants reached the two- to four-leaf; however, this level of treatment did not receive a glyphosate application.

Table 3.2. Estimation of regression parameters and glyphosate dose required for 50% (ED_{50}) and 90% (ED_{90}) reduction in the biomass of the glyphosate-resistant (GR) NY Palmer amaranth population 14 days after treatment under different temperature regimes.

Temperature (C day/night)	Glyphosate			
	Regression parameters (±SE)[b]		ED_{50} (±SE)[a]	ED_{90} (±SE)[a]
	b	d	g ae ha^{-1}	
Low (25/15)	1.74 (±0.67)	92.79 (±6.61)	674.14 (±150.98)	2390.46 (±1019.39)
Medium (30/20)	1.40 (±0.41)	94.55 (±7.69)	450.70 (±123.93)	2155.27 (±836.52)
High (35/25)	1.62 (±0.56)	89.92 (±5.73)	1169.14 (±258.52)	4523.60 (±2037.03)

[a]Abbreviations: ED_{50}, the effective dose of glyphosate required to reduce biomass relative to the non-treated control (NTC) by 50%; ED_{90}, the effective dose of glyphosate required to reduce biomass relative to the NTC by 90%; ae, acid equivalent; SE, standard error.

[b]Regression parameters were estimated using a three-parameter log-logistic model, $Y = \frac{d}{1+\exp[b(\log x - \log e)]}$, where b represents the slope of the curve at the inflection point, d represents the upper limit, and e represents the dose of glyphosate needed to cause 50% biomass reduction (ED_{50}) compared to the non-treated control (NTC).

Figures

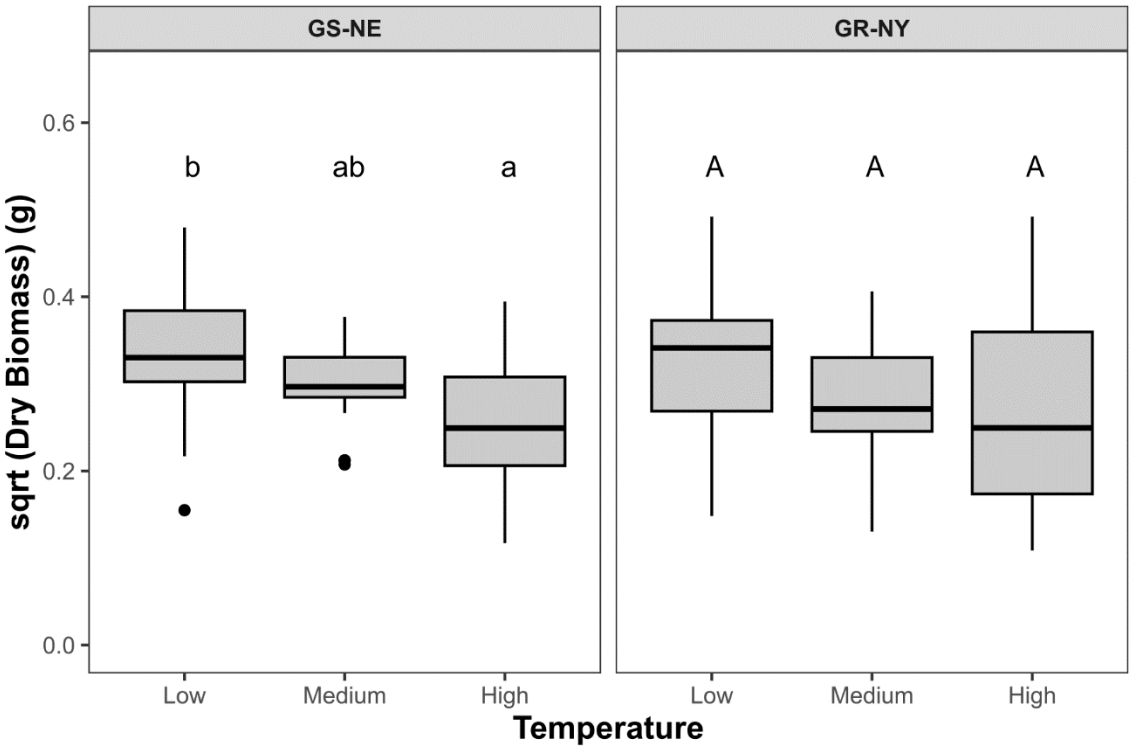

Figure 3.1. Square-root transformed dry biomass of glyphosate-susceptible (GS-NE) and glyphosate-resistant (GR-NY) Palmer amaranth populations under different temperature regimes (Low, 25/15 C; Medium, 30/20 C; High, 35/25 C) 14 days after treatment. Treatments with the same letters did not significantly differ according to Tukey's pairwise comparisons difference ($\alpha = 0.05$).

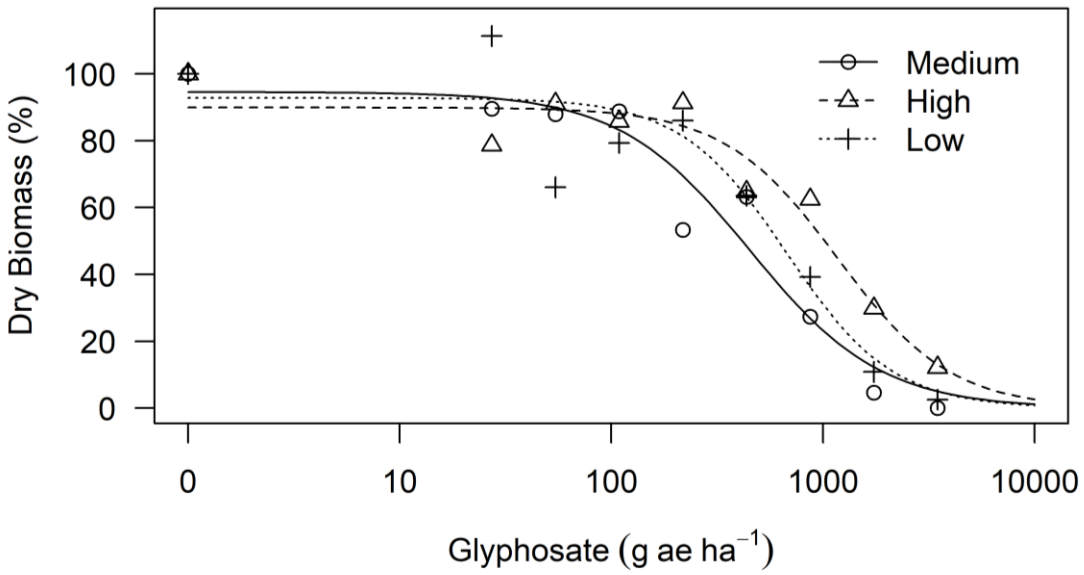

Figure 3.2. Dose-response curves of a glyphosate-resistant NY Palmer amaranth population under different temperature regimes (Low, 25/15 C; Medium, 30/20 C; High, 35/25 C) showing percent biomass 14 days after treatment. Percent biomass was calculated with the following equation: $\text{Percent biomass} = \frac{\text{DBEU}}{\overline{\text{DBNTC}}} \text{x } 100$ where DBEU represents the biomass of the experimental unit and $\overline{\text{DBNTC}}$ represents the mean biomass of ten non-treated control replicates.