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Distribution of glufosinate resistance and glutamine synthetase copy number variation among Palmer amaranth (*Amaranthus palmeri*) accessions in northeast Arkansas

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Research Article

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Abstract

The presence of glufosinate-resistant Palmer amaranth (*Amaranthus palmeri* S. Watson) is of concern for Arkansas farmers. The objective of this study was to understand the distribution of glufosinate resistance among *A. palmeri* accessions collected in 2023 from locations surrounding MSR2 (a highly glufosinate-resistant accession) in 2020, focusing on the distance and direction patterns. Additionally, the cytosolic (*GS1*) and chloroplastic (*GS2*) glutamine synthetase copy number were quantified in glufosinate survivors. In 2023, a total of 66 *A. palmeri* samples were collected within a 15-km radius of MSR2. *Amaranthus palmeri* seedlings were treated with glufosinate at 590 g ai ha^{-1}. Plant tissues were collected, and gene copy number assays were conducted with survivors from accessions showing less than 96% mortality. Glufosinate provided ≥80% mortality in most of the accessions evaluated. Nonetheless, a few accessions showed low mortality rates, with values as low as 34%. Within and among accessions, there was no variation for *GS1.1* and *GS1.2*, while the *GS2.1* and *GS2.2* copy numbers varied greatly. There was no evidence that the geographic distance between samples and MSR2 impacted mortality or gene copy number. However, there was strong evidence that direction, relative to MSR2, affected both mortality and *GS2.1* copies. Samples collected north from MSR2 showed lower average mortality rates (83%) with a higher number of *GS2.1* copies (2.3). For comparison, average mortality ranged from 90% to 95% and *GS2.1* copy number ranged from 1 to 1.2 in the other directions. The predominant summer and fall wind directions do not explain the movement of resistance in a specific direction. These findings indicate that there are multiple *A. palmeri* accessions capable of surviving a label recommended use rate of glufosinate in northeast Arkansas, and resistance distribution needs to be further investigated.

Introduction

The management of Palmer amaranth (*Amaranthus palmeri* S. Watson) has become an obstacle for crop production in many countries, including the United States (Chahal et al. 2015; Gazziero et al. 2023; Matzrafi et al. 2025). The presence of *A. palmeri* was reported to impact row crop growth and reduce yield up to 91% due to its aggressive competitive nature, depending on the density and time of emergence (Klingaman and Oliver 1994; Massinga et al. 2001; Morgan et al. 2001). Overall, chemical control with herbicides is the most common method to manage weeds in crop fields in the United States (Zimdahl and Basinger 2024). However, *A. palmeri* has evolved resistance to herbicides from nine site-of-action groups, and single accessions carrying resistance to herbicides from six and seven sites of action have been documented (Carvalho-Moore et al. 2025b; Heap 2025; Shyam et al. 2021). Although diversified herbicide programs overlapping preemergence and postemergence herbicides with varied sites of action are recommended to manage herbicide-resistant accessions (Norsworthy et al. 2012), satisfactory control with herbicides is compromised in areas infested with *A. palmeri* accessions harboring multiple resistance.

Glufosinate (Herbicide Resistance Action Committee [HRAC]/Weed Science Society of America [WSSA] Group 10) is one of the effective postemergence herbicide options available to

control herbicide-resistant *A. palmeri* (Cahoon et al. 2015; Singh et al. 2023). Susceptible biotypes are controlled by glufosinate through the inhibition of the isoforms of the enzyme glutamine synthetase (cytosolic: *GS1*; chloroplastic: *GS2*) and the production of high levels of reactive oxygen species. These events are followed by rapid cell death (Bayer et al. 1972; Takano et al. 2019, 2020). The continuous use of glufosinate has led to the evolution of resistance in a few monocotyledon species, such as goosegrass [*Eleusine indica* (L.) Gaertn.] and Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) (Heap 2025). In dicots, the first case of glufosinate resistance was reported in *A. palmeri* accessions from Arkansas (Priess et al. 2022), with additional glufosinate-resistant accessions later encountered in Missouri and North Carolina (Jones et al. 2024; Noguera et al. 2022).

Dispersal of weeds and potential introduction of herbicide resistance in areas with susceptible plants can occur through the aid of wind, water, humans, animals, or machinery within and between fields via pollen, seeds, or vegetative structures (Bagavathiannan et al. 2013; Jasieniuk et al. 1996; Zimdahl and Basinger 2024). In dioecious species like *A. palmeri*, the reproductive organs are separated into distinct male and female individuals and have an obligatory outcrossing behavior, which aids the movement of herbicide-resistance genes and other adaptative traits (Borgato et al. 2025; Sauer 1957; Sosnoskie et al. 2012; Stark et al. 2012; Thomson and Brunet 1990). Previous research has shown the potential of *A. palmeri* pollen to move into adjacent fields (Sosnoskie et al. 2012; Stark et al. 2012). Moreover, pollen from glyphosate-resistant (HRAC/WSSA Group 9) plants migrated up to 300 m into susceptible individuals, resulting in 20% of the progeny carrying resistance (Sosnoskie et al. 2012).

Besides pollen-mediated gene flow, herbicide resistance can also move spatially through seeds. There is evidence that glyphosate-resistant *A. palmeri* was introduced in the Pacific Northwest states, such as Idaho and Oregon, through birdfeed, livestock feed, manure, farm equipment, or roadsides contaminated by vehicles transiting between states (Adjesiwor et al. 2024). Herbicide-resistant *A. palmeri* was recently identified in northeastern states like New York and Connecticut (Aulakh et al. 2021, 2024; Butler-Jones et al. 2024). Furthermore, importing contaminated machinery or grain has been suggested as the entry route for *A. palmeri* accessions already resistant to glyphosate or acetolactate synthase–inhibiting herbicides (HRAC/WSSA Group 2) in South America and European countries (Gazziero et al. 2023; Manicardi et al. 2023, 2025; Matzrafi et al. 2025). Although only a limited number of seeds entered through the aforementioned routes, each emerged *A. palmeri* female plant has the potential to produce thousands of seeds (Borgato et al. 2025; Keeley et al. 1987; Webster and Grey 2015). Previous work conducted with glyphosate-resistant *A. palmeri* showed that 20,000 seeds in a square meter, which is less than what a single female plant can produce, led to field areas up to 0.77 ha completely infested with resistance in less than 2 yr (Norsworthy et al. 2014). Regardless of the entry route or the spreading mechanism, *A. palmeri* is highly mobile and adaptable to different environments.

Herbicide-resistant *A. palmeri* biotypes can quickly infest previously "resistance-free" fields. Even though glufosinate resistance in *A. palmeri* has been reported in isolated areas, great concern exists regarding the spread of the resistant biotype. The accession MSR2 was collected in 2020 from a cotton (*Gossypium hirsutum* L.) field in Mississippi County, AR, USA, and it was identified as highly resistant to glufosinate (24-fold) when compared with susceptible standards (Priess et al. 2022). Additionally, the amplification and overexpression of *GS2* was identified as a resistance mechanism for the MSR2 accession (Carvalho-Moore et al. 2022). In the following years, upon visiting fields adjacent to where MSR2 was collected in 2020, *A. palmeri* plants escaping herbicide-centric control programs were frequently observed. Although the amount of glufosinate sprayed in Mississippi County is not available, it is believed that a significant portion of corn (*Zea mays* L.), cotton, and soybean [*Glycine max* (L.) Merr.] fields in this area receive an in-crop application of this herbicide based on conversations with farmers and county extension specialists.

The initial hypothesis is that accessions collected closer to the collection site of MSR2 will likely carry similar glufosinate-resistance levels and mechanisms. Therefore, the objective of this study was to quantify the distribution of glufosinate resistance among *A. palmeri* accessions collected in 2023 within a 15-km radius surrounding the collection site for MSR2 in 2020, focusing on the distance and direction patterns. Additionally, the cytosolic (*GS1*) and chloroplastic (*GS2*) glutamine synthetase copy number were quantified in selected glufosinate survivors to identify any similarity in the resistance mechanism.

Materials and Methods

Collection of Amaranthus palmeri Accessions

The accessions assessed in this study were collected in 2023 from a 15-km radius around the MSR2 accession, which was identified in 2020 in Mississippi County, AR, USA (35.826167°N, 90.240389°W). It is important to acknowledge that another glufosinate-resistant *A. palmeri* accession (MSR1) was located 5.5 km east (35.832444°N, 90.179805°W) of the MSR2 collection. Following the methodology proposed by Burgos et al. (2013) for sampling size for an obligate outcrossing species, a minimum of 5 and up to 10 *A. palmeri* female inflorescences were collected from a total of 66 six sampling points (Figure 1). The inflorescences from each sampling site were pooled together, forming an accession. Collection sites were randomly selected to better represent the geographic area around MSR2 and were located at least 300 m apart. Even though the priority was to collect seeds from *A. palmeri* plants left uncontrolled inside crop fields, this scenario was not feasible in all locations. Therefore, inflorescences were collected from *A. palmeri* plants located in 26 fields, 24 field edges (margin area of a field commonly used for boom calibration), and 16 field ditches (channels located adjacent to agricultural fields for drainage purposes).

Glufosinate Screening

The inflorescences collected were threshed and stored in a cold room at 4 C. The 66 accessions were planted in individual trays filled with potting mix (Sun Gro® Horticulture, Agawam, MA, USA). The seedlings were grown in greenhouses located at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, with 25 ± 5 C and 16-h photoperiod. At the cotyledon stage, *A. palmeri* seedlings were transplanted into 50-cell trays (Greenhouse Megastore, Danville, IL, USA) filled with potting mix with a depth of 5.9 cm and 110-cm^3 volume in each cell. Germination rates varied among accessions, which impacted the number of plants sprayed in each experimental run. A minimum of 70 and a maximum of 225 plants were screened per accession, divided into at least two experimental runs. A total of 7,922 *A. palmeri* plants were screened across the 66 accessions. A glufosinate-susceptible

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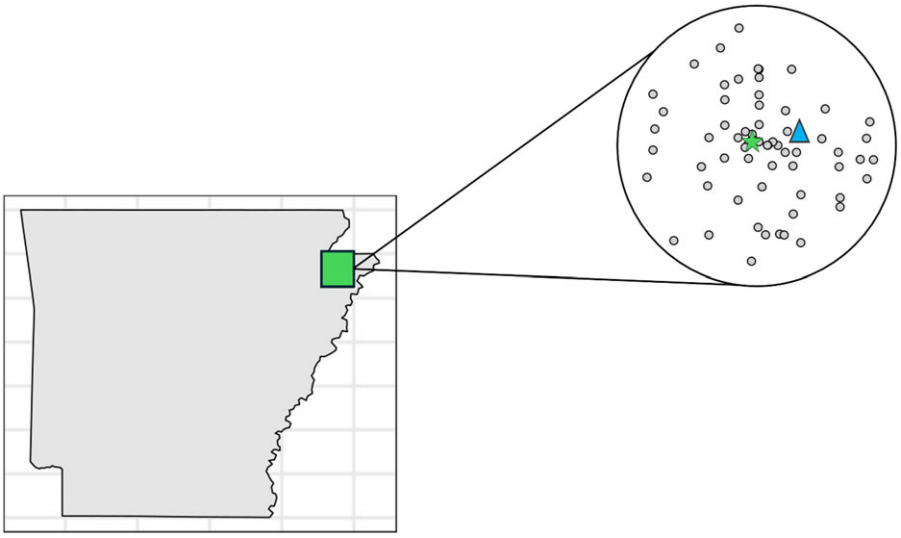

Figure 1. Map depicting the location where MSR2 (highly glufosinate-resistant accession) was collected and samples were collected. The square symbolizes where Mississippi County is located in Arkansas, the green star in the middle of the circle shows the location of MSR2 (35.826167°N, 90.240389°W), and the blue triangle shows the location of MSR1 (35.832444°N, 90.179805°W).

standard was also included to ensure efficacy. Plants were sprayed at the 5- to 7-leaf stage with glufosinate (Liberty®, BASF Ag Products, Research Triangle Park, NC, USA) at 590 g ai ha^{-1}. Treatments were applied using a two-nozzle spray chamber adjusted to deliver 187 L ha^{-1} at 1.6 km h^{-1} using 1100067 nozzles (TeeJet® Technologies, Glendale Heights, IL, USA).

The number of *A. palmeri* plants alive was counted before spraying and 21 d after glufosinate treatment (DAT) to calculate mortality. Mortality was calculated using Equation 1:

$$\text{Mortality}(\%) = \left[\frac{\text{No. of plants alive prior to treatment} - \text{no. of plants alive at 21 DAT}}{\text{No. of plants alive prior to treatment}}\right] \times 100 \quad [1]$$

Wind speed (m s^{-1}) and direction (blowing from) data from the MSR2 collection site were obtained from the National Aeronautics and Space Administration (NASA) Prediction of Worldwide Energy Resources (POWER) project v. 2.4.9 (NASA 2025). Following the pattern of *A. palmeri* pollen dispersion in Arkansas, data were used only from July, August, September, and October (Figure 2). Wind rose plots were constructed for each month of interest using the information of the years 2019 (a year before the report of putative resistance), 2020 (year of collection of MSR2), 2021, 2022, and 2023 (year of collection), using WR View Plot freeware v. 8.0.2 (Lakes Software, Waterloo, ONT, Canada).

Gene Copy Number Assay

Following the glufosinate screening, leaf samples were collected from survivors of accessions with mortality rates less than 96% ($n = 46$ accessions). Approximately 100 mg of leaf tissue was collected from a total of 251 survivors with a minimum of three biological replicates per selected accession, and genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Additionally, DNA from two well-characterized susceptible standards collected in South Carolina in 1986 (S1) and in Arkansas in 2001 (S2) were included for comparison. For each susceptible standard, a total of five biological replicates were used.

To quantify the *GS1* (*GS1.1* and *GS1.2*) and *GS2* (*GS2.1* and *GS2.2*) copy number variation among survivors, a nanowell-based digital PCR (dPCR) was conducted. The dPCR reaction volume (12 µl) consisted of 1.48 µl (6 ηg) of DNA, 0.48 µl (0.2 µM) of specific primers (Table 1), 0.25 µl (0.2 µM) of probe (biomers.net GmbH, Ulm, Germany), 3 µl of QIAcuity Probe PCR Kit mix (Qiagen, Hilden, Germany), and 3.92 µl PCR-Grade H_2O for dPCR. The assay was performed in a dPCR thermal cycler (QIAcuity One, 5plex Device, Qiagen, Hilden, Germany) in a 96-well nanoplate with 8,500 nanowells for each sample (QIAcuity Nanoplate 8.5k 96-well) under the following conditions: 2 min at 95 C and 55 cycles of 15 s of denaturation at 95 C; 40 s of annealing, elongation, and detection at 60 C. Partitions were imaged with the following conditions: FAM and HEX, 500-ms exposure time, gain set to 6; ROX 400-ms exposure time, gain set to 6. Qiagen's QIAcuity Software Suite (v. 2.1.8) was used to determine sample thresholds using positive, negative, and no-template control wells, as well as the copy number variation.

To determine the amplification of the *GS2.1* and *GS2.2* isoforms, TaqMan™ technology was used. A multiplex approach for the target and reference (*Actin*) genes (Table 1) was used in these assays. The real-time quantitative PCR (qPCR) was performed in a final volume of 25 µl with 6.25 µl of DNA, 1 µl (0.2 µM) of specific primers (Table 1), 0.25 µl (0.2 µM) of probe (biomers.net GmbH, Ulm, Germany), 12.5 µl of SensiFAST Real-Time PCR Kit (Meridian Bioscience, Luckenwalde, Germany), and 2.5 µl PCR-Grade H_2O. Three technical replicates were used for each sample. The assay was performed in a qPCR thermal cycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad Laboratories GmbH, Germany) under the following conditions: 5 min at 95 C and 35 cycles of 10 s of denaturation at 95 C; followed by 30 s at 60 C for annealing, elongation, and detection. The evaluation, according to the $2^{-\Delta\Delta C_T}$ method, was carried out with the software Bio-Rad CFX Maestro 2.2 v. 5.2.008.0222.

Data Analysis

Data visualization and analysis were conducted using R 4.4.1 (R Core Team 2024), JMP® Pro 18.0.2 (SAS Institute, Cary, NC,

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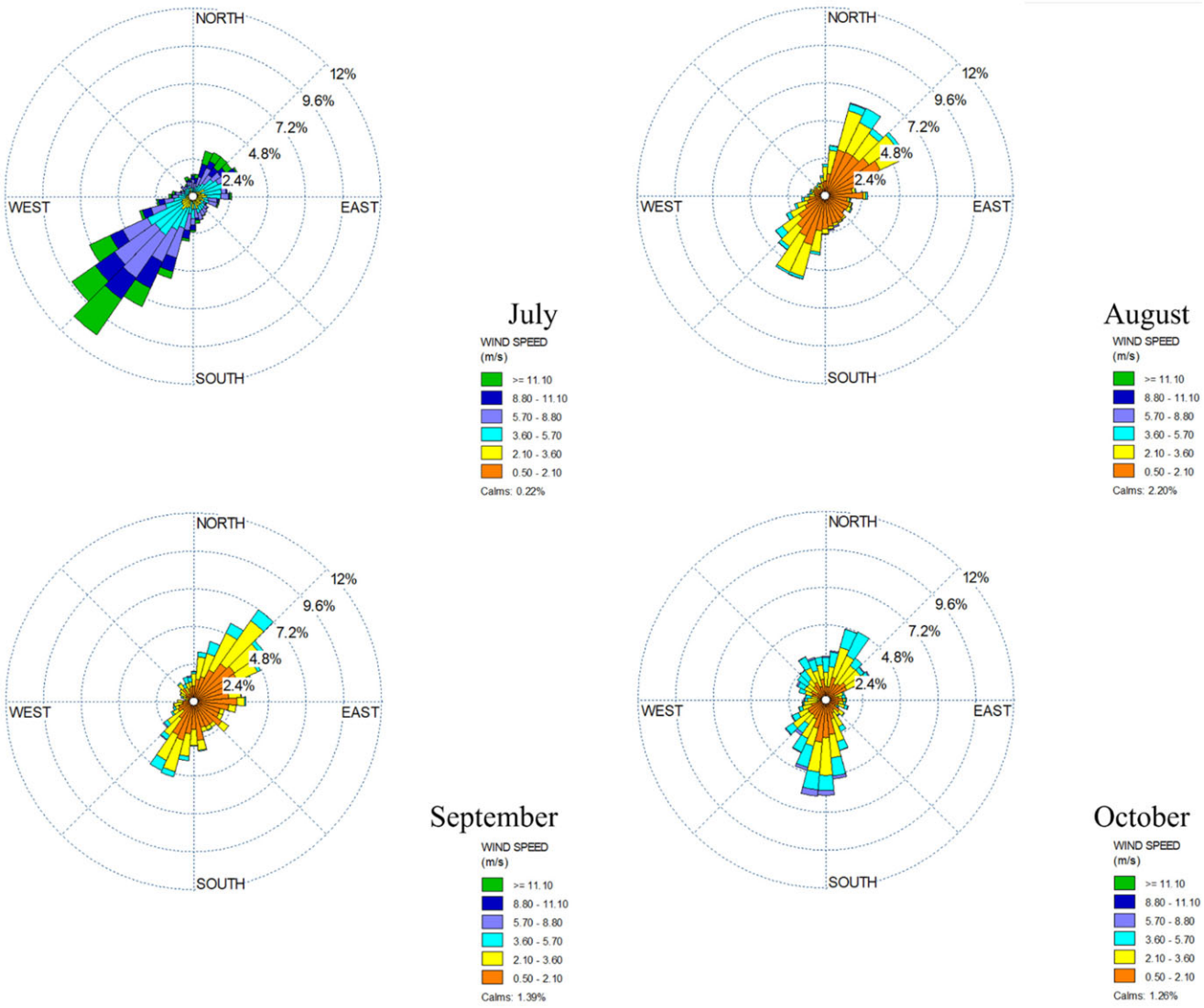

Figure 2. Wind rose plot showing the distribution and frequency (%) of monthly (July, August, September, and October) average weed speed (m s^{-1}) and direction (blowing from) from 2019 to 2023 at the MSR2 location. The data used to produce plots were obtained from the National Aeronautics and Space Administration (NASA) Prediction of Worldwide Energy Resources (POWER) project v. 2.4.9 (NASA 2025).

USA), and SigmaPlot 15.0 (Systat Software, San Jose, CA, USA). The relationships between the response variables, mortality and *GS2* (*GS2.1* and *GS2.2*) copy number, and the covariates, direction, and distance in relation to the location of MSR2 collection were investigated using generalized linear models (GLMs). The GLMs were fit using the glmmTMB (Brooks et al. 2017) library in the R environment. The mortality and copy number were modeled using a beta and a lognormal distribution, respectively (Gbur et al. 2012). Therefore, the link functions used in the modeling process were a logit function for the mortality response and a log function for the *GS2* copy number. The fitting process included building models in increasing order of complexity, going from including only an intercept to having direction, distance, and the interaction term between the two. Model residuals were visually inspected for patterns and spatial clustering. The generalized models can be described as follows:

$$\begin{aligned} g(Y_i) &= \Phi_i \\ \Phi_i &= \mathrm{X}_i\beta + \in_i \\ \in &\sim N(0, \Sigma) \end{aligned} \qquad [2]$$

where the response variable Y_i is mortality or gene copy number, $g(Y_i)$ is the link function, Φ_i models the linear combination of the covariate-specific parameters in the parameter vector β (i.e., direction and distance), and X_i is the observed data in the design matrix. The errors (ε_i) are assumed to be normally distributed and spatially related by the variance–covariance matrix Σ. The inclusion of covariates in the model was carried out using a model comparison approach between models containing all combinations of the covariates. The most parsimonious models were selected based on the lowest Akaike information criterion (AIC). When the selected model included the effect of covariate, multiple-comparison tests were conducted to compare

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Table 1. Digital and quantitative PCR primer information.

Primer[a]	Sequence	Modification
GS1.1-dPCR-forward	TGTGTGATGCCTATACTCCACA	Fam/BMN-Q535
GS1.1-dPCR-reverse	TACCATGGTTCCTCGGCAAC	
GS1.1-dPCR-probe	AGGAGAGCCAATCCCAACCAACA	
GS1.2-dPCR-forward	TGTGTGATGCATACACCCCG	ROX/BMN-590
GS1.2-dPCR-reverse	GACGTCGGGATGGCTAAAGA	
GS1.2-dPCR-probe	GCTGGAGAACCAATTCCAACAAACAAG	
GS2.1-qPCR-forward	AGGTTTGCTAGCAGAAACTACA	Fam/BMN-Q535
GS2.1-qPCR-reverse	GTTCAAGAATATGCGATACACGATTT	
GS2.1-qPCR-probe	GGGAACCAACACTTGAGGCTGA	
GS2.2-qPCR-forward	TGGTAACAGGTTTGCTCGCCGA	ROX/BMN-590
GS2.2-qPCR-reverse	TGGTTGGAATTACACATTAAGAGCGAGT	
GS2.2-qPCR-probe	CCCACACTTGAGGCCGAGTCACTTGCAGC	
Actin-qPCR/dPCR-forward	GCGGAAAGCTAAGCGTGAAC	Hex/BMN-Q535
Actin- qPCR/dPCR-reverse	TCAGACCTGCTCTGGAGTCA	
Actin- qPCR/dPCR-probe	GGAGGAAAAGGCGGATGCTGCA	

[a]Abbreviations: *GS1.1* and *GS1.2*, cytosolic glutamine synthetase isoforms; *GS2.1* and *GS2.2*, chloroplastic glutamine synthetase isoforms; qPCR, real time quantitative PCR; dPCR, digital PCR.

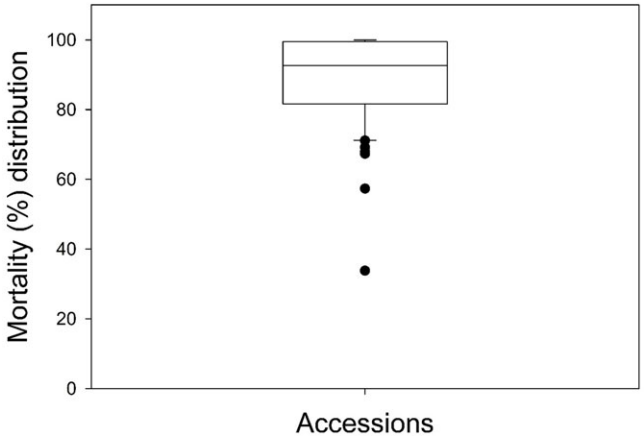

Figure 3. Mortality distribution from *Amaranthus palmeri* accessions ($n = 66$ accessions) collected around MSR2 (highly glufosinate-resistant accession). The box plot was generated using the mortality data collected from 66 accessions, with the center line representing the median, box limits representing the upper and lower quartiles, whiskers representing the 1.5× interquartile, and points representing the outliers.

the expected means for different cardinal directions using Bonferroni's adjustment for error rate control.

Results and Discussion

Glufosinate mortality varied among the *A. palmeri* accessions, ranging from 100% to 34% (Figure 3). Out of 66 accessions, high efficacy (mortality ≥ 99%) was observed for 20 accessions, and glufosinate resistance is unlikely to be present in these fields. According to Frans et al. (1986), a satisfactory response is observed when a herbicide provides ≥80% control of the weed species studied. Using this scale, glufosinate obtained satisfactory control of most of the *A. palmeri* accessions (51 out of 66 samples) collected around the MSR2 collection site. However, it is important to note that the growing environment (temperature, humidity, and light availability) and spraying conditions for accessions in the greenhouse were optimal, which may not always be representative of field conditions. Glufosinate may underperform in some of these areas. Concerningly, there were 15 accessions having a mortality of <80%.

Different from the initial hypothesis, the distance from MSR2, at least out to 15 km, did not influence the mortality response (Table 2). For mortality, the most parsimonious model (Model 2) detected the covariate direction as a positive response predictor for glufosinate mortality (Table 2; Figure 4). Accessions collected north (315° to 45°) relative to MSR2 tended to have lower average mortality (83%), which was statistically different from accessions collected east of it (95%). Recent multistate screenings showed that glufosinate obtained satisfactory control of several accessions of *A. palmeri* or its relative, waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Adjesiwor et al. 2024; Mahoney et al. 2020; D Singh et al. 2023; N Singh et al. 2024; Williams et al. 2024). However, the result of this study shows that putative glufosinate-resistant *A. palmeri* is likely distributed into a larger number of locations across the investigated range than previously determined, highlighting the need for region-specific resistance management efforts. Like MSR2, the accession MSR1 was also detected in 2020. As previously mentioned, the MSR1 collection site was located only 5.5 km east of the MSR2 location. The presence of two *A. palmeri* accessions harboring glufosinate resistance in proximity hints to the possibility of additional fields infested with resistant biotypes as early as 2020, albeit unreported.

Besides glufosinate mortality, assays were conducted to estimate the copy number of glutamine synthetase isoforms in survivors ($n = 251$ survivors) of selected accessions ($n = 46$ accessions). No variation was observed in the susceptible samples for any of the genes tested (Figure 5). Within and among accessions, *A. palmeri* survivors showed no copy number variation for *GS1.1* and *GS1.2*, while the *GS2.1* and *GS2.2* copy numbers varied considerably (Figure 5). Values for *GS2.1* and *GS2.2* ranged from 0.8 to 42 and 0.8 to 18 copies, respectively. Similar to mortality, the distance from MSR2 did not influence the copy number of either *GS2* isoform (Tables 3 and 4). For *GS2.1*, the most parsimonious model (Model 2) detected the covariate direction as a positive response predictor (Table 3; Figure 6). Accessions collected north from MSR2 had a higher average number of *GS2.1* copies, statistically different from accessions collected in any other direction relative to MSR2. For comparison, the *GS2.1* averaged 2.3 copies for survivors from accessions collected north of MSR2, and it ranged from 1 to 1.2 copies for survivors in the other directions (east, south, or west). For *GS2.2*, the model with the best fit (Model 1) did not include distance or direction relative to the MSR2

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Table 2. Models generated for mortality.[a]

Model	df	AIC	BIC	Intercept	Direction	Distance	Direction × distance
1	4	−589.6662	−580.9076	Yes	No	No	No
2	7	−591.7389	−576.4113	Yes	Yes	No	No
3	8	−589.7390	−572.2218	Yes	Yes	Yes	No
4	5	−587.8403	−576.8920	Yes	No	Yes	No
5	11	−585.0362	−560.9500	Yes	Yes	Yes	Yes

[a]Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion. Yes or No indicates whether a parameter was or was not included in the model fit, respectively.

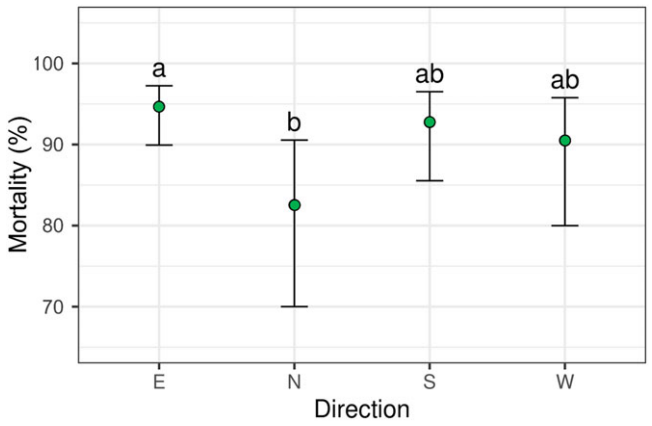

Figure 4. Glufosinate mortality (%) of *Amaranthus palmeri* accessions ($n = 66$ accessions) collected around MSR2 (highly glufosinate-resistant accession). Bars with the same lowercase letter are not statistically different according to multiple comparisons tests ($\alpha = 0.05$) using Bonferroni's adjustment for error rate control. Abbreviations: E, east (46° to 135° from MSR2); N, north (316° to 45° from MSR2); S, south (136° to 225°); W, west (226° to 315°).

collection site as covariates (Table 4), which indicates that there is no covariate influencing this response.

The covariate direction was a positive predictor for both mortality and *GS2.1* copy number, with survivors collected north of MSR2 exhibiting the lowest accession average mortality (83%; Figure 4) and highest *GS2.1* copy number (2.3 copies; Figure 6). Conversely, Sosnoskie et al. (2012) observed that direction did not affect resistance transfer via pollen from resistant to susceptible *A. palmeri* plants. In the same study, distance significantly impacted the resistance spreading, with a higher percentage of resistant individuals being at closer distances (up to 5 m). A different study evaluating pollen dispersal in cotton fields observed that 82% of pollen captured was within 2 m of the *A. palmeri* source with no correlation to direction (Stark et al. 2012). Interestingly, when examining the 5-yr average predominant wind direction for months when *A. palmeri* pollen dispersal will likely occur in Arkansas (July, August, September, and October), wind blowing toward the north of the MSR2 collection site was observed only near the end of the crop season in October (Figure 2). Therefore, wind patterns in this region may not be involved in the movement of resistance, assuming that MSR2 was the origin of resistance. The absence of a distance-dependency effect and strong evidence that direction influences the glufosinate mortality response in this study suggest that glufosinate resistance may be spreading through routes beyond localized pollen or seed dispersal.

Gene flow in plants can occur via pollen or seed dispersal (Ennos 1994), and herbicide resistance migration can be transmitted over long distances in *A. palmeri*. In a study evaluating the pollen-mediated movement of glyphosate resistance between susceptible and resistant *A. palmeri* biotypes, moderate outcrossing (20%) occurred at 300 m (Sosnoskie et al. 2012). Similarly, the pollen of *A. tuberculatus* remained viable to at least 800 m up to 120 h after dispersal (Liu et al. 2012). Besides the mobility via pollen, *A. palmeri* seeds are small and easily transported as well. For instance, the entry of herbicide-resistant *A. palmeri* to different countries has been linked to imported grain or machinery contaminated with seeds (Gazziero et al. 2023; Manicardi et al. 2023). The spread of contaminated residues in production areas is also a possibility. In Arkansas, viable *A. palmeri* seeds were found in composted cotton gin trash, which is usually spread onto fields during fallow months (Norsworthy et al. 2009). Seeds of *Amaranthus* species, including *A. palmeri*, were also present in the surface water of irrigation canals and were recovered from the digestive tracts of migratory birds (Farmer et al. 2017; Kelley and Bruns 1975; Wilson 1980). Although the wind has less impact on the dispersal of *A. palmeri* seeds compared with pollen, the introduction of this species in previously non-infested areas in Texas was connected to a hurricane in 1980 (Menges 1987).

An extrachromosomal circular DNA structure co-amplifying both *GS2.1* and *GS2.2* isoforms has been characterized and validated in MSR2 plants (Carvalho-Moore et al. 2025a). Despite being collected near the site for MSR2, only four accessions showed amplification of both isoforms among the survivors evaluated (data not shown). This result hints that the existence of additional arrangements might be driving the amplification of the *GS2* gene. In fact, different amplification patterns were identified in the aforementioned MSR1 glufosinate-resistant accession and in an accession from Missouri, where only the *GS2.1* isoform showed gene amplification (Carvalho-Moore et al. 2025a; Noguera et al. 2022). Additionally, survivors from 27 accessions (out of 46 selected accessions), with mortality ranging from 57% to 96%, did not show amplification of any of the *GS2* isoforms (data not shown). This result suggests that an additional resistance mechanism, other than *GS2* amplification, might be involved.

It is important to re-emphasize that the MSR2 accession was collected in 2020, whereas the accessions analyzed in this study were collected in 2023. As a result, there is a gap in knowledge regarding the management practices used in this region from 2020 to 2023, which may have impacted the selection and spread of resistant mechanisms and resistant individuals. Moreover, volunteer *A. palmeri* plants were observed on roadsides throughout the collection region (PC-M, personal observations). Previous studies have shown that *A. palmeri* accessions collected from roadsides, field edges, or ditches harbored herbicide resistance, reflective of chemical failures that often occurred in adjacent fields, and acted as carriers of resistance (Bagavathiannan and Norsworthy 2016; Vieira et al. 2018). Moreover, the recurring exposure to sublethal herbicide doses in field edges can increase the tolerance of problematic weeds (Tehranchian et al. 2017; Vila-Aiub and Ghersa 2005). Zero

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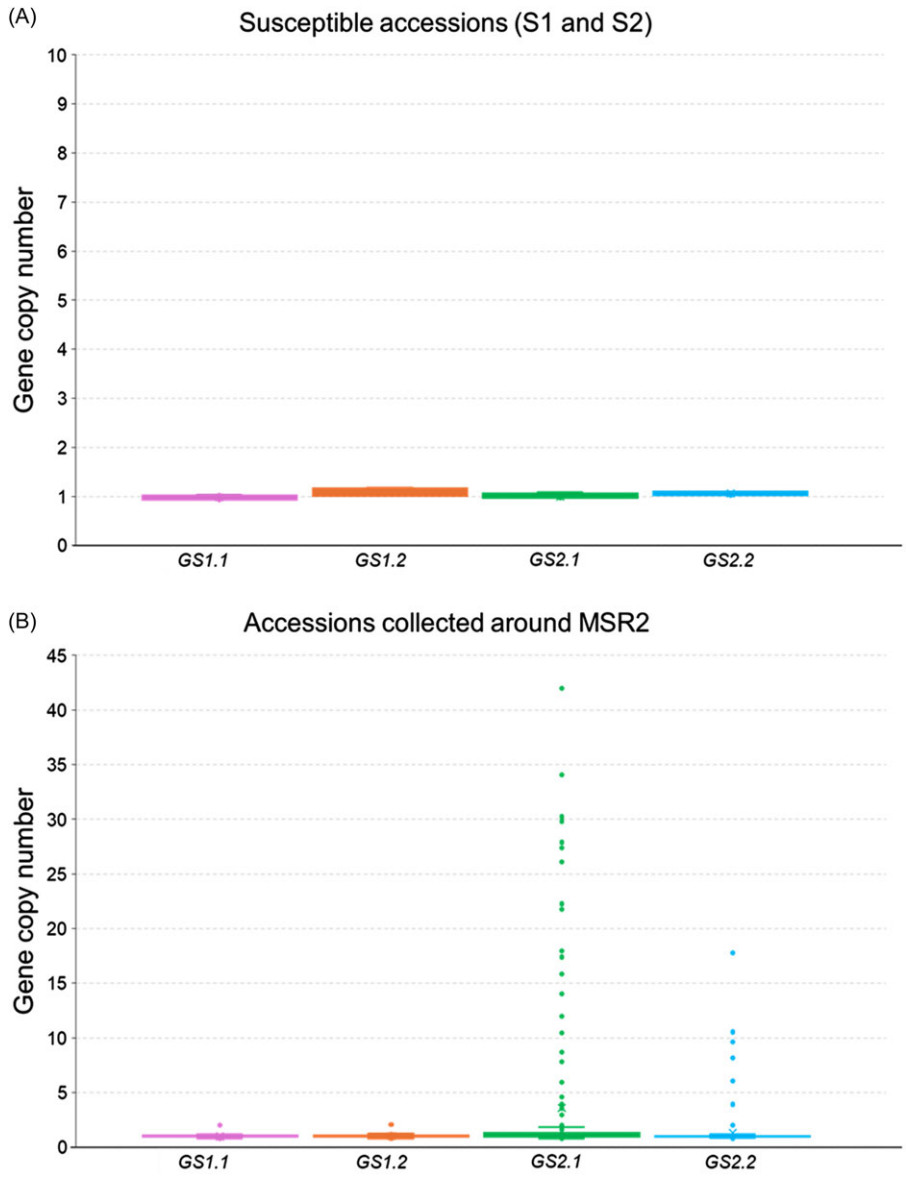

Figure 5. Glutamine synthetase copy number distribution among (A) *Amaranthus palmeri* survivors from accessions ($n = 46$ accessions) collected around MSR2 (highly glufosinate-resistant accession) following glufosinate screening; (B) nontreated plants from susceptible standards (S1 and S2). Abbreviations: *GS1.1* and *GS1.2*, cytosolic glutamine synthetase isoforms; *GS2.1* and *GS2.2*, chloroplastic glutamine synthetase.

Table 3. Models generated for *GS2.1*.[a]

Model	df	AIC	BIC	Intercept	Direction	Distance	Direction × distance
1	4	143.53430	149.86837	Yes	No	No	No
2	8	84.56127	97.22942	Yes	Yes	No	No
3	8	132.42655	145.09470	Yes	Yes	Yes	No
4	3	140.65209	145.40265	Yes	No	Yes	No
5	9	132.05494	146.30661	Yes	Yes	Yes	Yes

[a]Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion. Yes or No indicates whether a parameter was or was not included in the model fit, respectively.

tolerance is strongly recommended to manage resistant populations, especially species with high seed production (Keeley et al. 1987; Norsworthy et al. 2012, 2014). Hand weeding is practiced by some growers within the radius of fields sampled here (JK Norsworthy, personal observation).

Glufosinate is a valuable postemergence herbicide. However, the findings presented here show that putative glufosinate-resistant *A. palmeri* populations are present in more areas than initially detected and are a threat to the stewardship of this and other technologies. Ideally, management practices to minimize

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Table 4. Models generated for *GS2.2*.[a]

Model	df	AIC	BIC	Intercept	Direction	Distance	Direction × distance
1	4	60.29336	66.62744	Yes	No	No	No
2	7	66.22757	77.31220	Yes	Yes	No	No
3	8	68.02120	80.68935	Yes	Yes	Yes	No
4	5	62.08460	70.00220	Yes	No	Yes	No
5	9	69.32819	83.57986	Yes	Yes	Yes	Yes

[a]Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion. Yes or No indicates whether a parameter was or was not included in the model fit, respectively.

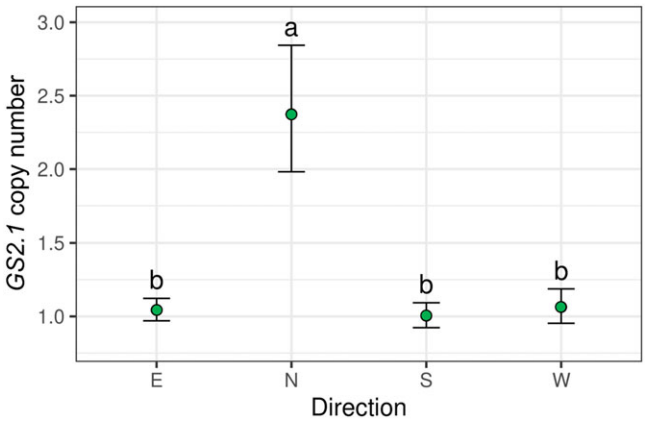

Figure 6. Chloroplastic glutamine synthetase isoform (*GS2.1*) copy number of *Amaranthus palmeri* survivors from accessions ($n = 46$ accessions) collected around MSR2 (highly glufosinate-resistant accession). Means followed by the same lowercase letter are not statistically different according to multiple comparisons tests ($\alpha = 0.05$) using Bonferroni's adjustment for error rate control.

farther movement of the resistant biotype need to be applied soon, because resistance management is easier with smaller or localized populations (Adjesiwor et al. 2024; Norsworthy et al. 2012). In 2021, the state of Minnesota reported the complete eradication of *A. palmeri* infestations that were detected in 2016. Yu et al. (2021) reported an aggressive protocol that included intensive scouting, area burning, torching, and herbicide applications, as well as regulatory support and collaboration with agencies of interest. Although this is an ambitious and utopian approach for Arkansas due to the high presence of *A. palmeri* in the state, the high collaboration and communication between different entities, followed by rapid response, is valuable. Zero tolerance with the physical removal of any *A. palmeri* field escapes and control of plants in nonagricultural areas (roadsides and ditches) is crucial to reduce resistance spreading and perpetuation by avoiding seed deposition and pollen migration (Norsworthy et al. 2012; Sosnoskie et al. 2012; Vieira et al. 2018; Webster and Nichols 2012). Crop and herbicide rotation and a foundational residual program are recommended, especially in the fields where putative glufosinate resistance was detected in *A. palmeri*. Future investigations should broaden the evaluated radius to assess the extent of glufosinate resistance among *A. palmeri* accessions throughout Arkansas. Also, it is crucial to unravel the additional mechanisms involved in the response of accessions with low mortality and no variation in gene copy number.

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