Unraveling the mechanism of resistance in a glufosinate-resistant Palmer amaranth (Amaranthus palmeri) accession

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Abstract

Glufosinate resistance in Palmer amaranth (Amaranthus palmeri S. Watson) was recently detected in three accessions from Arkansas, USA. Amaranthus palmeri is the first and only broadleaf weed species resistant to this herbicide, and the resistance mechanism is still unclear. A previous study characterized the glufosinate resistance level in the accessions from Arkansas. A highly glufosinate-resistant accession was further used to investigate the mechanism conferring glufosinate resistance in A. palmeri. Experiments were designed to sequence the herbicide target enzyme cytotoxic and chloroplastic glutamine synthetase isozymes (GS1 and GS2, respectively) and quantify copy number and expression. Absorption, translocation, and metabolism of glufosinate using the 14C-labeled herbicide were also evaluated in the resistant and susceptible accessions. The glufosinate-resistant accession had an increase in copy number and expression of GS2 compared with susceptible plants. All accessions showed only one GS1 copy and no differences in expression. No mutations were identified in GS1 or GS2. Absorption (54% to 60%) and metabolism (13% to 21%) were not different between the glufosinate-resistant and glufosinate-susceptible accessions. Most residues of glufosinate (94% to 98%) were present in the treated leaf. Glufosinate translocation to tissues above the treated leaf and in the roots was not different among accessions. However, glufosinate translocation to tissues below the treated leaf (not including roots) was greater in the resistant A. palmeri (2%) compared with the susceptible (less than 1%) accessions. The findings of this paper strongly indicate that gene amplification and increased expression of the chloroplastic glutamine synthetase enzyme are the mechanisms conferring glufosinate resistance in the A. palmeri accession investigated. Thus far, no additional resistance mechanism was observed, but further investigations are ongoing.

Introduction

Glufosinate-ammonium is a broad-spectrum, nonselective herbicide labeled to control dicotyledonous and certain monocotyledons. In the United States, this herbicide was first registered in 1993 by AgrEvo under the commercial names Finale® and Rely® (Bijman 2001; Hoerlein 1994). Glufosinate is one of the foundational postemergence herbicides in LibertyLink® (BASF, Florham Park, NJ, USA), Enlist™ (Corteva Agriscience, Indianapolis, IN, USA), and XtendFlex® (Bayer CropScience, Pittsburgh, PA, USA) systems, in which crops contain the glufosinate-resistant trait. Glufosinate controls plants by inhibiting the glutamine synthetase enzyme, which synthesizes glutamine from glutamate with photosynthetic ammonia. Because the herbicide is a chemical analogue to glutamate, it competes with this amino acid to bind to the enzyme. Inhibition of glutamine synthetase eventually leads to ammonia accumulation, amino acid deplecentration, detrimental accumulation of reactive oxygen species, lipid peroxidation, and, ultimately, cell death (Bayer et al. 1972; Takano et al. 2019, 2020; Wendler et al. 1990; Wild and Manderscheid 1984). In plants, glutamine synthetase has two major isozymes in different compartments: one located in the cytosol (GS1) and the other located in the plastids (GS2) (Mann et al. 1979). GS1 is associated to nitrogen assimilation that will generate glutamine for nitrogen transport inside plants, while GS2 is involved in...
the reassociation of ammonium from the photosynthetic pathway and other plant processes (Edwards et al. 1990; Kamachi et al. 1991; Wallsgrove et al. 1987).

Compared with other frequently used nonselective herbicides such as glyphosate and paraquat, glufosinate has a relatively lower number of resistant weed species. Currently, only five weed species have been confirmed resistant to glufosinate. These weeds are goosegrass [Eleusine indica (L.) Gaertn.], Italian ryegrass [Lolium perenne L. ssp. multiflorum (Lam.) Husnot], perennial ryegrass (Lolium perenne L.), rigid ryegrass (Lolium rigidum Gaudin), and Palmer amaranth (Amaranthus palmeri S. Watson). A. palmeri is the first and only broadleaf weed resistant to glufosinate (Avila-Garcia and Mallory-Smith 2011; Gaudin), and Palmer amaranth (Amaranthus palmeri S. Watson). A. palmeri is resistant to herbicides targeting eight other sites of action, which makes this weed extremely challenging to control (Priess et al. 2022). Additionally, two susceptible (accession (Glu-R1) were collected in 2020 from a cotton field where glufosinate applications failed to control A. palmeri plants. Seeds from these fields were collected, and glufosinate resistance was confirmed through dose–response experiments (Priess et al. 2022). In addition to glufosinate, A. palmeri is resistant to herbicides targeting eight other sites of action, which makes this weed extremely challenging to control (Heap 2022b). Although the resistance has been confirmed, the resistance mechanism in glufosinate-resistant A. palmeri remains unrevealed.

The possible mechanisms of resistance are divided into target-site and non–target-site mechanisms. Target-site resistance encompasses any alteration in the target enzyme that will prevent herbicide binding, such as amino acid/nucleotide change. Gene amplification and overexpression of the targeted protein are also considered to be target-site resistance mechanisms. Non–target site resistance is any plant mechanism that reduces the quantity of herbicide reaching the target site (Dèlye et al. 2013; Powles and Yu 2010). It is crucial to understand the basis of herbicide resistance and the biology of a weed population. With this knowledge, scientists can design and apply proper strategies to limit the spread of resistance (Norsworthy et al. 2012). Glufosinate resistance was characterized in three A. palmeri accessions from Arkansas by Priess et al. (2022), and a high resistance level was observed in one of these accessions (resistance/susceptibility ratio = 24). In this study, this highly glufosinate-resistant accession was further used to investigate the mechanism conferring resistance to glufosinate.

Materials and Methods

One A. palmeri accession previously confirmed to have high glufosinate resistance (resistance/susceptibility ratio = 24) was selected to conduct the experiments described. The seeds from the resistant accession (Glu-R1) were collected in 2020 from a cotton (Gossypium hirsutum L.) field where glufosinate applications failed to provide control (Priess et al. 2022). Additionally, two susceptible A. palmeri accessions collected in South Carolina in 1986 (S1) and in Arkansas in 2001 (S2) were used for comparison. Seedlings were established in a greenhouse at 25 ± 5 C and 16-h day at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, USA.

Glutamine Synthetase Sequencing

Illumina sequencing was conducted on RNA from Glu-R1 survivors and S1 nontreated plants to identify the presence of G5I and G52 mutations possibly correlated with target-site resistance. Approximately 1 g of leaf tissue was collected from glufosinate-resistant and glufosinate-susceptible A. palmeri accessions, frozen using liquid nitrogen, and then ground to fine powder. The finely powdered leaf tissue of each accession was processed using the RNaseq Plant Mini Kit (Qiagen, Hilden, Germany) to extract RNA. The quality and quantity of RNA were determined using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). RNA samples were immediately stored at −80 C until further processing for transcriptomic sequencing. RNA samples of the Glu-R1 and S1 accessions of A. palmeri were analyzed using the Agilent Bioanalyzer 2100 instrument (Agilent Technologies, Palo Alto, CA, USA) to determine the RNA integrity number. Prepared libraries were run on Illumina NovaSeq 6000 instrument (Novogene, Beijing, China) to produce 150-bp paired-end reads. The sequence read data were evaluated to determine the percentage of reads containing adapters, reads containing N >10% (N represents the base that cannot be determined), and reads of low quality (Qscore ≤ 5) before releasing the data.

Paired-end reads were assembled using Trinity (https://github.com/trinityrnaseq), with standard flags of Trimmomatic (Bolger et al. 2014). Assembled contigs were annotated using Transotate (https://trinotate.github.io), and peptide sequences were produced using TransDecoder (http://transdecoder.github.io). Glutamine synthetase nucleotide and peptide sequences were extracted from fasta files using the TrinotateExtractor (http://github.com/mcelrjo/trinotateExtractor) based on Blast annotation in the Trinotate output file. Protein sequences were aligned within each accession to eliminate redundant sequences and were sorted within each accession to refine annotation to specific glutamine synthetase orthologues based on Blast annotation. The Glu-R1 and S1 protein sequences derived from TransDecoder were aligned for G5I and G52 to identify any amino acid differences. Protein sequences were aligned to reference protein sequences of glutamine synthetase cytosolic and chloroplastic isozymes from A. palmeri (NCBI accession GFQGQ01042326.1 and Heap [2022a], respectively). The G5I and G52 isozymes of cantaloupe (Cucumis melo L.) (NCBI accessions NP_001284433.1 and NP_001284439.1, respectively) were included as an unrelated species to demonstrate the sequence homology across diverse taxa. Illumina sequencing reads for Glu-R1 and S1 were submitted as NCBI BioProject PRJNA831848.

Read mapping was performed to identify numerical differences in expression between Glu-R1 and S1 accessions. Illumina sequencing of the Glu-R1 biotype generated 22,582,008 paired-end reads, while sequencing of the S1 biotype generated 10,883,584 paired-end reads. Reads were mapped using the CLC Genomics Workbench (CLCbio, Seoul, Republic of Korea) read mapping tool with the following settings: no masking, match score 1, mismatch cost 2, linear gap cost for insertions and deletions, insertion cost 3, deletion cost 3, length fraction of 0.5, similarity fraction of 0.8, and no global alignment. Read counts, average coverage of reads, and the percent of the total reads mapped are presented in Supplementary Table 1.

Glutamine Synthetase Gene Copy Number Quantification

Gene copy number assay was conducted with nontreated plants from the susceptible accessions (S1 and S2), and glufosinate survivors from the resistant accession (Glu-R1) sprayed with glufosinate.
Table 1. Primer pairs used to quantify relative copy number and gene expression by real-time polymerase chain reaction in Amaranthus palmeri accessions.

| Targeted gene | Primer sequence | Amplicon | Efficiency |
|--------------|----------------|----------|------------|
| GS1a         | F 5'-CTGGAGTGCTGGTCTGCTG-3' R 5'-GTTGCGTCTTGGGTGCACGC-3' | ~116 | 94.3 |
| GS2a         | F 5'-ATCGGTCCTTGTCTACGGGCTG-3' R 5'-TCTCGGCGGCAACGTTG-3' | 121 | 102.9 |
| GS1b         | F 5'-AACATCCGCTGACTGAAAGCAG-3' R 5'-AGGCAAGCGGTTGATCGGTGCT-3' | 172 | — |
| GS2b         | F 5'-AGAGGACCTGGCCATCTGGTGGC-3' R 5'-TCTCAGAAACAACTTTGTTG-3' | 133 | — |
| CCR          | F 5'-CGAGGAGGAAATAGCAAGAAATG-3' R 5'-GTCTTTGACGGGTGCGTAAAC-3' | 116 | — |
| PPAN         | F 5'-TGCCATTTTTGTGGTGGTG-3' R 5'-GACATGCAGGCCTCAAGTCTG-3' | 113 | — |

*GISA (cytoplasmic) and GS2a (chloroplastic) glutamine synthetase in gene copy number experiment; GS1b (cytoplasmic) and GS2b (chloroplastic) glutamine synthetase in gene expression experiment; CCR, Cinnamoyl-CoA reductase; PPAN, pan-Peter Pan-like.

Absorption and Translocation of Glufosinate

Absorption and translocation experiments of 14C-labeled glufosinate were conducted in resistant (Glu-R1) and susceptible (S1 and S2) accessions. *Amaranthus palmeri* seedlings were transplanted into 7-cm-diameter plastic pots filled with potting mix (Sun Gro® Horticulture, Agawam, MA, USA). Each accession had three replications that consisted of one plant per pot in each replication. The experiment was organized in a completely randomized design and repeated twice. At the 6- to 8-leaf stage, plants received an overspray of nonradioactive glufosinate at 656 g ai ha⁻¹ at the 5- to 7-leaf stage. Around 100 mg of leaf tissue was collected per plant from four plants of each accession, and genomic DNA was extracted using the E.Z.N.A.® Plant DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer’s directions. After extraction, DNA was quantified using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and diluted with deionized water to 10 ng μl⁻¹. A quantitative real-time polymerase chain reaction (qPCR) was conducted to quantify the cytoplasmatic (GS1) and chloroplastic (GS2) glutamine synthetase copy number. The primers GS1a and GS2a were designed to quantify gene copy number for GS1 or GS2, respectively (Table 1).

The qPCR reaction mixture (20 μl) consisted of 10 μl of 2X SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 0.8 μl each of 10 μM forward and reverse primers (Table 1), 5.9 μl of deionized water, and 25 ng of genomic DNA. The assay was conducted in a CFX96 Real-Time System (Bio-Rad Laboratories) under the following conditions: 98 C for 3 min, 40 cycles of 98 C for 10 s, and 60 C for 30 s. Melting curves were created by increasing the temperature from 65 C to 95 C, 0.5 C every 5 s to ensure specific amplification.

Each biological sample (total of four per accession) had two technical replicates in each primer pair, and the experiment was repeated in time. No-template controls (DNA substituted by deionized water) were included in each plate. Quantification cycles (Cq) were produced by CFX Maestro software (Bio-Rad Laboratories), and genomic copy numbers of GS1 and GS2 were calculated using a modified version of the 2⁻ΔΔCq method (Livak and Schmittgen 2001). Fold increase in GS1 and GS2 was assessed relative to two reference genes (single gene copy) previously used in A. palmeri, Cinnamoyl-CoA reductase (CCR), and Pan Peter Pan-like (PPAN) (González-Torralva and Norsworthy 2021; Salas et al. 2012).
cocktail (Ultima Gold™, PerkinElmer, Waltham, MA, USA) and analyzed with a Tri-Carb 2900TR Liquid Scintillation Analyzer (LSA; PerkinElmer). Absorption was calculated by subtracting the [14C]glufosinate activity in the rinsate of the treated leaf at the sampling time from the [14C]glufosinate activity in the rinsate of the treated leaf at the initial time. The initial [14C]glufosinate recovery was 95%, and it was obtained by washing treated leaves soon after spot testing.

Each plant section was individually placed into a paper envelope and then dried in a freeze-dryer (Botanique Preservation Equipment, Phoenix, AZ, USA) at −50 C for 72 h. The dried plant parts were individually combusted to 14CO2 by a biological oxidizer (Model OX-700, R.J. Harvey Instruments, Tappan, NY, USA) at 900 C for 3 min. The 14CO2 gas was entrapped into 15 ml of 14C-trapping cocktail (R.J. Harvey Instrument). The 14C activity in the vials was analyzed using the LSA. Translocation was calculated as the proportion of the [14C]glufosinate measured in each plant part relative to the total [14C]glufosinate absorbed after 48 h.

Metabolism of Glufosinate

Metabolism experiments were conducted twice on the same dates on which the absorption/translocation experiment was being performed. Amaranthus palmeri plant sample preparation, non-radiolabeled herbicide spray, and 14C-labeled herbicide treatment were done following the same methods as used in the absorption/translocation experiment. At 48 h after 14C-labeled herbicide treatment, the treated leaf was thoroughly rinsed with 15 to 20 ml of methanol in water. Subsequently, the sample was left at 4 C in a refrigerator for 1 h and then centrifuged at 8000 × g for 5 min. The 14CO2 gas was entrapped into 15 ml of 14C-trapping cocktail (R.J. Harvey Instrument). The 14C activity in the vials was analyzed using the LSA. Translocation was calculated as the proportion of the [14C]glufosinate measured in each plant part relative to the total [14C]glufosinate absorbed after 48 h.

Data Analysis

All data collected were subjected to ANOVA in JMP Pro v. 15 (SAS Institute, Cary, NC, USA). The experimental runs were not significant across the experiments and were thus set as a random effect in the subsequent statistical model statement. If significant, means from gene copy number and gene expression assays were separated using Fisher’s protected LSD at α = 0.05. Results of absorption, translocation, and metabolism were also subjected to ANOVA and separated using Tukey’s honestly significant difference (HSD) at α = 0.05.

Results and Discussion

Glutamine Synthetase Sequencing, Relative Copy Number, and Expression

Protein isoforms were compared to identify any amino acid substitutions present in the resistant (Glu-R1) in comparison to the susceptible (S1) A. palmeri accession and reference sequences (A. palmeri and C. melo). Based on amino acid sequences, one isoform of cytosolic resistant (Glu-R1) and susceptible (S1) was identified and aligned with the reference cytosolic GS (Supplementary Figure 1). The sequences did not assemble correctly prior to 306 due to low alignment quality past this point of the sequencing. While differences between the C. melo reference and Glu-R1 and S1 were observed, no differences were observed between the A. palmeri accessions (reference, Glu-R1, and S1). For chloroplastic GS, only one isoform was identified (Supplementary Figure 2). The Glu-R1 GS isoform (GSChl_Glu-R1) contained one amino acid substitution (Gly-20-Ser) not present in GS2 from S1 or the A. palmeri reference. However, Blastp alignment inspection of this region identified that this position shows high amino acid polymorphism across different species. Due to this lack of evolutionary conservation, it is unlikely that this substitution would constitute a target-site resistance, but additional studies such as cloning vectors need to be conducted to prove this hypothesis. Little numerical difference was observed in GS1 between Glu-R1 and S1 reads (Supplementary Table 1). For GS1, 0.013% to 0.035% of total reads mapped to either accession with no obvious numerical differences. For GS2, greater numerical difference was observed between the biotype reads. Glu-R1 reads mapped to 0.079% to 0.242% of the total reads, while S1 reads only mapped to 0.018% of total reads. The mapping difference translates to 4.4 to 13.4 times greater expression for Glu-R1 than S1.

Sequence data for both GS1 and GS2 suggest that a point mutation does not contribute to glufosinate resistance in the Glu-R1 accession. Working with a glufosinate-resistant L. perenne accession, Avila-Garcia et al. (2012) identified an amino acid substitution of aspartate for asparagine in the GS2 gene, which was initially considered the resistance mechanism. However, further investigation led to the conclusion that this alteration in GS2 could not account for glufosinate resistance in this specific accession (Brunharo et al. 2019). In a recent investigation
conducted with glufosinate-resistant *E. indica*, Zhang et al. (2022) identified the substitution of a serine for glycine at the 59th position of GS1 in the resistant accession, which is likely the resistance mechanism in this accession. This substitution was also encountered in glufosinate-resistant *E. indica* from different regions and countries. Mutation in the target enzyme might confer glufosinate resistance in the other *A. palmeri* accessions not tested in this study.

Gene copy number and gene expression assays were conducted to detect any differences in the cytoplasmatic and chloroplastic glutamine synthetase (GS1 and GS2, respectively) among the *A. palmeri* biotypes. Regarding the GS1 copy number relative to the reference primers, there was no difference between susceptible and resistant plants, and all accessions had approximately one GS1 copy (Figure 1A). Similarly, GS1 expression was not different within the accessions (Figure 1B). Based on these results, glufosinate resistance in *A. palmeri* did not involve gene amplification or increased expression of cytosolic glutamine synthetase. The cytosolic isoform of the GS enzyme is essential in the assimilation and transport of nitrogen throughout the plant. GS1 and GS2 enzyme activities greatly vary among species, plant sections, and plant stages (Bernard and Habash 2009; Brugiere et al. 2000; Habash et al. 2001; McNally et al. 1983; Miflin and Habash 2002; Woo et al. 1982). After applications of glufosinate to *A. palmeri* seedlings from Colorado, higher GS1 expression in old leaf or root tissues compared with young leaf tissues was found. Glufosinate has no soil activity and is recommended to be applied when *A. palmeri* plants are small and young; therefore, gene amplification or overexpression of GS1 might not impact resistance due to low GS1 expression in this plant stage.

GS2 copy number and expression were different within the glufosinate-resistant and glufosinate-susceptible accessions. Calculated against *CCR* and *PPAN* reference genes, accession Glu-R1 had 85 and 86 copies, respectively, while the two susceptible accessions had 2 GS2 copies (Figure 2A). For gene expression, accession Glu-R1 showed 15 and 31 times relative GS2 expression increase relative to *CCR* and *PPAN*, respectively (Figure 2B). The susceptible accessions showed no increase in GS2 expression. In a dose–response experiment, accession Glu-R1 showed 24-fold glufosinate resistance (Priess et al. 2022). The resistance fold

![Figure 1. Cytoplasmatic glutamine synthetase copy number (A) and expression (B) relative to Cinnamoyl-CoA reductase (CCR) and peter Pan-like (PPAN) reference genes in glufosinate-resistant (Glu-R1) and glufosinate-susceptible (S1 and S2) accessions. Error bars represent standard errors of the means (n = 8 and n = 6). Means were subjected to ANOVA, and P-values were generated using JMP Pro v. 15 (SAS Institute, Cary, NC).](https://doi.org/10.1017/wsc.2022.31 Published online by Cambridge University Press)
Figure 2. Chloroplastic glutamine synthetase copy number (A) and expression (B) relative to Cinnamoyl-CoA reductase (CCR) and Peter Pan-like (PPAN) reference genes in glufosinate-resistant (Glu-R1) and glufosinate-susceptible (S1 and S2) accessions. Error bars represent standard errors of the means (n = 8 and n = 6). Means were subjected to ANOVA, and P-values were generated using JMP Pro v. 15 (SAS Institute, Cary, NC). Means displayed with different uppercase letters are different according to Fisher’s protected LSD test at α = 0.05.

Figure 3. Absorption of [14C]glufosinate by glufosinate-resistant (Glu-R1) and glufosinate-susceptible (S1 and S2) accessions assessed at 48 h after application of radiolabeled herbicide. Error bars represent standard errors of the means (n = 6). Means were subjected to ANOVA, and P-values were generated using JMP Pro v. 15 (SAS Institute, Cary, NC).
obtained by Priess et al. (2022) and the relative GS2 expression obtained in this study for accession Glu-R1 are similar, suggesting that high copy number and expression may confer high resistance. Therefore, gene amplification and overexpression of GS2 enzyme is likely the mechanism conferring glufosinate resistance in the *A. palmeri* accessions investigated in this study. Further experiments, such as examinations of enzyme activity and inheritance of this trait, remain to be conducted. This is the first report of increase in GS2 copy number or expression in a glufosinate-resistant species.

Herbicide resistance by gene amplification and overexpression has been previously reported in *A. palmeri*. Resistance to glyphosate can be due to an increased copy number of the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; Gaines et al. 2016; Godar et al. 2015). Gene amplification has also been observed in large crabgrass (*Digitaria sanguinalis* (L.) Scop.) resistant to acetyl-CoA carboxylase inhibitors (Laforest et al. 2017). Although seldom identified as a herbicide-resistance mechanism, increase in gene copy number has been reported in several cases of fungicide or insecticide resistance (Anthony et al. 1998; Cattel et al. 2021; Elmore et al. 2015; Heckel 2022; Puinean et al. 2010).

Interestingly, the gene amplification data strongly suggest that the GS2 gene in *A. palmeri* has two copies natively in susceptible plants (Figure 2A). A future experiment involving other *A. palmeri* accessions from different regions might further investigate this finding. GS2 duplication has been observed before in black cottonwood (*Populus balsamifera* L. ssp. *trichocarpa* (Torr. & A. Gray ex Hook.) Brayshaw), barrelclover (*Medicago truncatula* Gaertn.), and some green algae species (Castro-Rodríguez et al. 2011; Ghoshroy et al. 2010; Seabra et al. 2010).

**[^14C]Glufosinate Absorption, Translocation, and Metabolism**

No difference in glufosinate absorption was observed among resistant and susceptible accessions (Figure 3). Absorption ranged from 54% to 60% in the accessions, similar to the magnitude (59% to 85%) reported in previous studies with *A. palmeri* (Everman et al. 2009b; Meyer et al. 2020).

Most of the [14C]glufosinate absorbed remained in the treated leaf: 94% for Glu-R1 and 98% for susceptible accessions (P < 0.05) (Figure 4). Translocation of [14C]glufosinate to tissues above the treated leaf (1% to 1.9%) and roots (<1% to 1.4%) showed no difference among accessions. However, glufosinate translocation to tissues below the treated leaf (not including roots) was slightly greater in the Glu-R1 accession (2%) than in the susceptible accessions (<1%). Although the herbicide translocation to tissues below the treated leaf was statistically significant among the accessions, this negligible difference (1%) seems to be insufficient to explain the mechanism of glufosinate resistance. The low translocation (<6%) of [14C]glufosinate observed in this study was likely due to the localized phytotoxicity and rapid tissue necrosis caused by glufosinate, which possibly restrain translocation to other plants parts (Beriault et al. 1999; Steckel et al. 1997). Low glufosinate translocation has been previously observed in *A. palmeri* and other weed species such as pitted morningglory (*Ipomoea lacunosa* L.) (Everman et al. 2009a, 2009b; Meyer et al. 2020; Steckel et al. 1997).

![Figure 4. Translocation of [14C]glufosinate by glufosinate-resistant (Glu-R1) and susceptible (S1 and S2) accessions assessed at 48 h after application by plant section. ABT, above-treated leaf; TL, treated leaf; BTL, below treated leaf. Error bars represent standard errors of the means (n = 6). Means were subjected to ANOVA, and P-values were generated using JMP Pro v. 15 (SAS Institute, Cary, NC). Means displayed with different uppercase letters are different according to Tukey’s honestly significant difference (HSD) test at α = 0.05.](https://doi.org/10.1017/wsc.2022.31)
Metabolism did not differ between the glufosinate-resistant and glufosinate-susceptible accessions, and the accessions metabolized 13% to 21% of the absorbed glufosinate at 48 h after treatment (Figure 5; Supplementary Figure 3). Previous studies reported total glufosinate metabolites in \( A. \) \( \text{palmeri} \) ranging from 31% to 62% of the total \(^{14}\text{C}\) absorbed (Everman et al. 2009; Meyer et al. 2020). Metabolism varied from 20% to 30% in the broadleaf weeds common lambsquarters (\( \text{Chenopodium album} \) (L.)) and sicklepod [\( \text{Senna obtusifolia} \) (L.) Irwin & Barneby], respectively (Everman et al. 2009a; Pline et al. 1999). Metabolism results observed in this study demonstrate that a mechanism enhancing herbicide metabolism was not involved in evolution of glufosinate resistance in the tested \( A. \) \( \text{palmeri} \).

The overall results of uptake, translocation, and metabolism show that the resistance evolution to glufosinate in \( A. \) \( \text{palmeri} \) is not attributable to the non–target site resistance mechanisms investigated in this study. A similar result was reported in glufosinate-resistant \( E. \) \( \text{indica} \), in which the resistant accession had no changes in uptake, translocation, or enhanced metabolism (Jalaludin et al. 2017). In contrast, one glufosinate-resistant \( L. \) \( \text{perenne} \) population from Oregon showed increased metabolism compared with the susceptible standard (Brunharo et al. 2019).

Another non–target site resistance mechanism is herbicide degradation by glutathione conjugation (Powles and Yu 2010). However, in a previous study conducted with the addition of 4-chloro-7-nitrobenzofurazan (NBD-Cl), a glutathione S-transferase inhibitor, to glufosinate, there was no difference in mortality when resistant \( A. \) \( \text{palmeri} \) was treated with only glufosinate or glufosinate plus NBD-Cl (Carvalho-Moore et al. 2021). Other investigations into areas such as the influence of the addition of cytochrome P450 inhibitors or reactive oxygen species accumulation in different biotypes remain to be conducted.

In conclusion, the results obtained strongly indicate that glufosinate resistance in the investigated \( A. \) \( \text{palmeri} \) accession from Arkansas is likely a result of increased chloroplastic glutamine synthetase copy number and overexpression. No alterations were observed in the cytosolic glutamine synthetase isofrom. There was no change observed in absorption, translocation, or metabolism; therefore, it can be concluded that these three non–target site resistance mechanisms do not influence the glufosinate resistance level in the resistant accession assessed in this study. Future efforts will focus on the heritability of this mechanism across generations, correlation between gene expression and enzyme activity, and alternative control methods targeting this problematic accession. Along with resistance mechanism investigations, glufosinate screenings with \( A. \) \( \text{palmeri} \) field accessions that have survived one or more applications of glufosinate have been conducted yearly for the past 4 yr at the University of Arkansas System Division of Agriculture. These screenings aim to identify other potentially glufosinate-resistant accessions and provide farmers rapid identification of problematic areas, consequently minimizing the dissemination of a resistant accession to neighboring fields.

**Supplementary material.** To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2022.31

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**Figure 5.** Metabolism of \(^{14}\text{C}\)glufosinate by glufosinate-resistant (Glu-R1) and glufosinate-susceptible (S1 and S2) accessions assessed at 48 h after application. Error bars represent standard errors of the means (\( n = 6 \)). Means were subjected to ANOVA, and P-values were generated using JMP Pro v. 15 (SAS Institute, Cary, NC).
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