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Evaluation of ALS-resistant yellow nutsedge (*Cyperus esculentus*) in Georgia peanut

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Research Article

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Abstract

Accounting for 53% of U.S. peanuts (*Arachis hypogaea* L.), Georgia is the top peanut-producing state, with approximately 1.42 billion kg produced in 2023. Peanut producers often use the acetolactate synthase (ALS) imidazolinone herbicide imazapic, but reduced yellow nutsedge (*Cyperus esculentus* L.) control was reported in Georgia peanuts after 4 yr of continuous imazapic use. This study aimed to determine the level of resistance ($LD_{50,}$ I_{50}, and GR_{50}) and potential cross-resistance for the suspected resistant population and to identify the associated genetic mutations conferring resistance. A susceptible biotype was treated with 0, 0.0088, 0.0175, 0.035, 0.07, 0.14, 0.28, and 0.56 kg ai ha^{-1}, and a resistant biotype was sprayed with 0, 0.07, 0.14, 0.28, 0.56, 1.13, 2.26, and 4.5 kg ai ha^{-1} of imazapic. To determine whether the suspected resistant biotype was cross-resistant to halosulfuron-methyl, an ALS herbicide used to control *Cyperus* spp., both biotypes were treated with 0, 0.0117, 0.0233, 0.0466, 0.0933, 0.187, 0.373, and 0.746 g ai ha^{-1} of halosulfuron-methyl. Plants were rated for injury at 7, 14, and 28 d after treatment (DAT), and aboveground biomass was harvested at 28 DAT. For imazapic, LD_{50} was 0.041 and 1.503 kg ai ha^{-1} and the GR_{50} was estimated to be 0.0128 and 1.853 kg ha^{-1} for Sus and Res biotypes, respectively, indicating 36- and 145-fold increase in resistance of the Res biotype for I_{50} and GR_{50}, respectively. Both biotypes responded similarly to applications of halosulfuron-methyl, with biomass reduction at rates greater than 0.023 kg ai ha^{-1}. Transcriptome profiles revealed a mutation in the target-site gene of the resistant biotype causing an amino acid substitution from alanine to valine at position 205 (Ala-205-Val). Growers should continue to rotate chemistries and implement integrated weed management approaches for control of *C. esculentus*, as the use of imazapic over consecutive years has led to resistance in *C. esculentus*.

Introduction

Georgia is the top peanut (*Arachis hypogaea* L.)-producing state, accounting for 53% of U.S. peanut production. Approximately 1.42 billion kg of peanuts were produced in 2023 (USDA-NASS 2024), and average state yields are between 4,751 kg ha^{-1} and 6,359 kg ha^{-1} (UGA 2024). Peanuts are the second most valuable row crop in Georgia behind cotton, with a production value of US$783 million (USDA-NASS 2024). Given lucrative peanut market prices, consistent production of peanuts has led to regular use of herbicides to maximize yields and reduce weed competition. Common weed species prevalent in peanut production in the southeastern United States are sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby], Florida beggarweed [*Desmodium tortuosum* (Sw.) DC.], Palmer amaranth (*Amaranthus palmeri* S. Watson), and yellow nutsedge (*Cyperus esculentus* L.) (Van Wychen 2022).

Cyperus esculentus is a C_4 species that predominately reproduces asexually and proliferates through the production of extensive underground systems of rhizomes, tubers, and basal bulbs. Its rapid emergence and vegetative growth patterns impede early peanut canopy coverage, leading to reduced peanut yields (Drost and Doll 1980; Holt and Orcutt 1991; Keeley 1987; Willis et al. 1980). The basal bulb of *C. esculentus* acts as a primary site for initial leafy shoots and subterranean growth into rhizomes, allowing a single plant to spread throughout an area (Jansen 1971). *C. esculentus* tubers often go dormant for many years, but most tubers sprout during the subsequent growing season (Stoller and Sweet 1987). Prolific tuber production ensures plant survival under adverse field conditions and possible regrowth after herbicide applications. The complex vegetative shoot and rhizome systems give *C. esculentus* a competitive advantage. Leafy plant development and vegetative shoot systems promote rapid colonizing with a high level of genetic and adaptive variation due to heterogeneity in morphological traits (Bhowmilk 1997;

Figure 1. Size comparison of *Cyperus esculentus* tubers (left) and peanut seeds (right).

Holt 1994; Tehrenchian et al. 2015). *Cyperus esculentus* tubers 10 cm below from the soil surface escape the range that common management practices reach during the peanut growing season, supplementing rapid colonization (Ryck et al. 2020). Peanut yield was reduced by 13 kg ha^{-1} when peanuts were in competition with 68 *C. esculentus* plants m^{-2} (Johnson and Mullinix 2003). In addition to reducing yields, *C. esculentus* tubers are not easily separated at peanut harvest and are a major contaminant during shelling and cleaning of peanuts, as they are similar in size to shelled peanuts (Pattee and Young 1982) (Figure 1).

The most common herbicides used for postemergence control of *C. esculentus* in peanut production include bentazon (photosystem II inhibitor), imazapic (acetolactate synthase [ALS] inhibitor), and *S*-metolachlor (very-long-chain fatty-acid inhibitor) (UGA 2021). Early work on imazapic (previously known as AC 263,222) showed control of common problematic weeds in peanut such as *D. tortuosum*, *S. obtusifolia*, purple nutsedge (*Cyperus rotundus* L.), *C. esculentus*, *Amaranthus* spp., and weedy *Ipomoea* spp., along with favorable peanut safety at rates of 0.04 to 0.07 kg ha^{-1} (Grichar and Nester 1997; Richburg et al. 1995, 1996). Imazapic is predominantly applied postemergence 30 d after planting (DAP) on peanuts, with little to no crop injury. It has long residual activity while providing weed control for several weeks after application and has been shown to effectively control susceptible *C. esculentus* tubers (Grey and Wehtje 2005; Grichar 2002). Although imazapic is highly effective in peanut, there are label rotation restrictions of 18 and 9 mo for cotton (*Gossypium hirsutum* L.) and field corn (*Zea mays* L.), respectively, both of which are commonly grown in rotation with peanuts in the region. These rotation restrictions can be problematic for growers due to concerns about reduced crop stand in the subsequent crop. Continuous cropping systems with little to no herbicide site of action rotations and poor stewardship can lead to resistant weed species.

Cyperus esculentus Resistance

With limited tools available to producers for weed control in peanuts, *C. esculentus* becomes challenging to control. Although *C. esculentus* is less likely to become resistant due to its asexually reproducing nature and lower seed viability, evolution of resistance is not uncommon (Bagavathiannan et al. 2015; Lapham and Drennan 1990). Within the past decade, a case of ALS-resistant *C. esculentus* in Arkansas rice (*Oryza sativa* L.) production to halosulfuron-methyl was reported (Heap 2023a). Other instances of *C. esculentus* cross-resistant to the ALS herbicides azimsulfuron and halosulfuron-methyl have been recorded in Italy (Heap 2023b). The *C. esculentus* biotype resistant to halosulfuron-methyl discovered in Arkansas rice production had >2,714-fold resistance to halosulfuron-methyl compared with susceptible biotypes, as well as cross-resistance to imazethapyr, imazamox, bensulfuron, pyrithiobac, and penoxsulam (Tehranchian et al. 2014). Other species in the Cyperaceae family have also been reported to have cross-resistance to ALS herbicides. Rice flatsedge (*Cyperus iria* L.) has cross-resistance to bispyribac-sodium, halosulfuron, imazamox, imazethapyr, and penoxsulam in rice production (Heap 2024a), and annual sedge (*Cyperus compressus* L.), has cross-resistance to halosulfuron-methyl, imazapic, sulfometuron-methyl, and trifloxysulfuron-sodium in turf production (Heap 2024b). Efforts have been made to encourage producers not to apply multiple applications of imazapic in a single year to prevent or delay the evolution of further weed resistance (Prostko 2022).

Imazapic has become a reliable tool for producers to control nutsedge in Georgia peanuts but should not be relied upon year after year. Recent reports of a peanut producer from Webster County, GA, being unable to control *C. esculentus* after successive imazapic use has raised concern. Therefore, to understand whether there is ALS-resistant *C. esculentus* in Georgia and determine the possibility of it being cross-resistant, the objectives of our research are: (1) determine the level of resistance of this *C. esculentus* biotype to imazapic, (2) evaluate possible cross-resistance to halosulfuron-methyl, and (3) identify the mutation conferring resistance to imazapic or other ALS herbicides.

Materials and Methods

Initial Biotype Identification and Propagation

Suspected resistant (Res) *C. esculentus* tubers were collected from a field in Webster County, GA, following four consecutive years of peanuts, which is not common practice in the region. Each season imazapic was used, a reduction of control increased year after year. Tubers were collected from emerged plants in August of 2019 and 2020 in the infested field using a peanut inverter to dig up plants and tubers, after which the tubers were removed by hand and placed in a plastic bag and transported in a cooler with ice packs to remove field heat. Following collection, tubers were air-dried at room temperature and stored at 4.5 C in a refrigerator to break dormancy (Beckie et al. 2000). Due to COVID-19 restrictions and lack of labor, tubers were held in a 4.5 C refrigerator until spring 2021 for tubers dug in 2020. To determine resistance, susceptible (Sus) biotype samples from Azlin Seed (Azlin Seed Service, 112 Lilac Drive, Leland, MS 38756) were used for comparison. Sus and Res biotypes were soaked in water for 24 h before planting to allow for tuber imbibition and improve germination. Once soaked, the tubers were placed in plastic trays (55.5 by 26.5 by 5.5 cm) filled with sterile potting media (Sta Green®, Sta Green Inc., 3902 Lakeview Parkway, Rowlett, TX 75088) and maintained at 21 C day/night temperature under a 15-h photoperiod in greenhouse settings for optimal germination of Sus and Res biotypes.

Vegetative Whole-Plant Assay

The trials were initiated in 2019 and conducted in 2021 under greenhouse conditions, with each herbicide screen containing 15 replications per treatment for the Sus biotype and 12

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Table 1. Herbicide dose of imazapic and halosulfuron-methyl to determine I_{50} and GR_{50} of suspected acetolactate synthase (ALS)-resistant *Cyperus esculentus*[a].

Res biotype		Sus biotype	
Imazapic[b]	Halosulfuron-methyl[c]	Imazapic	Halosulfuron-methyl
kg ai ha^{-1}			
0	0	0	0
0.07	0.012	0.009	0.012
0.14	0.023	0.018	0.023
0.28	0.047	0.035	0.047
0.56	0.093	0.07	0.093
1.13	0.187	0.14	0.187
2.26	0.373	0.28	0.373
4.50	0.750	0.56	0.750

[a]Abbreviations: ALS, acetolactate synthase; Gr_{50}, 50% effective dose for growth reduction; I_{50}, 50% visual injury; Res, suspected resistant *C. esculentus* biotype from Webster County, GA; Sus, known susceptible *C. esculentus* biotype from Azlin Seed.
[b]ALS herbicide that *C. esculentus* biotype is suspected to be resistant to.
[c]ALS herbicide that *C. esculentus* biotype is suspected to not be resistant to.

replications per treatment for the Res biotype, due to limited tuber availability. Based on preliminary germination studies, Res tubers were planted 18 d before Sus tubers so that plants would reach the desired phenological stage at the same time. Res *C. esculentus* biotypes have been reported to have longer dormancy length and emerge later compared with Sus biotypes due to low early-growth seedling vigor (Bagavathiannan et al. 2015). Sprouted Res and Sus *C. esculentus* plants at the 2- to 3-leaf stage were transplanted into the center of plastic greenhouse pots (9 by 9 by 9 cm) containing potting mix as previously described. When plants reached the 4- to 5-leaf stage, herbicide applications of imazapic or halosulfuron-methyl treatments were assigned to individual plants of the Res and Sus biotypes per dose based on the recommended rate. Fifteen individual plants were set aside to serve as a nontreated check for the Sus biotype, and 12 for the *Res* biotype.

Dose Response

Rates of imazapic were based on preliminary screening data and were 0, 0.0088, 0.0175, 0.035, 0.07, 0.14, 0.28, and 0.56 kg ai ha^{-1} for the Sus biotype and 0, 0.07, 0.14, 0.28, 0.56, 1.13, 2.26, and 4.52 kg ai ha^{-1} for the Res biotype to evaluate possible resistance to imazapic (Table 1). The field recommended rate for imazapic is 0.28 kg ai ha^{-1}.

To evaluate possible ALS cross-resistance, as was reported in Arkansas rice production (Tehranchian et al. 2014), rates of halosulfuron-methyl at 0, 0.0117, 0.0233, 0.0466, 0.093, 0.187, 0.373, and 0.75 kg ai ha^{-1} for the Sus and Res biotypes were used based on the 0.035 kg ai ha^{-1} recommended rate. Both herbicide applications included crop oil concentrate at 1% v/v (CNI Agri-Oil, 800 Business Park Drive, Highway 82 West, Leesburg, GA 31763) and were applied to *C. esculentus* plants with 4 to 5 leaves. Herbicides were applied using a Generation III Research Spray Chamber (DeVries Manufacturing, 86956 MN-251, Hollandale, MN 56045) calibrated to apply 190 L ha^{-1} through a TeeJet® TP8002 brass nozzle to ensure uniform coverage (TeeJet Technologies, 200 W North Avenue, Glendale Heights, IL 60139). After being sprayed, plants were placed back into the greenhouse for evaluation. At 1, 2, and 4 wk after treatment (WAT) visual injury was assessed to calculate I_{50} (50% visual injury). At 4 WAT, plants were rated as dead (complete necrosis) or alive to calculate LD_{50}; aboveground Res and Sus *C. esculentus* shoot biomass was harvested, oven-dried at 60 C for 72 h, and weighed; and percent dry weight reduction, or 50% effective dose for growth reduction (GR_{50}), compared with nontreated control was calculated. Additional data collection of plant height and mortality also occurred at 4 WAT.

Statistical Analyses of Dose Response

Dose–response analysis was performed using JMP v. 16 software (SAS Institute, 920 SAS Campus Drive, Cary, NC 27513). An initial ANOVA was conducted using the mixed procedure, with herbicide, dose, and biotype as fixed effects and replication as random to determine whether global effects were significant ($\alpha \geq 0.05$). Data were then graphed using SigmaPlot v. 15.0 (Grafiti LLC, 405 Waverly St., Palo Alto, CA 94301). GR_{50} data were obtained from a comparison of imazapic- and halosulfuron-methyl-treated Res and Sus *C. esculentus* (*T*) dry weight and the nontreated control (*C*), using the following equation:

$$\text{Growth reduction (\%)} = \left[1 - \left(\frac{T}{C}\right)\right] \times 100 \quad [1]$$

Percent damage from herbicide dose response was determined using ANOVA, while the interaction between biotypes and herbicide doses was analyzed based on a P-value < 0.05.

The herbicide doses resulting in 50% growth reduction (GR_{50}) and 50% mortality (LD_{50}) were obtained by a nonlinear regression using the four-parameter log-logistic dose–response equation(s):

$$Y = c + \frac{d - c}{\left[1 + \left(\frac{X}{GR_{50}}\right)^b\right]} \quad [2]$$

$$Y = c + \frac{d - c}{\left[1 + \left(\frac{X}{LD_{50}}\right)^b\right]} \quad [3]$$

where *c* and *d* denote lower and upper limits, respectively; *b* is the response curve slope; *X* is the independent variable (herbicide dose rate); and *Y* is biomass or mortality (Seefeldt et al. 1995). The data from visual injury assessments were used to calculate I_{50} estimate values for imazapic in Res and Sus biotypes.

RNA Extraction

Res and the Sus biotypes were transplanted in pots and grown under control conditions at the Auburn University Weed Science greenhouse in Auburn, AL. Freshly collected leaf tissues of about 100 mg from six individual plants of each biotype were flash frozen in liquid nitrogen and ground using a mortar and pestle. RNA extraction was done using the RNeasy Plant Mini Kit (Qiagen, Kalkvaerksvej 5, 11, 8000 Aarhus, Denmark) following the manufacturer's instructions. DNA digestion was performed using the TURBO DNA-free™ Kit (Applied Biosystems, Inc., 850 Lincoln Center Dr, Foster City, CA 94404) to eliminate any genomic DNA content in the samples. RNA concentration and quality were checked on a NanoDrop 2000 (Thermo Fisher Scientific, 168 3rd Ave, Waltham, MA 02451), and RNA integrity was determined using electrophoresis in 20 g L^{-1} agarose gel. Single RNA samples from Res and Sus biotypes were sent to Novogene (Novogene Corporation Inc., 8801 Folsom Blvd, Suite 290, Sacramento, CA 95826) for transcriptomic sequencing. At Novogene, the samples were tested for RNA quality using a bioanalyzer instrument (Agilent 2100, Agilent Technologies, 5301

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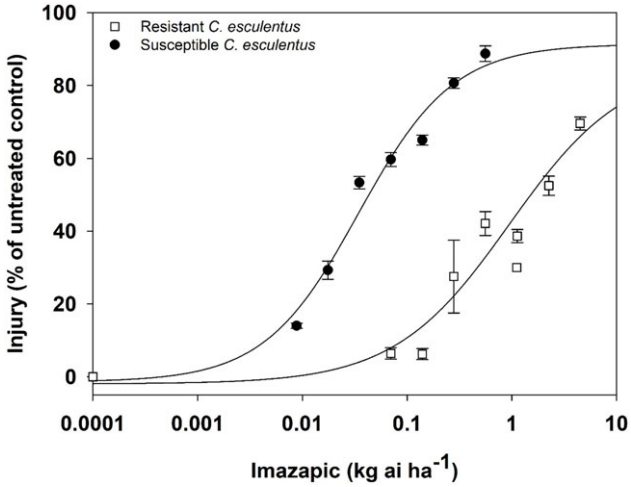

Figure 2. Injury of suspected resistant and known susceptible *Cyperus esculentus* in response to imazapic applications.

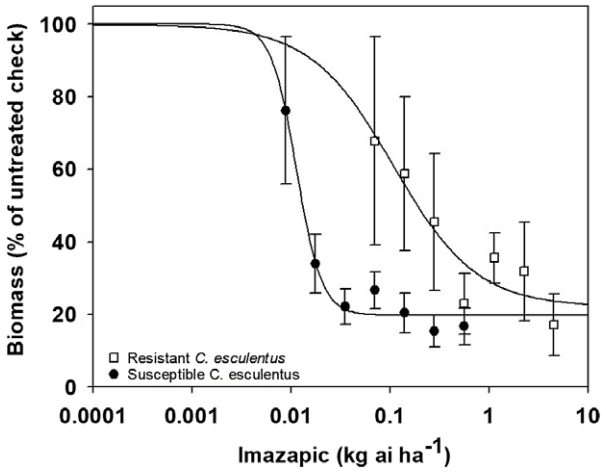

Figure 4. Biomass response of suspected resistant and known susceptible *Cyperus esculentus* to imazapic applications.

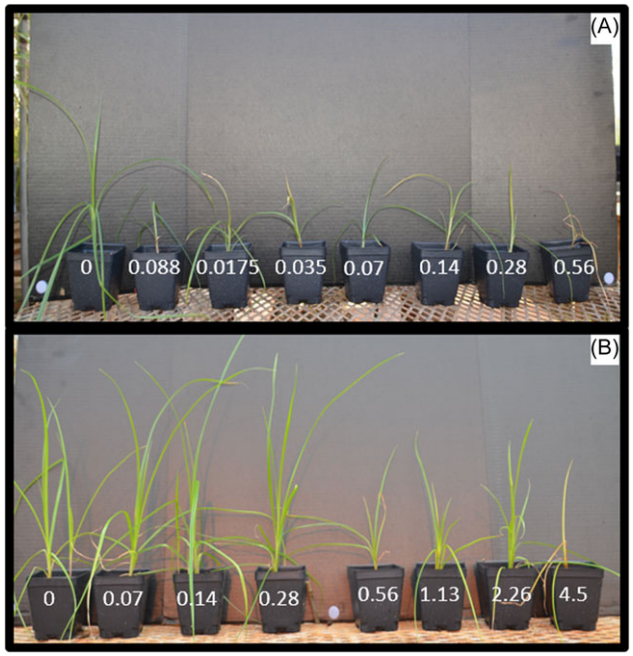

Figure 3. Response of known susceptible *Cyperus esculentus* (A) and suspected resistant *C. esculentus* (at 28 DAT) to applications of imazapic (B). Numbers indicate imazapic rate (kg ai ha^{-1}).

STevens Creek Blvd, Santa Clara, CA 95051) and then proceeded for library preparation for transcriptome sequencing. Prepared libraries were further checked for quality before being pooled into one tube and then run on an Illumina NovaSeq 6000 (Illuina, Inc., 5200 Illumina Way, San Diego, CA 92122) instrument to produce 150-bp paired-end reads. At the end of run, we received at least 53M raw reads for each sample (8 G raw data/sample).

Transcriptome Profiling

Transcriptome data were analyzed using the Qiagen CLC Genomics Workbench 20.0 (Qiagen). The transcriptome data of Res and Sus biotypes were separately mapped to the *ALS* gene sequence of small flower umbrella sedge (*Cyperus difformis L.)* (GenBank accession no. EF061294.2) and compared to identify potential singular-nucleotide polymorphism (SNP) that can be associated with herbicide resistance. The mapping parameters assigned for assembling the reads to the *ALS* gene were Mismatch cost = 3, Insertion cost = 3, Deletion cost = 3, Length fraction = 0.95, and Similarity fraction = 0.95. To avoid false-positive identification of the SNP, the parameters for variants calling were Minimum coverage = 30 and Variant probability = 90. Further, it was observed that the *ALS* gene was in a heterozygous state, so SNP was only called if the minor allele frequency was >5%.

Results and Discussion

Dose Response

Visual injury estimates (I_{50}) for Sus *C. esculentus* exceeded 50% at imazapic doses of 0.041 kg ai ha^{-1} with obvious chlorosis, while necrosis occurred with higher doses. These results are consistent with previous studies to control *C. esculentus* (Grichar and Nester 1997). In contrast, the minimum imazapic dose required for I_{50} for the Res biotype was 1.503 kg ai ha^{-1} (Figure 2). At the highest dose (4.5 kg ai ha^{-1}), Res plants exhibited chlorosis but did not develop necrosis. Imazapic doses less than 1.503 kg ai ha^{-1} (well above the recommended rate) caused little injury (chlorosis/necrosis) at 7 and 14 DAT, and Res biotypes almost completely recovered and continued to grow and produce new, normal tissue by the time plants were harvested, suggesting the occurrence of a *C. esculentus*-resistant biotype (Figure 3).

When compared with the Sus biotype, the Res biotype exhibited relatively high levels of resistance to imazapic at 0.56 kg ai ha^{-1} (Figure 4). GR_{50} values for Res and Sus biotypes were 1.853 and 0.0128 kg ai ha^{-1}, respectively, indicating that the Res biotype was approximately 145-fold more resistant to imazapic relative to the Sus biotypes. The highest applied dose of imazapic (4.5 kg ai ha^{-1}) caused serious chlorosis but did not completely kill a majority of Res biotypes; however, biomass was reduced by roughly 80% relative to nontreated Res biotypes. The lethal dose to kill 50% of Res and Sus biotypes (LD_{50}) coincides with the GR_{50}; Sus biotype LD_{50} levels

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Table 2. Missense mutations were identified in both susceptible and resistant *Cyperus esculentus* in the transcriptome data.

Amino acid position[a]	SNP position[b]	Major allele[c]	Minor allele[d]	Amino acid change
343	3	GAT	GAG	Asp-343-Glu
593	1	AAT	GAT	Asn-593-Asp
616	1	ACA	TCA	Thr-616-Ser

[a]Amino acid position is based on acetolactate synthase (ALS) protein sequence of *Arabidopsis thaliana*.
[b]Single-nucleotide polymorphism (SNP) number is base number on the codon.
[c]Major allele is the allele with more read counts in susceptible and resistant transcriptome data.
[d]Minor allele is the one with fewer read counts in susceptible and resistant transcriptome data.

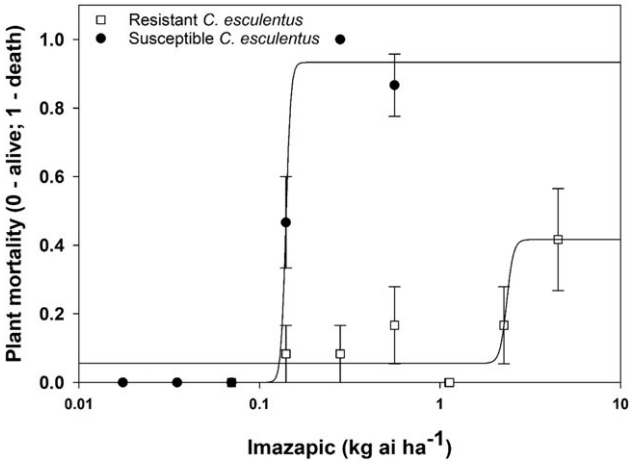

Figure 5. Mortality response of suspected resistant and known susceptible *Cyperus esculentus* to imazapic applications.

require a dose of 0.14 kg ai ha^{-1} of imazapic, while Res biotypes never reach 50% kill with even the highest dose (4.5 kg ai ha^{-1}) (Figure 5).

Cross-resistance

There are reports of *Cyperus* spp. cross-resistant to ALS herbicides, such as *C. difformis*, *C. iria*, and *C. esculentus* within the United States (Heap 2023b). Cross-resistance to halosulfuron-methyl and imazapic in Georgia peanut production, did not occur, and Res and Sus biotypes were both controlled by halosulfuron-methyl doses greater than 0.023 kg ha^{-1} (Figure 6), implying that producers can still control *C. esculentus* using ALS herbicides in rotational crops. However, due to the use of ALS herbicides across production systems in Georgia and the southeastern United States, other methods of management such as crop rotation and rotation of herbicide modes of action should be utilized. This minimizes the risk of placing further selection pressure on this population and others in peanut-growing regions of the United States.

Transcriptome

Transcriptome profiling found a total of 28 SNPs common in Res and Sus biotypes, with three nonsynonymous SNPs causing amino

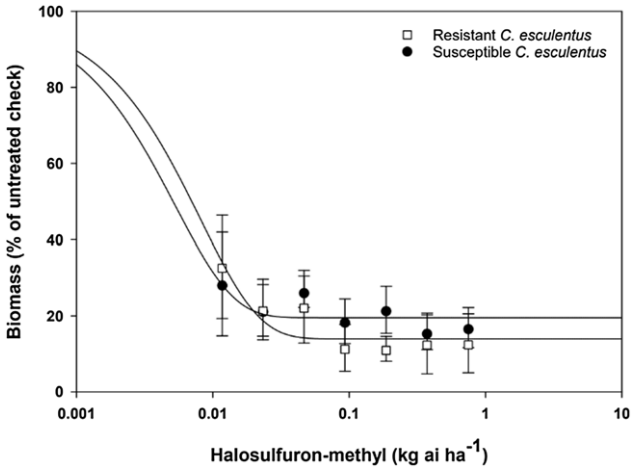

Figure 6. Biomass response of suspected resistant and known susceptible *Cyperus esculentus* to halosulfuron-methyl applications.

acid changes (Table 2). Similar results were observed in a previous study of ALS resistance in *C. esculentus* (McCullough et al. 2016). *Cyperus esculentus* propagates by an asexual method in the form of tubers, which results in minimal genetic variability. Such a high number of heterozygous loci needs further evaluation to ascertain the potential presence of multiple copies of the *ALS* gene within the *C. esculentus* genome. Additionally, two mutations were found only in the Res biotype. One of the mutations caused a change in amino acid from alanine to valine (Figure 7; Table 3). This mutation corresponds to the previously reported mutation change Ala-205-Val in redroot pigweed (*Amaranthus retroflexus* L.) that confers resistance to ALS herbicides (McNaughton et al. 2005), suggesting a target-site mutation in the gene causes resistance in *C. esculentus*. This is different from the ALS resistant *C. esculentus* previously reported in Arkansas, which has a Trp-574-Leu mutation (Heap 2024c; Tehranchian et al. 2015).

In summary, transcriptome profiling and dose–response experiments revealed that imazapic resistance in the Res biotype was due to a mutation causing alanine to change to valine. Although consecutive years of peanuts in the same field is not a common practice in Georgia or in other peanut-producing regions of the country, the findings of this study indicate that consecutive use of imazapic can result in the development of herbicide resistance in *C. esculentus*. Phenotypic and physiological characteristics of Res *C. esculentus* biotypes can produce extensive networks of rhizomes and basal bulbs with delayed emergence and lengthened dormancy compared with Sus biotypes due to low early-growth seedling vigor (Bagavathiannan et al. 2015; Tehranchian et al. 2015). Producers applying imazapic post-emergence (30 DAP) might spray the initial flush of Sus *C. esculentus* while the resistant flush emerges afterward, as seen in the more slowly emerging Res biotype in our study. Therefore, to manage herbicide resistance in *C. esculentus*, multiple tools and management approaches are necessary. Cultural practices such as crop rotation, cover crops, and tillage methods can reduce weed pressure, and rotating herbicide mechanisms of action help prevent herbicide resistance.

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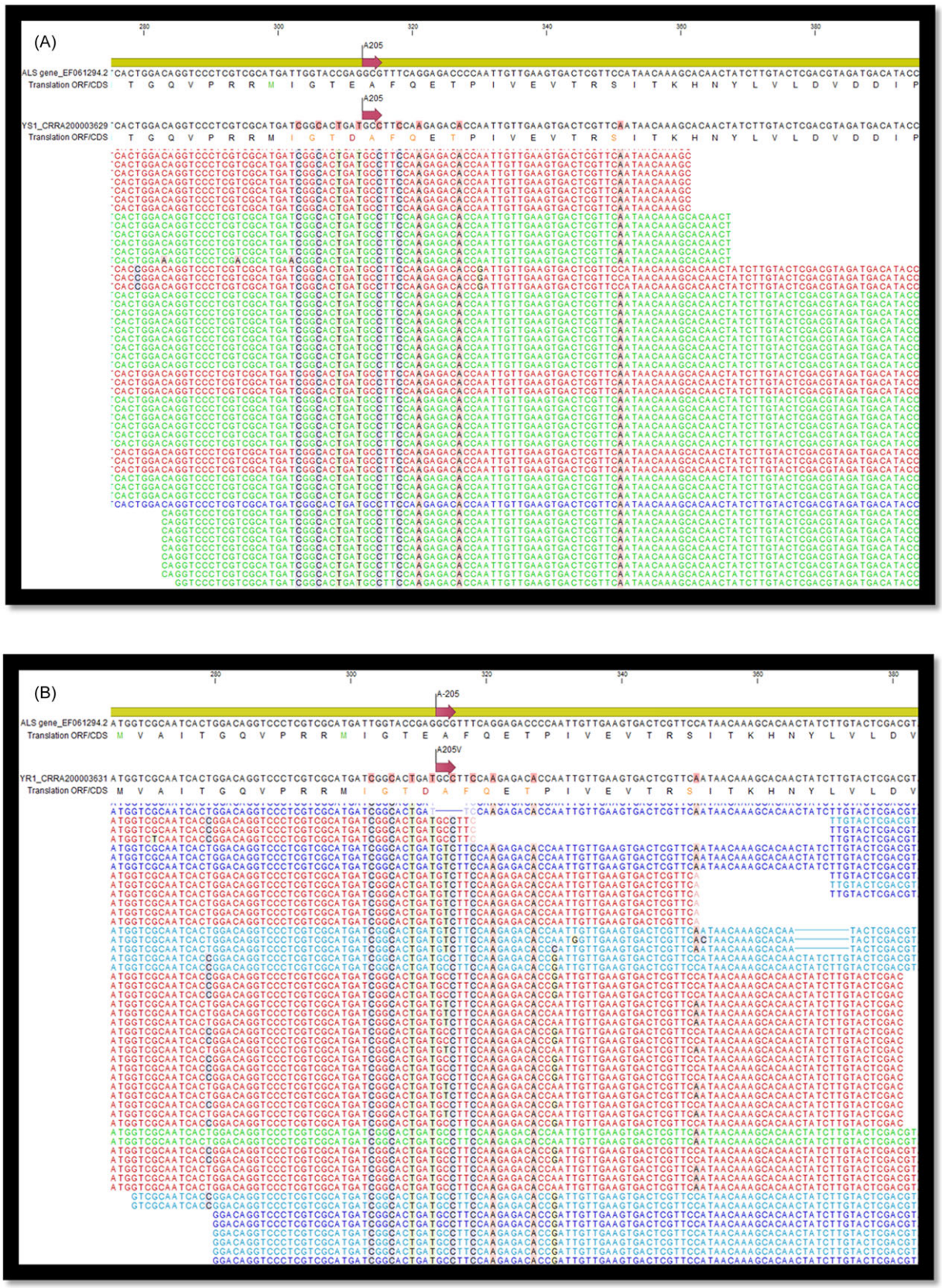

Figure 7. Alignment of Illumina sequencing reads from susceptible (Sus) biotype (A) and resistant (Res) biotype (B) of *Cyperus esculentus* to acetolactate synthase (ALS) gene from *Cyperus difformis* (GenBank accession no. EF061294.2) focusing on target-site mutation Ala-205-Val. The horizontal line color codes highlight mismatches in the nucleotide sequences of mapped reads compared with the reference sequence: red for adenine (A), blue for cytosine (C), yellow for guanine (G), and green for thymine (T).

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Table 3. Mutations exclusively found in transcriptome data of resistant biotype of *Cyperus esculentus*.

Amino acid position[a]	SNP position[b]	Reference	Polymorphism	Freq[c]	Amino acid change
177	3	T	C	0.22	na
205	2	C	T	0.34	Ala-205-Val

[a]Amino acid position is based on acetolactate synthase (ALS) protein sequence of *Arabidopsis thaliana*.
[b]Single-nucleotide polymorphism (SNP) number is base number on the codon.
[c]Frequency (Freq) is the number of mapped reads carrying the polymorphic nucleotide divided by total number of mapped reads carrying both alleles.

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