This is a “preproof” accepted article for Weed Science. This version may be subject to change in the production process, *and does not include access to supplementary material*.
DOI: 10.1017/wsc.2024.87

Evaluation of ALS-Resistant Yellow Nutsedge (*Cyperus esculentus*) in Georgia Peanut

Hannah C. Lindell[1], Eric P. Prostko[2], Scott McElroy[3], Jinesh D. Patel[4], Jason D. Blankenship[5], Timothy L. Grey[6], and Nicholas T. Basinger[7]

[1]Graduate Research Assistant; Department of Crop and Soil Sciences, University of Georgia, Athens, GA, 30602

[2]Professor and Extension Weed Specialist, Department of Crop and Soil Sciences, University of Georgia, Tifton, GA, 31793

[3]Professor in Weed Science and Turf, Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL, 36849

[4]Research Associate, Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL, 36849

[5]Former Randolph County Extension Agent, University of Georgia Cooperative Extension, P.O. Box 282, 413 Blakely Street, Cuthbert, GA 39840.

[6]Professor, Department of Crop and Soil Sciences, University of Georgia, Tifton GA, 31793

[7]Associate Professor, Department of Crop and Soil Sciences, University of Georgia, Athens, GA, 30602

ABSTRACT

Accounting for 53% of United States 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 ALS imidazolinone herbicide imazapic but reduced yellow nutsedge (*Cyperus esculentus* L.) control was reported in Georgia peanuts after four-years of continuous imazapic use. This study aimed to determine the level of resistance ($LD_{50,}$ I_{50}, and GR_{50}), potential cross-resistance for the suspected resistant population, and 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 if the suspected resistant biotype was cross-resistant to halosulfuron-methyl, an ALS herbicide used to control nutsedge 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 7, 14, and 28 days after treatment (DAT), and above-ground biomass 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 ai 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 reductionat 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 (Ala205Val). 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*.

Keyword: ALS resistance, base pair change, cross-resistance, GR_{50}, halosulfuron-methyl, herbicide resistance, I_{50}, imazapic, LD_{50}

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

INTRODUCTION

Georgia is the top peanut (*Arachis hypogaea* L.) producing state, accounting for 53% of United States peanut production. Approximately 1.42 billion kgs 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 783 million US dollars (USDA -NASS 2024). Considering 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 US 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. 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 (Tehrenchian *et al*. 2015; Holt 1994; Bhowmilk 1997). *Cyperus esculentus* tubers below 10 cm from the soil surface escape the range that common management practices do not 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 nutsedge plants per 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

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

shelling and cleaning of peanuts due to their similar size of shelled peanuts (Figure 1) (Davidson et al. 1982).

The most common herbicides used for postemergence control of *C. esculentus* in peanut production include bentazon (PSII inhibitor), imazapic (ALS inhibitor), and *S*-metolachlor (VLCFA inhibitor) (UGA 2022). 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 (*Cyperus rotundus* L.), and *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, UGA 2022). Imazapic is predominantly applied postemergence 30 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 for cotton (*Gossypium hirsutum* L.) and field corn (*Zea mays* L.), 18 and 9 months, respectively, 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.

Resistance of C. esculentus. With limited tools producers can use 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, it 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 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 to susceptible biotypes as well as cross-resistance to imazethapyr, imazamox, bensulfuron, pyrithiobiac, bensulfuron, 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-

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

resistance to bispyribac-sodium, uron-methyl, 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-Na 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, Georgia unable to control *C. esculentus* after successive imazapic use has raised concern. Therefore, to understand whether there are ALS-resistant *C. esculentus* in Georgia and its possibility for cross-resistance, 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 back and transported in a cooler with icepacks to remove field heat. Following the collection, tubers were air-dried at room temperature and stored at 4.5 ºC in a refrigerator to break dormancy (Beckie et al. 2000). Due to COVID-19 restrictions and lack of labor, tubers were held in 4.5 ºC fridge until spring 2021 for tubers dug in 2020. To determine resistance, susceptible (*Sus*) biotype samples from Azlin Seed (Azlin Seed Service 112 Lilac Dr, Leland, MS 38756) were used for comparison. *Sus* and *Res* biotypes were soaked in water for 24 hours prior to planting allowing 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,

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

Rowlett, TX, 75088) and maintained at 21 ºC 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 susceptible and 12 replications per treatment for resistant biotypes, due to limited tuber availability. Based on preliminary germination studies, *Res* tubers were planted 18 days prior to *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 to *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 both *Sus* and twelve plants for the *Res* biotypes.

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 *Sus* and 0, 0.07, 0.14, 0.28, 0.56, 1.13, 2.26, and 4.52 kg ai ha^{-1} for the *Res* biotypes to evaluate possible resistance to imazapic (Table 1). The field recommended rate is 0.28 kg ai ha^{-1} for imazapic.

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 were applied with crop oil concentrate at 1% v/v (CNI Agri-Oil, CNI, Leesburg, GA 31763), and to *C. esculentus* plants with 4 to 5 leaves. Herbicides were applied with a generation III research spray chamber (DeVries Manufacturing, Hollandale, MN) calibrated to apply 190 L ha^{-1} through a TeeJet TP8002 brass nozzle to ensure uniform coverage (TeeJet Technologies, 200 W North Ave, Glendale Heights, Illinois, 60139). After spraying plants were placed back into the greenhouse for evaluation. At 1, 2, and 4 weeks after treatment (WAT) visual injury was assessed to calculate I_{50}, and at 4 WAT plants were rated as dead (complete necrosis) or alive to calculate LD_{50}, and aboveground *Res* and *Sus C.*

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

esculentus shoot biomass was harvested and oven dried at 60 °C for 72 hours, weighed and percent dry weight reduction, or 50% effective dose for growth-reduction (GR_{50}), compared with nontreated control 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 16 software (JMP 2021). An initial ANOVA was conducted using the mixed procedure with herbicide, dose, and biotype as fixed effects and replication as random to determine if global effects were significant ($\alpha \geq 0.05$). Data were then graphed using Sigmaplot v15.0 (Grafiti LLC, Palo Alto, CA). Growth-reduction by 50% data was 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:

$$growth\ reduction\ (\%) = \left[1 - \left(\frac{T}{C}\right)\right] X\ 100$$

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):

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

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

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} (50% visual injury) estimate values for imazapic in *Res* and *Sus* biotypes.

RNA extraction. *Res* and the *Sus* biotype were transplanted in pots and grown under control conditions at the Auburn University Weed Science greenhouse in Auburn, AL. Freshly collected

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

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) and following manufacturer instructions. DNA digestion was performed using turbo DNA-free kit (Applied Biosystems) to eliminate any genomic DNA content in the samples. RNA concentration and quality were checked on Nanodrop 2000 (ThermoFisher Sci., Waltham, MA), and RNA integrity was determined using electrophoresis in 2% (w/v) agarose gel. Single RNA samples from resistant and susceptible biotypes were sent to Novogene for transcriptomic sequencing. At Novogene, the samples were tested for RNA quality using a bioanalyzer instrument (Agilent 2100) and then proceeded for library preparation. Prepared libraries were further checked for quality before pooling into one tube and then run-on Illumina NovaSeq 6000 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 QIAGEN CLC Genomics Workbench 20.0 (QIAGEN, Aarhus, Denmark). The transcriptome data of resistant and susceptible biotypes were separately mapped to the acetolactate synthase (ALS) gene sequence of small flower umbrella sedge (*Cyperus difformis* L.) (GenBank accession number: EF061294.2) and compared to identify potential single 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

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

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 3a, 3b).

When compared to 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 non-treated *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 require a dose of 0.14 kg ai ha^{-1} of imazapic, whilst *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 sedges cross-resistant to ALS inhibitors, 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 ai ha^{-1} (Figure 6), implying that producers can still control *C. esculentus* using ALS inhibitors and other herbicides in rotaional crops. However, due to the use of ALS herbicides across production systems in Georgia and the southeastern US, 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 US.

Transcriptome. Transcriptome profiling found a total of 28 SNPs common in resistance and susceptible biotypes, with three non-synonymous SNPs causing amino acid change (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 resistant

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

biotype. One of the mutations caused a change in amino acid from Alanine to Valine (Figure 7a, 7b, Table 3). This mutation corresponds to previously reported mutation change Ala205Val in redroot pigweed (*Amaranthus retroflexus* L.) that confers resistance to ALS herbicide resistance (McNaughton *et al.* 2005), suggesting a target site mutation in the gene causes resistance in *C. esculentus*. This is contrary to previous ALS resistant *C. esculentus* from Arkansas, with mutation changing from tryptophan 574 to leucine (Tehranchian et al 2015; Heap 2024c).

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 resistant *C. esculentus* biotypes can produce extensive networks of rhizomes and basal bulbs with delayed emergence and lengthened dormancy compared to susceptible biotypes due to low early-growth seedling vigor (Bagavathiannan et al. 2015; Tehranchian 2015). Producers applying imazapic postemergence, 30 DAP might spray the initial flush of susceptible *C. esculentus* while the resistant flush emerges afterwards as seen in the more slowly emerging resistant 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.

Acknowledgments

We would also like to thank Francisco Rigodanzo, Jared Baker, Wayne Dillard, Logan Dyer, and David Weisberger for their assistance in propagule collection, study initiation and maintenance, and data collection.

Funding

The authors would like to thank the Georgia Commodity Commission for Peanuts under grant number UGA-47-19/21.

Competing Interests

The authors declare none.

References

Bagavathiannan MV, Norsworthy JK, Tehranchian P, Riar DS (2015) ALS-inhibitor resistance in yellow nutsedge (*Cyperus esculentus* L.): I. Phenotypic differences. Weed Sci 63:810–818

Beckie HJ, Heap IM, Smeda RJ, Hall LM (2000) Screening for herbicide resistance in weeds. Weed Technol 14:428-445

Bhowmilk PC (1997) Weed biology: importance to weed management. Weed Sci. 45:349-356

Davidson JI, Jr, Whitaker TB, Dickens JW (1982) Grading, cleaning, storage, shelling, and marketing of peanuts in the United States. Peanut Sci Technol pp 571-623

Drost DC, Doll JD (1980) The allelopathic effect of yellow nutsedge (*Cyperus esculentus*) on corn (*Zea mays*) and soybeans (*Glycine max*). Weed Sci 28:229-233Grey TL and Wehtje GR (2005) Residual herbicide weed control systems in peanut. Weed Technol 19:560-567

Grey TL, Wehtje GR (2005) Residual herbicide weed control systems in peanut. Weed Technol 19:560-567

Grichar WJ (2002) Effect of continuous imidazolinone herbicide use on yellow nutsedge (*Cyperus esculentus*) populations in peanut. Weed Technol 16:880-884

Grichar WJ and Nester PR (1997) Nutsedge (Cyperus spp.) Control in Peanut (Arachis hypogaea) with AC 263,222 and Imazethapyr. Weed Technol 11:714-719

Heap (2023a) Herbicide Resistant Yellow Nutsedge Globally (*Cyperus esculentus*). https://www.weedscience.org/Pages/Species.aspx. Accessed: March 30, 2023

Heap, I. (2023b). The International Survey of Herbicide Resistant Weeds. Online. Available: http://www.weedscience.org. Accessed on April 6, 2023

Heap, I (2024a) Herbicide Resistant Rice Flatsedge Globally (*Cyperus iria*). http://weedscience.org/Pages/Species.aspx. Accessed: August 5, 2024

Heap, I (2024b) Herbicide Resistant Annual Sedge Globally (*Cyperus compressus*). http://weedscience.org/Pages/Species.aspx. Accessed: August 5, 2024

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

Heap, I (2024c) Yellow Nutsedge (Cyperus esculentus) with group B/2 resistance: (inhibition of acetolactate synthase). http://weedscience.org/Pages/MutationDetailDisplay.aspx?SubstitutionID=1237. Accessed: August 5, 2024

Holt JS (1994) Genetic variation in life history traits in yellow nutsedge (*Cyperus esculentus*) from California. Weed Sci 42:378-384

Holt JS and Orcutt DR (1991) Functional relationships of growth and competitiveness in perennial weeds and cotton (*Gossypium hirsutum*). Weed Sci 39:575-584

Jansen LL (1971) Morphology and photoperiodic responses of yellow nutsedge. Weed Sci 19:210-219

JMP® (2021) Version 16.0, SAS Institute Inc., Cary, NC

Johnson WC, Mullinix BG Jr (2003) Yellow nutsedge (*Cyperus esculentus*) interface in peanut (*Arachis hypogaea*). Peanut Sci 30:14-18

Keeley PE (1987) Interference and interaction of purple and yellow nutsedges (*Cyperus rotundus and C. esculentus*) with crops. Weed Technol 1:74-81

Lapham J, Drennan DSH (1990) The fate of yellow nutsedge (*Cyperus esculentus*) seed and seedlings in soil. Weed Sci 38:125-128

McCullough PE, McElroy JS, Yu J, Zhang H,. Miller TB, Chen S, Johnston CR, Czarnota MA (2016) ALS-resistant spotted spurge (*Chamaesyce maculata*) confirmed in Georgia. Weed Sci 64: 216-222

McNaughton, KE, Letarte J, Lee EA, Tardif FJ (2005) Mutations in ALS confer herbicide resistance in redroot pigweed (*Amaranthus retroflexus*) and Powell amaranth (*Amaranthus powellii*). Weed Sci 53: 17-22

Prostko EP (2022) 2022 Peanut/Field Corn/Soybean Weed Control Recommendations. The University of Georgia. Athens, GA https://site.extension.uga.edu/plowpoints/2022/02/2022-uga-peanut-field-corn-soybean-weed-control/ Accessed: 6/20/2023

Richburg JW, Wilcut JW, Colvin DL, Wiley GR (1996) Weed management in Southeastern peanut (*Arachis hypogaea*) with AC 263,222. Weed Technol 10:145-152

Richburg JW, Wilcut JW, Wiley GR (1995) AC 263,222 and imazethapyr rates and mixtures for weed management in peanut (*Arachis hypogaea*). Weed Technol 9:801-806

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

Ryck SD, Reheul D, Cauwer BD (2020) Impacts of herbicide sequences and vertical tuber distribution on the chemical control of yellow nutsedge (*Cyperus esculentus* L.) Weed Res 61:454-464

Seefeldt SS, Jensen JE, Feurst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:218–227

Stoller EW, Sweet RD (1987) Biology and life cycle of purple and yellow nutsedge (*Cyperus rotundus and Cyperus esculentus*). Weed Technol 1:66-73

Tehranchian P, Norswaorhty JK, Bagathiannan MV, Riar DS (2015) Acetolactate synthase-inhibitor resistance in yellow nutsedge (*Cyperus esculentus)*: II-physiognomy and photoperiodic response. Weed Sci 63:819-827

Tehranchian P, Norsworthy JK, Nandula V, McElroy S, Chen S, Scott RC (2014) First report of resistance to acetolactate- synthase-inhibiting herbicides in yellow nutsedge (*Cyperus esculentus*) confirmation and characterization. Pest Manag Sci 71:1274-1280

Van Wychen L (2022) 2022 Survey of the Most Common and Troublesome Weeds in Broadleaf Crops, Fruits & Vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. Available: http://wssa.net/wp-content/uploads/2022 Weed-Survey Broadleaf crops.xlsx

[USDA] United State Department of Agriculture (2022) 2022 State Agriculture Overview-Georgia https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=GEORGIA. Accessed April 24, 2023

[USDA-NASS] US Department of Agriculture (2024) Crop Production 2023 Summary. National Agricultural Statistics Service. Washington, DC. 117 p

[USDA-NRCS] US Department of Agriculture (2021) The PLANTS Database (https://plants.usda.gov, 6 April 2023). National Plant Data Team, Greensboro, NC 27401-4901 USA

[UGA] University of Georgia Extension (2024) UGA Peanut Production – the 2024 pest management quick reference guide. Athens, GA: University of Georgia, AP-118

Willis GD, Hoagland RE, Paul RN (1980) Anatomy of yellow nutsedge (*Cyperus esculentus*). Weed Sci 28:432-437

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

Table 1. Herbicide dose of imazapic and halosulfuron-methyl to determine I_{50} and GR_{50} of suspected ALS resistant yellow nutsedge (*Cyperus esculentus*).

Res biotype[a]		*Sus* biotype[b]	
imazapic[c]	halosulfuron-methyl[d]	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] suspected resistant yellow nutsedge biotype from Webster County, GA

[b] known susceptible yellow nutsedge biotype from Azlin Seed

[c] suspected ALS herbicide that *C. esculentus* biotype is resistant to

[d] suspected ALS herbicide that *C. esculentus* biotype is not resistant to

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

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	Asp343Glu
593	1	AAT	GAT	Asn593Asp
616	1	ACA	TCA	Thr616Ser

[a] Amino acid position is based on ALS of *Arabidopsis thaliana*

[b] Single nucleotide polymorphism (SNP) number is base number on the codon.

[c] Major allele is the one with more read count in susceptible and resistance transcriptome data.

[d] Minor allele is the one with less read count in susceptible and resistance transcriptome data.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

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 [d]
177	3	T	C	0.22	na
205	2	C	T	0.34	Ala205Val

[a] Amino acid position is based on ALS of *Arabidopsis thaliana*

[b] Single nucleotide polymorphism (SNP) number is base number on the codon

[c] Freq is the number of mapped reads carrying the polymorphic nucleotide divided by total number of mapped reads is carrying both alleles.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

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

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

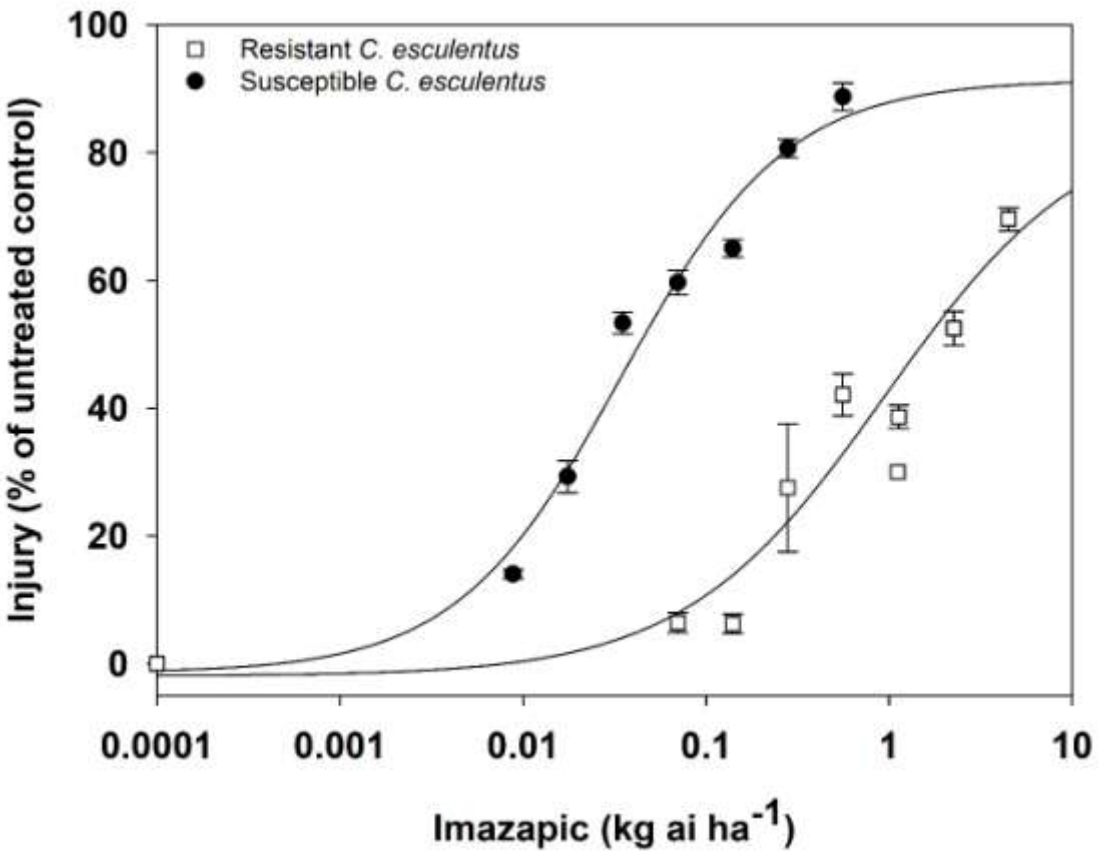

Figure 2. Injury of suspected resistant and known susceptible *C. esculentus* to applications of imazapic.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

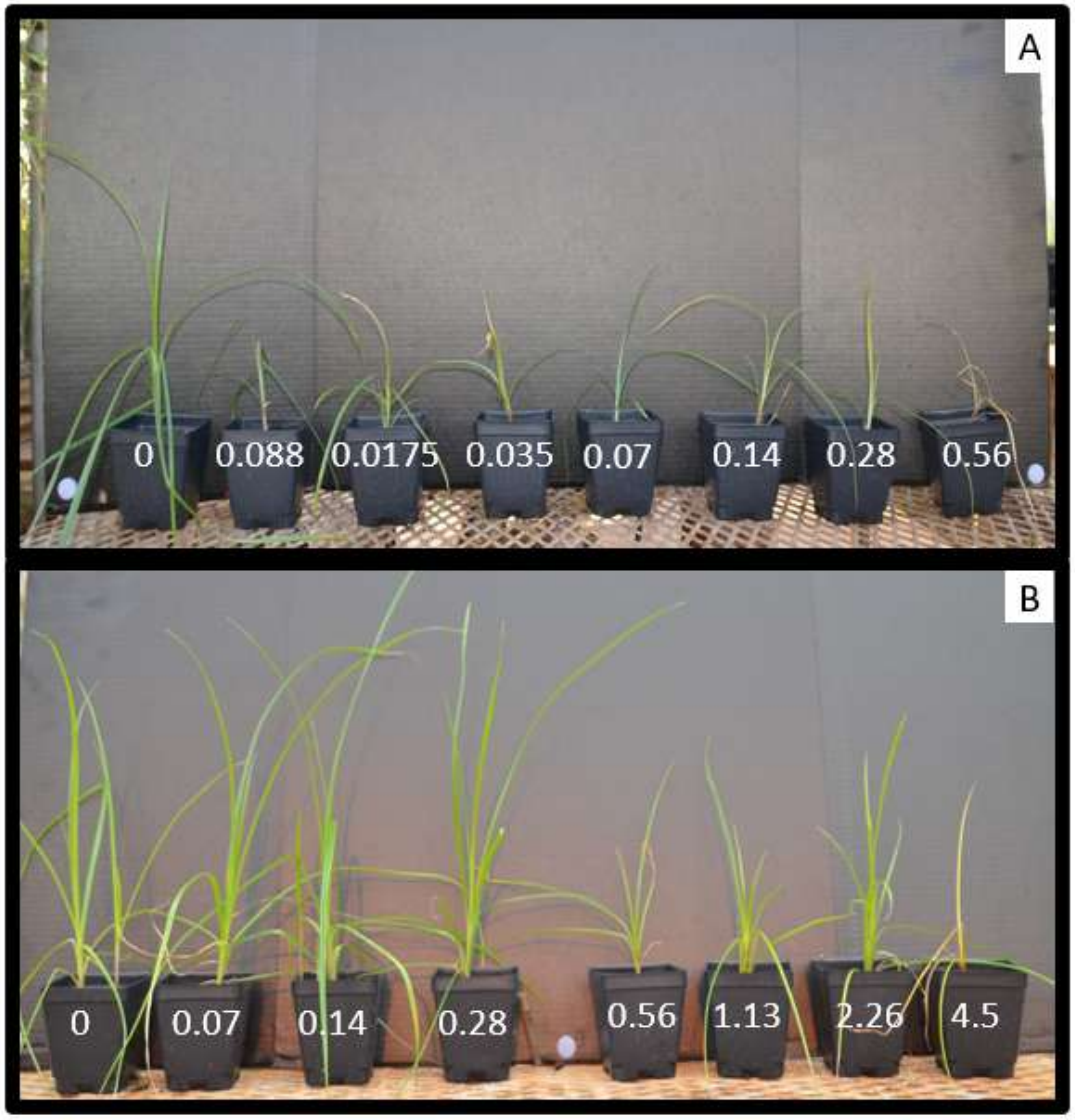

Figure 3. Response of known susceptible *C. esculentus* (Panel A) and suspected resistant *C. esculentus* (28 DAT) to applications of imazapic 28 DAT (Panel B). Numbers shown indicate rate of imazapic (kg ai ha^{-1}).

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

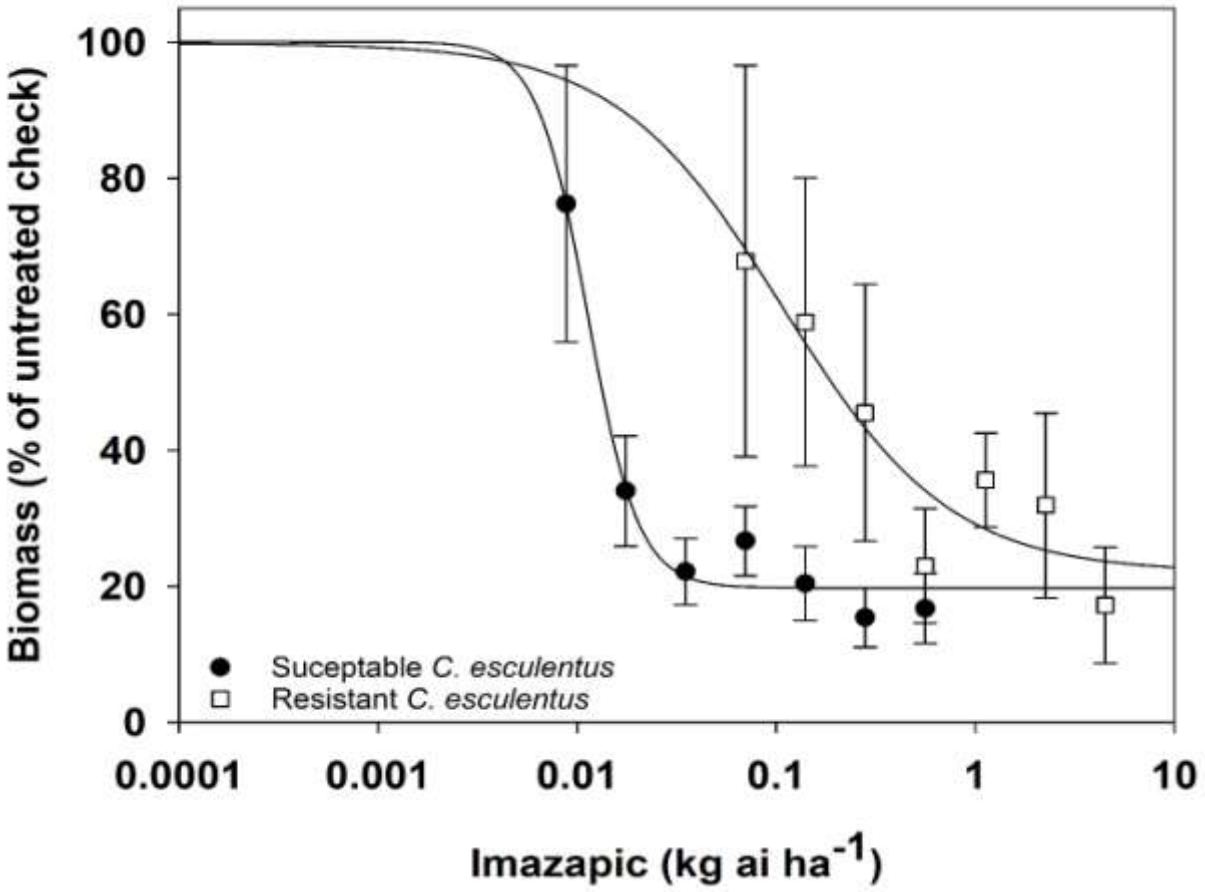

Figure 4. Biomass response of suspected resistant and known susceptible *C. esculentus* to applications of imazapic.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

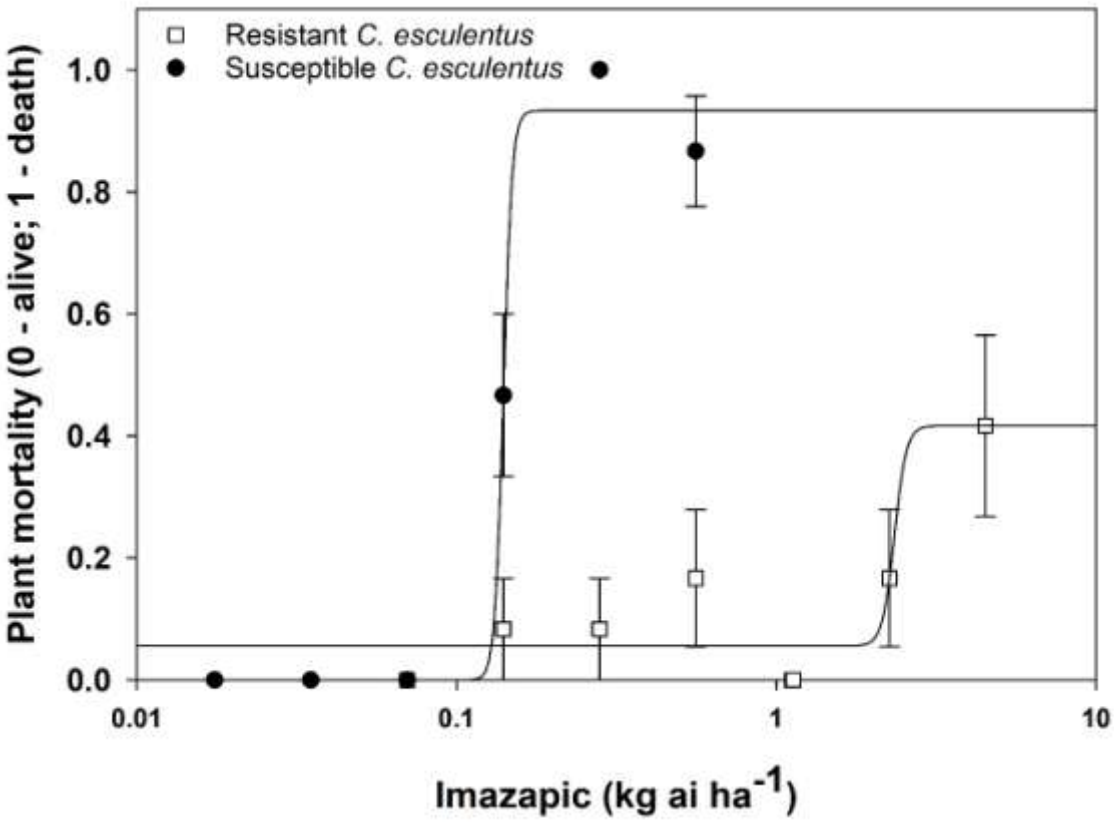

Figure 5. Mortality response of suspected resistant and known susceptible *C. esculentus* to applications of imazapic.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

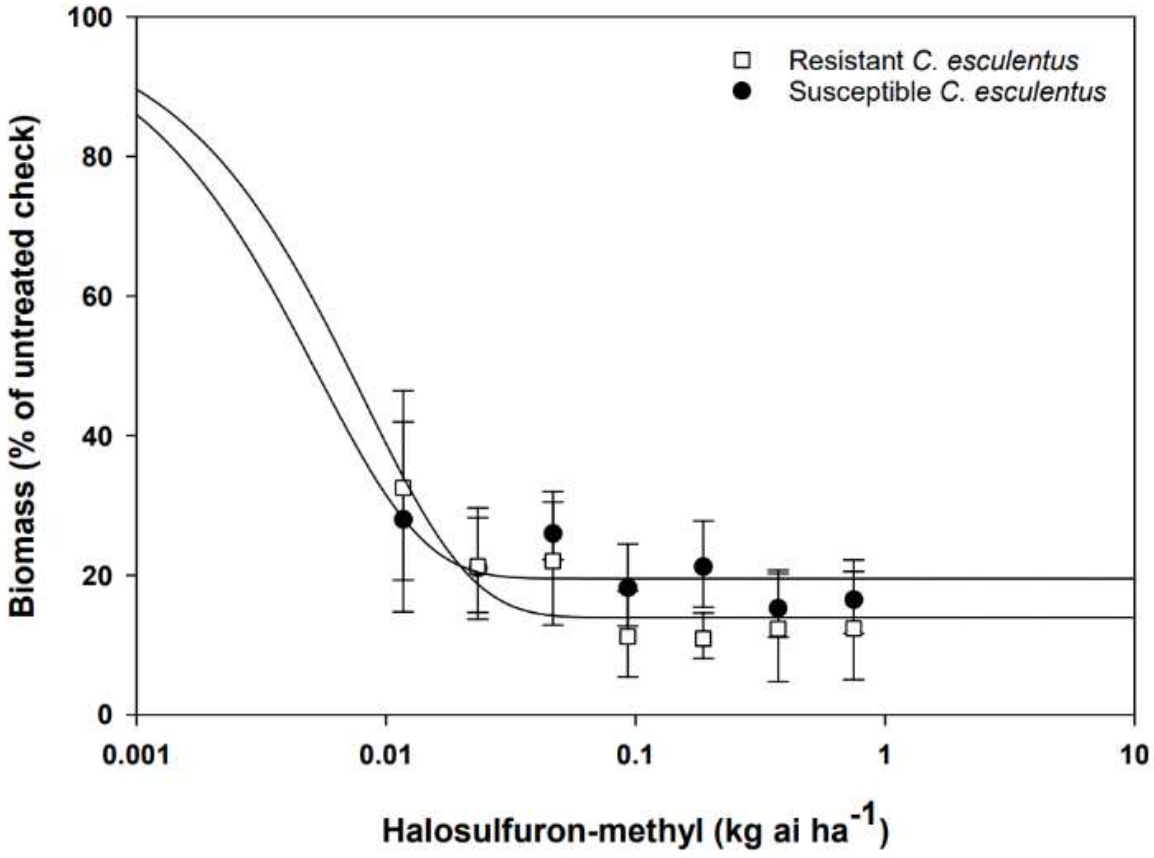

Figure 6. Biomass response of suspected resistant and known susceptible *C. esculentus* to applications of halosulfuron-methyl.

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press

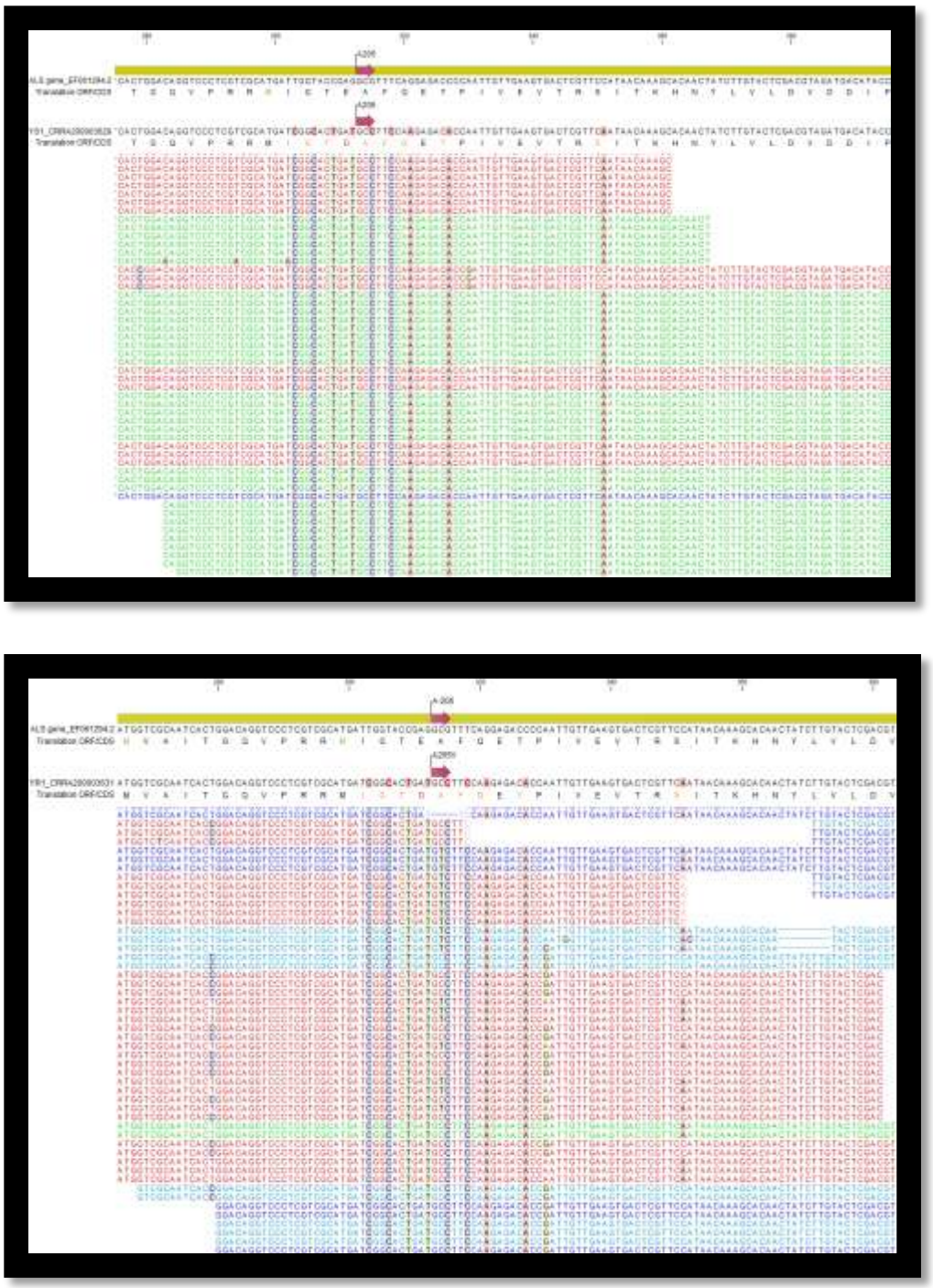

Figure 7. Alignment of Illumina sequencing reads from Susceptible (Sus) biotype (Panel A) and Resistant (Res) biotype (Panel B) of *C. esculentus* to ALS gene from *Cyperus difformis* (GenBank accession number: EF061294.2) focusing on target site mutation Ala205Val. The horizontal line color codes highlight mismatches in the nucleotide sequences of mapped reads compared to the reference sequence: red for Adenine (A), blue for Cytosine (C), yellow for Guanine (G), and green for Thymine (T).

https://doi.org/10.1017/wsc.2024.87 Published online by Cambridge University Press