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Agricultural Science and Production

B.S. in Agricultural Sciences

Special Graduation Project

The response of lettuce (*Lactuca sativa* L.) accessions to postemergence herbicides

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Abstract

This study evaluated the phytotoxic response of thirteen lettuce accessions, including commercial cultivars and experimental breeding lines from the University of Florida's Breeding Program (UF/IFAS), to twelve postemergence (POST) herbicides under controlled greenhouse conditions. The experiment was conducted at the Everglades Research and Education Center (EREC), University of Florida, using a completely randomized design with a factorial arrangement. Visual injury ratings and relative dry biomass were assessed 28 days after treatment. Significant differences were detected among genotypes, herbicides, and the genotype × herbicide interaction. The herbicides fomesafen, glufosinate, glyphosate, linuron, mesotrione, prometryn, and topramezone caused severe injury across all genotypes, indicating a lack of selectivity. In contrast, acetolactate synthase (ALS) inhibitors, particularly flumetsulam, resulted in minimal damage in several genotypes. Principal component analysis (PCA) identified distinct sensitivity groupings, highlighting Batavia Reine de Glaces, PI 491224, 10221, 49017, and H1098 as promising candidates for breeding programs due to their tolerance profiles. Overall, these findings provide valuable insights for the development of herbicide-tolerant lettuce cultivars and support sustainable weed management strategies.

Keywords: Leaf vegetables, phytotoxicity, plant breeding, tolerance, weed control.

Resumen

Este estudio evaluó la respuesta fitotóxica de trece accesiones de lechuga, que incluyeron cultivares comerciales y líneas experimentales del Programa de Mejoramiento Genético de la Universidad de Florida (UF/IFAS), frente a doce herbicidas postemergentes (POST) bajo condiciones controladas de invernadero. El experimento se llevó a cabo en el Everglades Research and Education Center (EREC) de la Universidad de Florida, utilizando un diseño completamente aleatorizado con arreglo factorial. A los 28 días después de la aplicación, se registraron porcentajes de daño visual y biomasa seca relativa. Los análisis revelaron diferencias significativas entre genotipo, herbicida y la interacción genotipo × herbicida. Los herbicidas fomesafen, glufosinate, glyphosate, linuron, mesotrione, prometryn y topramezone provocaron daños severos en todos los genotipos, lo que evidenció su falta de selectividad. En contraste, los inhibidores de la sintasa de acetolactato (ALS), particularmente flumetsulam, ocasionaron daños mínimos en varios genotipos. El análisis de componentes principales (PCA) permitió identificar agrupaciones de sensibilidad, destacando a Batavia Reine de Glaces, PI 491224, 10221, 49017 y H1098 como materiales prometedores para programas de mejoramiento debido a sus perfiles de tolerancia. En conjunto, los resultados aportan información relevante para el desarrollo de cultivares de lechuga tolerantes a herbicidas y respaldan estrategias de manejo sostenible de malezas

Palabras clave: Control de malezas, fitomejoramiento, fitotoxicidad, hortaliza de hoja, tolerancia.

Introduction

Lettuce (*Lactuca sativa* L.) is one of the most widely cultivated leafy vegetables worldwide, valued for its nutritional value, economic importance, and increasing consumer demand (Lal et al., 2024). In Florida, lettuce is a significant winter vegetable crop, with approximately 5,000 hectares in production and a farm gate value of $70 to $80 million annually (Sandoya & Lu, 2020).

The Everglades Agricultural Area (EAA), located just south of Lake Okeechobee in Palm Beach County, is a primary region for lettuce cultivation in Florida, characterized by organic-rich muck soils and a humid subtropical climate (Sandoya & Lu, 2020). These environmental conditions, while favorable for lettuce growth, also promote the proliferation of various weed species, leading to intense competition for resources (Odero & Wright, 2022). Common problematic weeds in the EAA include common lambsquarters (*Chenopodium album* L.), pigweed (*Amaranthus* spp.), common purslane (*Portulaca oleracea* L.), common ragweed (*Ambrosia artemisiifolia* L.), and American black nightshade (*Solanum americanum* Mill.) (Odero & Wright, 2022).

Effective weed control is a critical challenge in lettuce production, particularly in muck soils like those in the EAA, where mechanical weeding is labor-intensive, and herbicide options are limited due to crop sensitivity and the rotation with sugarcane, a monocot species unrelated to lettuce (Dusky et al., 1988). The delicate morphology, shallow root system, and slow early growth of lettuce make it particularly vulnerable to weed competition, which can significantly reduce yield and marketability (Dusky et al., 1995).

To address these challenges, a range of postemergence (POST) herbicides with diverse modes of action have been considered for weed management in lettuce cultivation. These include flumetsulam (Python®), imazamox (Raptor®), imazapyr (Cadre®), imazethapyr (Pursuit®), and rimsulfuron (Matrix®), which inhibit branched-chain amino acid synthesis and are known to suppress many broadleaf weeds in the region (Tan et al., 2005; U.S. Environmental Protection Agency [EPA], 2013). Photosystem II inhibitors such as linuron (Linex®) and prometryn (Caparol®), still used in vegetable production despite regulatory restrictions, were included for their relevance to weed control in organic-rich soils but are associated with crop bleaching and persistence concerns (EPA, 1995, 2002). Glyphosate (Roundup®) and glufosinate (Liberty®), which inhibit 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and glutamine synthetase, respectively, are chosen for their broad-spectrum use in and around crop fields, but they pose known risks of injury in lettuce (Minnesota Department of Agriculture [MDA], n.d.–b; Nagata et al., 1992). Finally, protoporphyrinogen oxidase (PPO) inhibitor fomesafen (Reflex®) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors mesotrione (Callisto®) and topramezone (Armezon®) were included for their postemergence activity on small-seeded broadleaf weeds like *Amaranthus spp*., which are problematic in South Florida (Boeri et al., 2021; MDA, n.d.–a; EPA, 2009a, 2009b).

These herbicides were selected based on their efficacy against the dominant weed species of the region and for their chemical diversity and potential to reveal differential tolerance among lettuce genotypes. Understanding how lettuce germplasm respond to these herbicides can help identify tolerant accessions and reduce reliance on trial-and-error approaches in commercial production systems (Leon & Tillman, 2015).

Herbicide injury in lettuce, including symptoms such as chlorosis, necrosis, stunting, and yield loss, has been widely reported when herbicides are misapplied or when varietal sensitivity is not considered (Dusky et al., 1988; Tan et al., 2005; Umeda, 2000). The risk is further exacerbated by the variability among POST herbicides in terms of selectivity, residual activity, and potential for crop injury. Despite this, comprehensive evaluations of herbicide effects across diverse lettuce germplasm remain limited (Leon & Tillman, 2015; Mou, 2011; Nagata et al., 1992). This knowledge gap is especially critical for breeding programs, where herbicide sensitivity can lead to the late-stage rejection of otherwise promising cultivars (Lusser et al., 2012; Prohens et al., 2008).

Research in other crops has demonstrated significant variation in herbicide tolerance among genotypes, highlighting the role of non–target site resistance mechanisms, such as enhanced metabolism or reduced translocation (Hanson et al., 2014; Leon & Tillman, 2015). Moreover, recent work by (Belisle et al., 2024) underscores the importance of evaluating lettuce accessions under stress conditions, such as heat tolerance and postharvest quality, further demonstrating the genetic diversity that can be leveraged in varietal development. These findings support the need to explore genotype performance under chemical stress conditions, such as herbicide exposure, in subtropical environments like the EAA.

Given this complexity, there is a critical need to evaluate how genetically distinct lettuce lines respond to these herbicides, both individually and collectively. It is hypothesized that sufficient variation exists among lettuce accessions to identify lines with broad-spectrum tolerance to multiple POST herbicide modes of action. Selecting such lines would not only enable the development of herbicide-tolerant cultivars but also enhance the efficiency of breeding programs and inform safer, more targeted herbicide use in commercial systems.

This study addresses the screening of thirteen lettuce accessions representing both commercial and experimental lines for their phytotoxic responses to twelve POST herbicides under greenhouse conditions. By characterizing differential herbicide tolerance, the study aims to identify high-performing lines with minimal injury symptoms and consistent tolerance profiles. The goal is to generate foundational data to support cultivar development, reduce crop loss risk, and improve the integration of chemical weed control strategies in sustainable lettuce production systems.

Materials and Methods

Study Location

Two experimental runs were conducted at the University of Florida’s Everglades Research and Education Center (EREC), located in Belle Glade, Florida (Lattitude 26°40'1.77"N and longitude 80°37'54.32"W). The first run was initiated on November 15, 2024, and the second conducted on December 23, 2024. Greenhouse conditions were maintained with a maximum daytime temperature of 30 °C and nighttime temperature of 20 °C. Relative humidity averaged approximately 60% and all trials were conducted under natural light. Thirteen lettuce genotypes including breeding lines from the UF/IFAS Breeding Program and commercial cultivars were evaluated in this experiment (Table 1).

Table 1

Lettuce genotypes evaluated for tolerance to different herbicides at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

Germplasm	Status	Type	Area of adaptation
60150	Breeding line	Crisphead	Florida
H1098	Breeding line	Crisphead	Florida
Batavia Reine des Glaces	Cultivar	Batavia	France
Cooper	Cultivar	Crisphead	Florida
49017	Breeding line	Crisphead	Florida
49019	Breeding line	Crisphead	Florida
10207	Breeding line	Romaine	Florida
10221	Breeding line	Romaine	Florida
60182	Breeding line	Romaine	Florida
60183	Breeding line	Romaine	Florida
45060	Breeding line	Latin	Florida
Floribibb	Cultivar	Latin/Bibb	Florida
PI 491224	Plant introduction	Romaine	Greece

Experimental Setup

Three seeds from each lettuce genotype were directly sown into 108 mm diameter and 105 mm deep pots filled with a commercial potting medium (Sun Gro® Professional Growing Mix, Sun Gro Horticulture, Agawam, MA, USA). A slow-release 14-14-14 fertilizer (Osmocote®; The Scotts Company, Marysville, OH, USA) was incorporated into the potting mix at rates of 140 g N kg^{-1}, 61 g P kg^{-1}, and 116 g K kg^{-1}. All pots were arranged on elevated greenhouse benches to ensure uniform exposure to light and ventilation (appendix A). Plants were irrigated daily, and seedlings typically emerged three to four days after planting. At 10 days after emergence, seedlings were thinned to one plant per pot, selecting individuals of uniform size across all genotypes.

Herbicide Treatments

Thirteen POST herbicides representing six different modes of action were included in the study (Table 2). Among them, imazethapyr was the only herbicide currently labeled for use in lettuce. Application rates were based on labeled recommendations for other crops. The selected herbicides were chosen for their chemical diversity and relevance to weed management in the EAA cropping systems. A non-treated control was included for each genotype within each herbicide treatment to allow for comparison.

Herbicide applications were made at the four-leaf stage of lettuce development, approximately 15 days after emergence, to simulate typical field application timing. Treatments were applied using a CO_2-pressurized moving-nozzle spray chamber (Generation II Spray Booth, Devries Manufacturing, Hollandale, MN) (appendix B) equipped with a TeeJet® 8002E nozzle tip (Spraying Systems, Wheaton, IL), calibrated to deliver 187 L ha^{-1} at 172 kPa.

The experiment followed a completely randomized design with a two-way factorial arrangement. The two factors were genotype (13 lettuce genotypes) and herbicide treatment (12 POST herbicides and untreated control). Each treatment combination was replicated four times. A total of 52 plants were evaluated for each lettuce genotype, distributed across thirteen herbicide treatments. This resulted in 676 experimental units (13 genotypes × 13 treatments × 4 replications), as illustrated in Figure 1.

Figure 1

Experimental design (completely randomized design with a factorial arrangement) used greenhouse experiments to evaluate the response of lettuce genotypes to POST herbicides at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

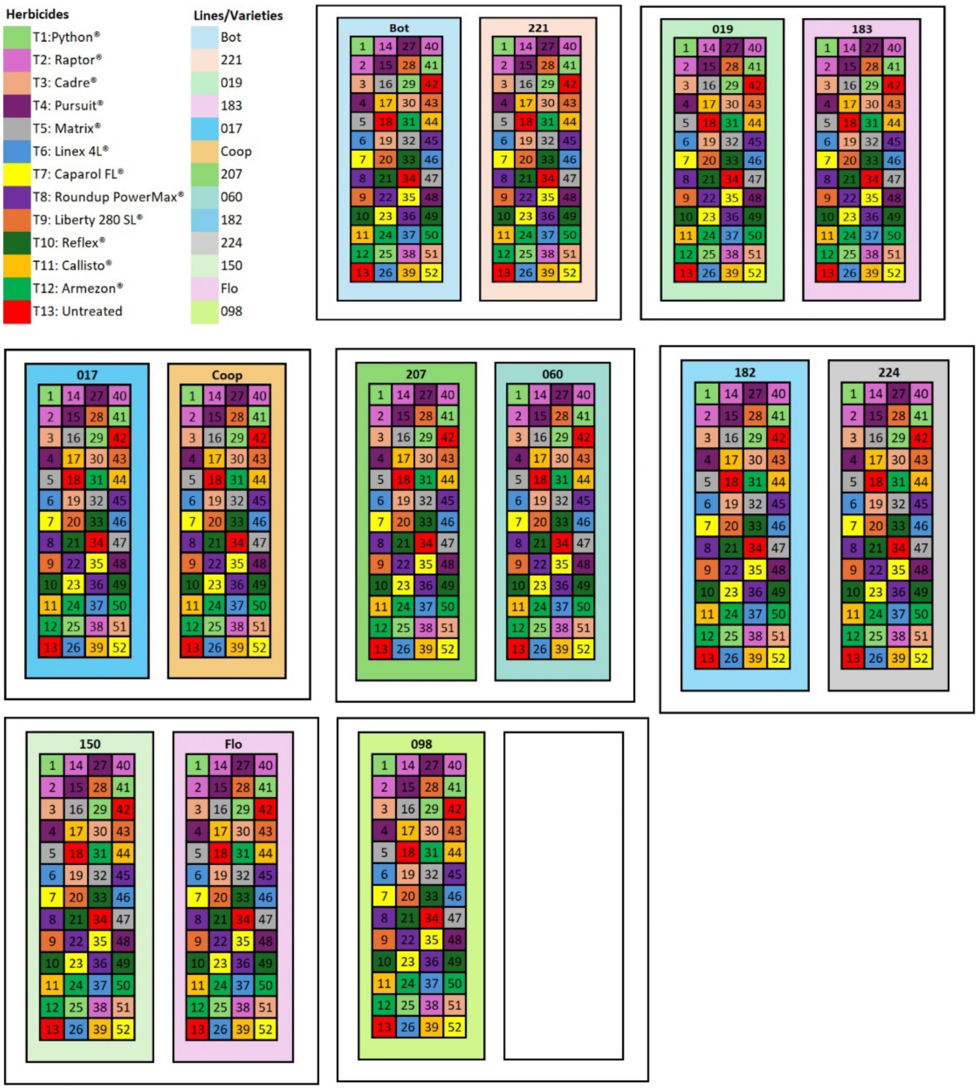


Table 2

Herbicides, modes of action, rates, and production information used in the herbicide tolerance screening of lettuce germplasm at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

Herbicide		HRAC Code[a]	Rate g ai ha^{-1}	Manufacturer
Common name	Tradename			
Flumetsulam	Python®	2	56	AMVAC Chemical Corporation, Newport Beach, CA, USA
Imazamox	Raptor®	2	35	BASF, Research Triangle Park, NC, USA
Imazapic	Cadre®	2	70	BASF, Research Triangle Park, NC, USA
Imazethapyr	Pursuit®	2	35	BASF, Research Triangle Park, NC, USA
Rimsulfuron	DuPont™ Matrix® SG	2	17.5	DuPont, Wilmington, DE, USA
Prometryn	Caparol® 4L	5	1120	Syngenta Crop Protection, Greensboro, NC, USA
Linuron	Linex® 4L	5	560	Tessenderlo Kerley, Inc., Phoenix, AZ, USA
Glyphosate	Roundup PowerMax® II	9	840	Bayer Crop Science, St. Lois, MO, USA
Glufosinate	Liberty®	10	450	Bayer Crop Science, St. Lois, MO, USA
Fomesafen	Reflex®	14	280	Syngenta Crop Protection, Greensboro, NC, USA
Mesotrione	Callisto®	27	105	Syngenta Crop Protection, Greensboro, NC, USA
Topramezone	Armezon®	27	25	BASF, Research Triangle Park, NC, USA

Note. [a](Herbicide Resistant Action Committee [HRAC], 2024), Group 2 = inhibition of acetolactate synthase; Group 5 = inhibition of photosynthesis II; Group 9 = inhibition of enolpyruvyl shikimate phosphate synthase; Group 10 = inhibition of glutamine synthetase; Group 14 = inhibition of protoporphyrinogen oxidase; Group 27 = inhibition of hydroxyphenyl pyruvate dioxygenase.

Data Collection

Lettuce injury was visually assessed 28 days after treatment (DAT) using a standardized percentage-based scale from 0% to 100%, where 0% indicated no visual injury and 100% represented complete plant death. Evaluations focused on common phytotoxic symptoms, including chlorosis, necrosis, stunting, or bleaching (appendix C).

The visual injury scale (Table 3) was developed based on the method described by the (Canadian Weed Science Society [CWSS], 2018) for assessing evaluation of crop tolerance (phytotoxicity). This method recommends percentage-based estimations of visible damage such as chlorosis, necrosis, or stunting, relative to untreated control plants.

Following visual evaluations, plants were harvested at the soil level at 28 DAT, and the aboveground biomass was oven-dried at 60 °C for 96 hours. After drying, the biomass was weighed to determine dry weight (appendix D), which provided a quantitative measure of herbicide effects on lettuce growth in addition to the visual symptom ratings. To control variability in the biomass, data values were converted to a percentage growth relative to the untreated control in each run for each genotype using Equation [1]:

$$RB = \frac{(DW_t)}{(DW_{nc})} \times 100 \qquad [1]$$

where RB is relative biomass, DW_t is the dry weight of treated genotype, and DW_{nc} is the dry weight of the nontreated genotype control.

Table 3

Visual rating scale for herbicide injury for lettuce accessions at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

% Injury	Description
0	No visible injury.
10–20	Very slight discoloration on few leaves.
30–40	Slight chlorosis or minor bleaching; minimal effect on plant vigor.
50–60	Clear chlorosis or bleaching; plant growth slightly affected.
70–80	Noticeable chlorosis, bleaching and/or necrosis; signs of stunting.
90	Severe bleaching, chlorosis, or necrosis; noticeable stunting; major portions of the plant visibly injured.
100	Complete plant death.

Statistical Analysis

Lettuce injury and relative biomass data were analyzed using ANOVA within a mixed-effects modeling framework, implemented in the LME4 package (Bates et al., 2022) in R (R Development Core Team, 2024). The *lmer* function was used to fit the model, with lettuce genotype, herbicide treatment, and their interaction specified as fixed effects. Experimental run and replication nested within run were treated as random effects. Where significant effects were detected, estimated marginal means were calculated, and pairwise comparisons were conducted using Tukey’s post hoc test (α = 0.05) using the EMMEANS package (Lenth et al., 2025). To visualize the injury or relative biomass patterns, a heatmap was generated in R using the mean injury and relative biomass scores. Genotypes were displayed on the y-axis and herbicides on the x-axis. Color intensity represented the magnitude of injury or relative biomass, with darker shades indicating greater injury or relative biomass. This visualization facilitated the identification of genotype-specific sensitivity and herbicide selectivity.

To explore genotype clustering based on herbicide response, two principal component analyses (PCA) were performed using the singular value decomposition method (*prcomp* function in R). Visualization of PCA results was carried out using GGPLOT and GGREPEL packages (Slowikowski, 2024; Wickham, 2016).

Results and Discussion

Lettuce Injury

ANOVA results showed a significant genotype, herbicide, and genotype x herbicide interaction for lettuce injury at 28 DAT (Table 4). Therefore, the injury is presented by herbicide and genotype.

Table 4

ANOVA results for herbicide, lettuce genotype, and their interactions on lettuce injury and relative dry biomass accumulation 28 days after treatment in greenhouse experiments at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

Source of variation	Injury	Relative dry biomass
Genotype	0.0325*	<0.0001***
Herbicide	<0.0001***	<0.0001***
Genotype* Herbicide	0.0062**	<0.0001***

Note. [a]Asterisks indicate probability levels: *Significant at P = 0.05; **significant at P = 0.001; ***significant at P < 0.0001.

The most injurious herbicides on lettuce were fomesafen, glufosinate, glyphosate, linuron, mesotrione, prometryn, and topramezone, all of which caused 84% to 100% injury 28 DAT (Table 5; Figure 2). Among these, fomesafen, a contact PPO inhibitor, resulted in 100% injury across all lettuce genotypes, making it the most damaging herbicide evaluated. The remaining herbicides in this group linuron and prometryn (photosystem II inhibitors), glyphosate (EPSPS inhibitor), glufosinate (glutamine synthetase inhibitor), mesotrione and topramezone (HPPD inhibitors) also caused consistently high levels of injury (89% to 100%) across genotypes, indicating a lack of crop selectivity and broad phytotoxicity.

In contrast, herbicides targeting ALS specifically flumetsulam, imazamox, imazapic, and imazethapyr were generally the least injurious, except for rimsulfuron, which caused 72% to 98% injury, indicating its limited safety for lettuce. Flumetsulam caused relatively minor injuries, ranging from 4% to 29%. The least injury from flumetsulam was observed in Batavia Reine des Glaces (1%), 10221 (3%), and PI 491224 (4%), representing a cultivar, breeding line, and plant introduction, respectively, all of which belong to romaine or Batavia lettuce types. In contrast, the highest injury from flumetsulam occurred in cultivar Cooper (29%) and breeding line 45060 (25%), indicating variability in genotype response. Injury from other ALS herbicides ranged from 9% to 45% for imazethapyr, 35% to 68% for imazamox, and 26% to 54% for imazapic, suggesting that while generally less phytotoxic, their safety varies significantly among genotypes.

Principal component analysis (PCA) of the genotype-by-herbicide injury matrix revealed that the first two principal components (PC1 and PC2) together accounted for approximately 45% of the total variance, with PC1 explaining 26.4% (eigenvalue = 3.16) and PC2 explaining 18.4% (eigenvalue = 2.20) (Figure 3). Genotypes Batavia Reine de Glaces, PI 491224, and 49017, located in the top right quadrant (PC1+, PC2+), showed a positive association with glyphosate and rimsulfuron, indicating increased sensitivity to these herbicides. Genotypes Cooper, 10207, and 60182, located in the bottom left quadrant (PC1−, PC2−), aligned with imazapic, imazethapyr, imazamox, glufosinate, and topramezone, suggesting heightened sensitivity, particularly to glufosinate and topramezone, which had stronger loading weights than the ALS herbicides. Breeding line 49019, located in the top left quadrant (PC1−, PC2+), was most closely associated with linuron and mesotrione, indicating a distinct injury profile separate from that of ALS inhibiting herbicides. Fomesafen, positioned in the bottom right quadrant (PC1+, PC2−), exhibited a strong and unique influence across genotypes, driving the most severe injury overall. The close alignment of 'Floribibb' with the fomesafen vector further highlights its exceptional sensitivity, distinguishing it clearly from all other genotypes. The observed strong directional separation of the different herbicides reinforces the central role of herbicide mode of action in shaping lettuce injury profiles.

The lettuce injury results underscore the variable sensitivity of lettuce genotypes to herbicides with differing modes of action. The pronounced injury caused by fomesafen, glufosinate, glyphosate, linuron, mesotrione, prometryn, and topramezone indicates a lack of selectivity, rendering these herbicides unsuitable for commercial lettuce production. Notably, the uniform and severe injury from fomesafen across all genotypes emphasizes the high risk associated with PPO inhibitors in lettuce systems. The ALS inhibitors exhibited the most selective injury profiles. Flumetsulam caused minimal injury across most genotypes (1% to as high as 29%), suggesting its potential for selective breeding or screening of lettuce lines for enhanced tolerance to ALS inhibiting herbicides.

Table 5

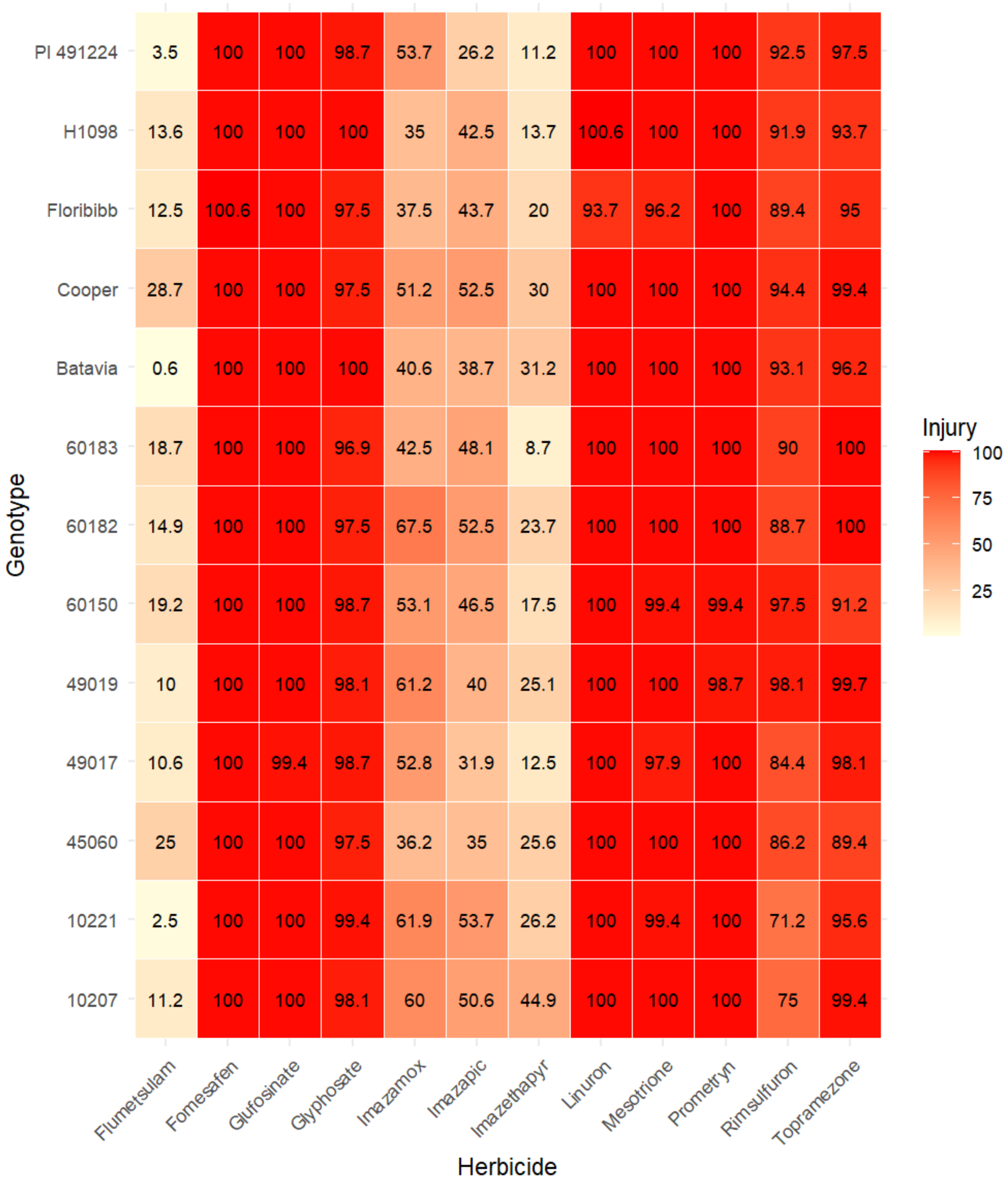
Lettuce genotype injury (percent) 28 days after treatment in response to different herbicides at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

Herbicide	Genotype												
	PI 491224	H1098	Floribibb	Cooper	Batavia	60183	60182	60150	49019	49017	45060	10221	10207
Flumetsulam	4 abA	14 abA	13 abA	29 bA	1 aA	19 abAB	15 abA	19 abA	10 abA	11 abA	25 abA	3 aA	11 ab A
Fomesafen	100 aC	100 aC	100 aC	100 aB	100 aC	100 aD	100 aD	100 aC	100 aD	100 aC	100 aB	100 aD	100 aD
Glufosinate	100 aC	100 aC	100 aC	100 aB	100 aC	100 aD	100 aD	100 aC	100 aD	99 aC	100 aB	100 aD	100 aD
Glyphosate	99 aC	100 aC	98 aC	98 aB	100 aC	97 aD	98 aD	99 aC	98 aD	99 aC	98 aB	99 aD	98 aD
Imazamox	54 a-dB	35 aAB	38 abcAB	51 a-dA	41 abcB	43 a-dBC	68 dBC	53 a-dB	61 bcdC	53 a-dB	36 ab	62 cdB	60 a-d AB
Imazapic	26 aA	43 abB	44 abB	53 bA	39 abB	48 abC	53 bB	46 abB	40 abBC	32 abAB	35 abA	54 bB	51 abBC
Imazethapyr	11 aA	14 aA	20 abAB	30 abA	31 abB	9 aA	24 abA	18 aA	25 abAB	13 aA	26 abA	26 abA	45 b B
Linuron	100 aC	100 aC	94 aC	100 aB	100 aC	100 aD	100 aD	100 aC	100 aD	100 aC	100 aB	100 aD	100 aD
Mesotrione	100 aC	100 aC	96 aC	100 aB	100 aC	100 aD	100 aD	99 aC	100 aD	98 aC	100 aB	99 aD	100 aD
Prometryn	100 aC	100 aC	100 aC	100 aB	100 aC	100 aD	100 aD	99 aC	99 aD	100 aC	100 aB	100 aD	100 aD
Rimsulfuron	93 abC	92 abC	89 abC	94 abB	93 abC	90 abD	89 abCD	98 bC	98 bD	84 aC	86 abB	71 aBC	75 a CD
Topramezone	98 aC	94 aC	95 aC	99 aB	96 aC	100 aD	100 aD	91 aC	100 aD	98 aC	89 aB	96 aCD	99 aD

Note. [a]Refer to Table 2 for herbicide rates and product information, [b]Means within a column followed by the same uppercase letters are not significantly different according to Tukey's test ($P < 0.05$), [c]Means within a row followed by the same lowercase letters are not significantly different according to Tukey's test ($P < 0.05$) and [d]Nontreated control data was not included in the analysis because there was no variance.

Figure 2

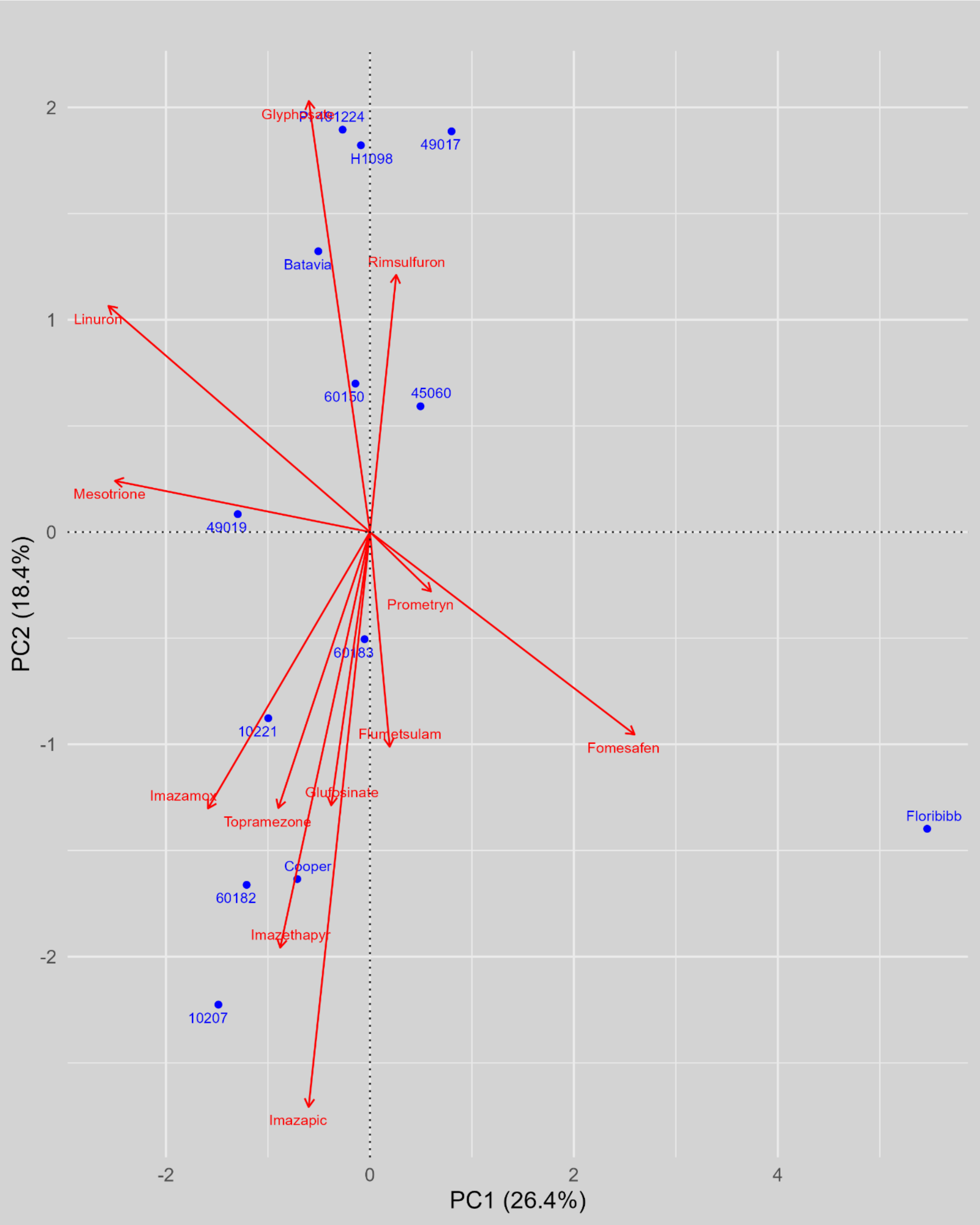
Heatmap showing lettuce genotype injury in response to different herbicides at 28 days after treatment at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.



Genotype	Flumetsulam	Fomesafen	Glufosinate	Glyphosate	Imazamox	Imazapic	Imazethapyr	Linuron	Mesotrione	Prometryn	Rimsulfuron	Topramezone
PI 491224	3.5	100	100	98.7	53.7	26.2	11.2	100	100	100	92.5	97.5
H1098	13.6	100	100	100	35	42.5	13.7	100.6	100	100	91.9	93.7
Floribibb	12.5	100.6	100	97.5	37.5	43.7	20	93.7	96.2	100	89.4	95
Cooper	28.7	100	100	97.5	51.2	52.5	30	100	100	100	94.4	99.4
Batavia	0.6	100	100	100	40.6	38.7	31.2	100	100	100	93.1	96.2
60183	18.7	100	100	96.9	42.5	48.1	8.7	100	100	100	90	100
60182	14.9	100	100	97.5	67.5	52.5	23.7	100	100	100	88.7	100
60150	19.2	100	100	98.7	53.1	46.5	17.5	100	99.4	99.4	97.5	91.2
49019	10	100	100	98.1	61.2	40	25.1	100	100	98.7	98.1	99.7
49017	10.6	100	99.4	98.7	52.8	31.9	12.5	100	97.9	100	84.4	98.1
45060	25	100	100	97.5	36.2	35	25.6	100	100	100	86.2	89.4
10221	2.5	100	100	99.4	61.9	53.7	26.2	100	99.4	100	71.2	95.6
10207	11.2	100	100	98.1	60	50.6	44.9	100	100	100	75	99.4

Figure 3

Principal component analysis (PCA) biplot illustrating injury of lettuce genotypes in response to herbicide treatments at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.



PCA was based on percent injury measured 28 days after treatment. Blue labels represent genotypes, and red arrows represent herbicide vectors, with arrow direction and length indicating the strength and orientation of each herbicide’s contribution to the principal components.

This finding is especially relevant for integrated weed management strategies, where use of herbicide-tolerant genotypes could facilitate the safe use of postemergence ALS inhibitors to broaden weed control options in lettuce.

Relative Biomass

ANOVA results showed a significant effect of genotype, herbicide, and genotype-by-herbicide interaction on lettuce relative biomass at 28 DAT (Table 4). Therefore, relative biomass data are presented by herbicide and genotype. The relative biomass of lettuce followed trends similar to those observed for injury. Overall, the most injurious herbicides including fomesafen, glufosinate, glyphosate, linuron, mesotrione, prometryn, and topramezone completely suppressed the growth of all genotypes (Table 6; Figure 4). This indicates a lack of crop selectivity and broad phytotoxicity across lettuce genotypes.

In contrast, herbicides targeting ALS specifically flumetsulam, imazamox, imazapic, and imazethapyr generally had less impact on relative biomass compared to herbicides with other modes of action. The relative biomass of lettuce treated with flumetsulam ranged from 33% to 98%, indicating variability in genotype tolerance. Breeding lines H1098, 10221, and 49017 maintained relative biomass values of 98%, 86%, and 83%, respectively, suggesting high levels of tolerance. For imazamox, imazapic, and imazethapyr, relative biomass ranged from 6% to 60%, 17% to 59%, and 15% to 83%, respectively. As with injury, rimsulfuron another ALS inhibitor, resulted in a greater biomass reduction compared to other ALS herbicides, highlighting its elevated phytotoxicity.

The PCA results revealed a clear separation of genotypes based on their relative biomass response to different herbicides. The first two principal components (PC1 and PC2) accounted for 55%

of the total variance, with PC1 explaining 31.1% (eigenvalue = 3.74) and PC2 explaining 24.0% (eigenvalue = 2.88) (Figure 5).

Table 6

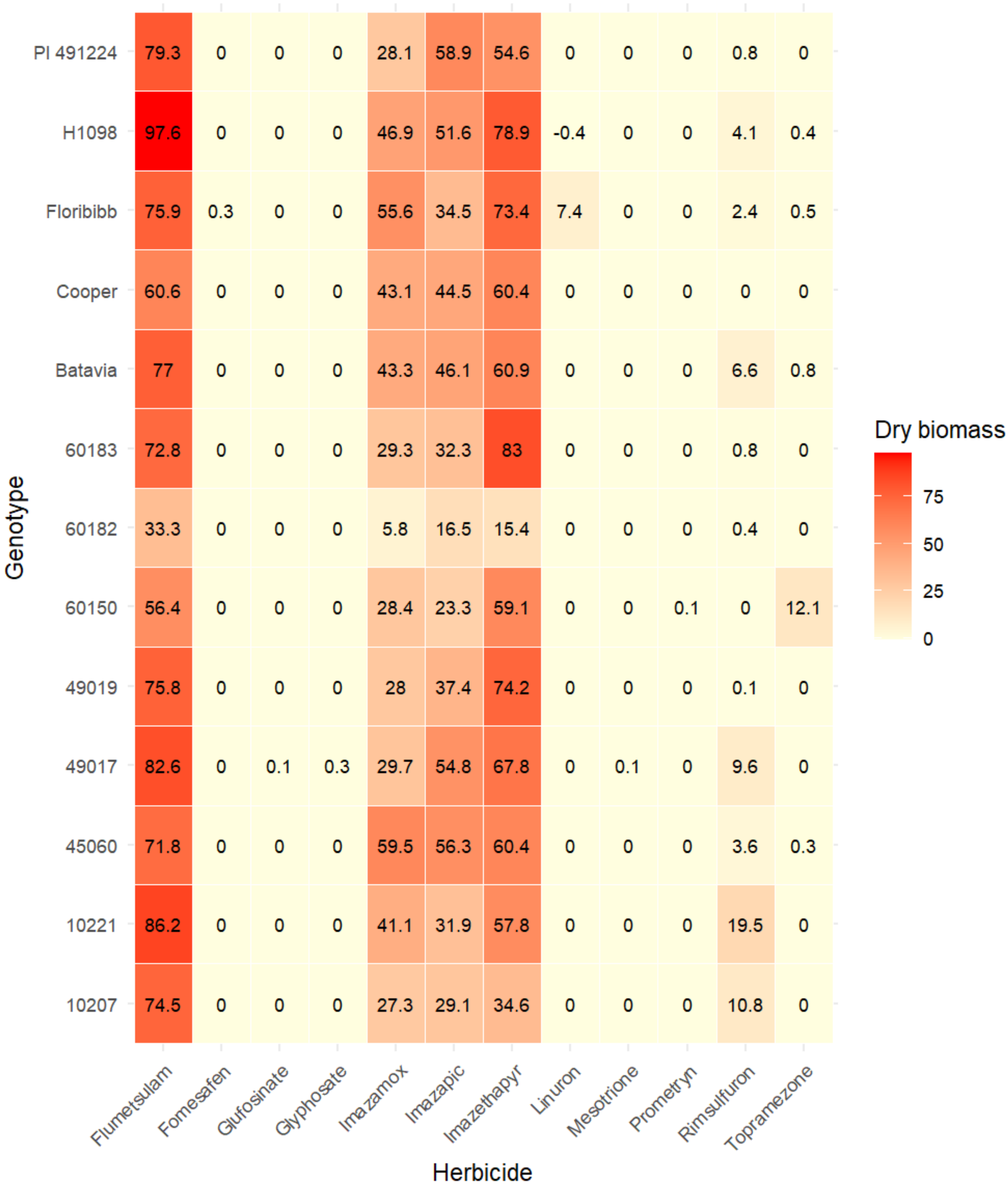
Lettuce genotype relative dry biomass (expressed as a percent of the untreated control) 28 days after treatment in response to different herbicides at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.

	Genotype												
Herbicide	PI 491224	H1098	Floribibb	Cooper	Batavia	60183	60182	60150	49019	49017	45060	10221	10207
Flumetsulam	79 bcC	98 cD	76 bcD	61 abB	77 bcC	73 bcC	33 aB	56 abBC	76 bcC	83 bcC	72 bcB	86 bcD	75 bcC
Fomesafen	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Glufosinate	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Glyphosate	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Imazamox	28 abAB	47 bcB	56 bcCD	43 bcB	43 bcB	29 abcAB	6 aAB	28 abAB	28 abAB	30 abcAB	60 cB	41 bcBC	27 abAB
Imazapic	59 cC	52 bcBC	35 abcBC	45 abcB	46 abcBC	32 abcB	17 aAB	23 abA	37 abcB	55 cBC	56 cB	32 abcBC	29 abcAB
Imazethapyr	55 bcBC	79 cCD	73 cD	60 bcB	61 bcBC	83 cC	15 aAB	59 bcC	74 cC	68 cC	60 bcB	58 bcCD	35 abB
Linuron	0 aA	0 aA	7 aAB	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Mesotrione	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Prometryn	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Rimsulfuron	1 aA	4 aA	2 aA	0 aA	7 aA	1 aA	0 aA	0 aA	0 aA	10 aA	4 aA	20 aAB	11 aAB
Topramezone	0 aA	0 aA	1 aA	0 aA	1 aA	0 aA	0 aA	12 aA	0 aA	0 aA	0 aA	0 aA	0 aA

Note. [a]Refer to Table 2 for herbicide rates and product information, [b]Means within a column followed by the same uppercase letters are not significantly different according to Tukey's test ($P < 0.05$), [c]Means within a row followed by the same lowercase letters are not significantly different according to Tukey's test ($P < 0.05$) and [d]Nontreated control data was not included in the analysis because there was no variance.

Figure 4

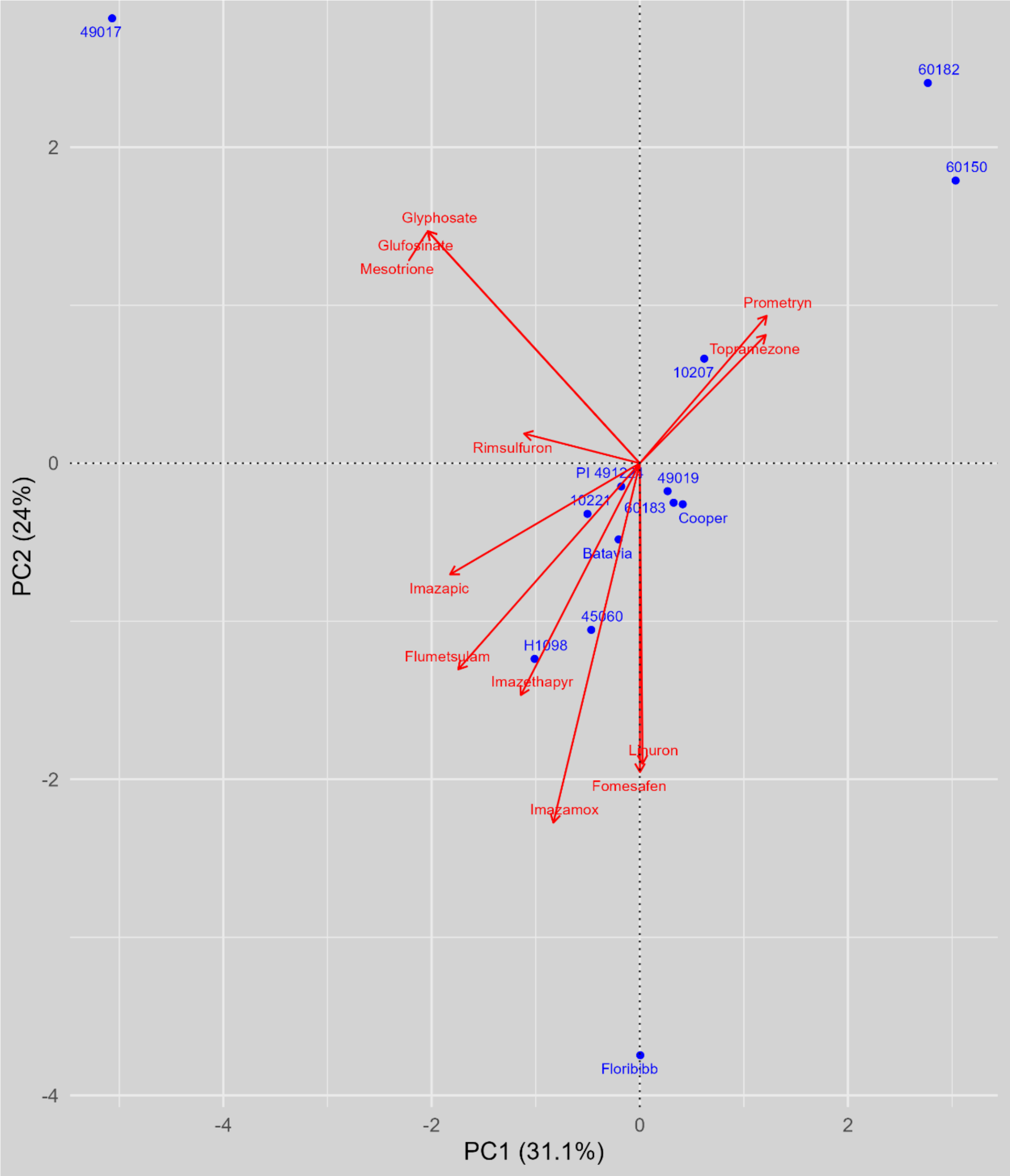
Heatmap showing lettuce genotype relative dry biomass (expressed as a percent of the untreated control) in response to different herbicides at 28 days after treatment at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.



Genotype	Flumetsulam	Fomesafen	Glufosinate	Glyphosate	Imazamox	Imazapic	Imazethapyr	Linuron	Mesotrione	Prometryn	Rimsulfuron	Topramezone
PI 491224	79.3	0	0	0	28.1	58.9	54.6	0	0	0	0.8	0
H1098	97.6	0	0	0	46.9	51.6	78.9	-0.4	0	0	4.1	0.4
Floribibb	75.9	0.3	0	0	55.6	34.5	73.4	7.4	0	0	2.4	0.5
Cooper	60.6	0	0	0	43.1	44.5	60.4	0	0	0	0	0
Batavia	77	0	0	0	43.3	46.1	60.9	0	0	0	6.6	0.8
60183	72.8	0	0	0	29.3	32.3	83	0	0	0	0.8	0
60182	33.3	0	0	0	5.8	16.5	15.4	0	0	0	0.4	0
60150	56.4	0	0	0	28.4	23.3	59.1	0	0	0.1	0	12.1
49019	75.8	0	0	0	28	37.4	74.2	0	0	0	0.1	0
49017	82.6	0	0.1	0.3	29.7	54.8	67.8	0	0.1	0	9.6	0
45060	71.8	0	0	0	59.5	56.3	60.4	0	0	0	3.6	0.3
10221	86.2	0	0	0	41.1	31.9	57.8	0	0	0	19.5	0
10207	74.5	0	0	0	27.3	29.1	34.6	0	0	0	10.8	0

Figure 5

Principal component analysis (PCA) biplot illustrating the relative biomass response of lettuce genotypes to herbicide treatment at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.



Note. Blue labels represent genotypes, and red arrows represent herbicide vectors, with arrow direction and length indicating the strength and orientation of each herbicide's contribution to the principal components.

This dimensionality reduction enabled visualization of genotype clustering and herbicide associations based on shared response profiles. PC1 primarily separated genotypes according to their sensitivity to herbicides with strong positive or negative loadings such as topramezone, mesotrione and prometryn on the positive axis, and glyphosate, and glufosinate on the negative axis. These herbicides represent distinct modes of action, including HPPD and PS II inhibition (positive PC1), and EPSPS and GS inhibition (negative PC1). PC2 further distinguished genotypes based on responses to herbicides like fomesafen, which loaded negatively, and it represented PPO inhibition, and linuron and rimsulfuron, which loaded positively and are associated with PS II and ALS inhibition, respectively.

The PCA biplot revealed clear genotype clustering based on herbicide sensitivity. Genotypes Floribibb, H1098, and 45060, located in the top right quadrant (PC1+, PC2+), were positively associated with topramezone, mesotrione, and prometryn, indicating increased sensitivity to HPPD and PS II inhibitors. Topramezone exhibited one of the strongest positive PC1 loadings, suggesting a pronounced biomass reduction effect in these genotypes. In contrast, genotypes 10207, 10221, and 60182, located in the bottom left quadrant (PC1−, PC2−), aligned with glyphosate, glufosinate, imazethapyr, and imazapic, suggesting heightened sensitivity to EPSP, GS, and ALS inhibitors. Glyphosate and glufosinate had high negative PC1 loadings, reinforcing their influence on this cluster.

Genotype Batavia Reine de Glaces, positioned in the bottom right quadrant (PC1+, PC2−), was most closely associated with fomesafen, indicating a distinct injury profile driven by PPO inhibition. This placement suggests that fomesafen had a unique and potent influence on biomass reduction in Batavia Reine de Glaces compared to other herbicides. Genotype 49019, located in the top left quadrant (PC1−, PC2+), clustered near linuron and rimsulfuron, suggesting a sensitivity pattern influenced by both PS II and ALS inhibition. The separation from other ALS herbicides implies a differentiated mechanism in 49019. Meanwhile, PI 491224, located near the origin of the biplot, showed limited association with any herbicide vector, indicating a relatively moderate biomass response across treatments. These results underscore the utility of PCA in elucidating genotype-by-herbicide interactions and identifying distinct sensitivity profiles. Such insights may inform breeding strategies or herbicide selection in integrated weed management programs.

This study revealed substantial variability in the phytotoxic responses of lettuce genotypes to a diverse set of postemergence herbicides, as evidenced by both visual injury assessments and relative biomass accumulation. The significant genotype × herbicide interaction highlights the crucial role of both genetic background and herbicide mode of action in determining crop tolerance. These findings emphasize the need for genotype-specific herbicide evaluations in lettuce, a crop known for its sensitivity to chemical injury.

Herbicides such as fomesafen, glufosinate, glyphosate, and topramezone caused consistently high levels of injury and biomass reduction across most genotypes, indicating broad-spectrum phytotoxicity and limited crop selectivity. These herbicides, representing PPO, GS, EPSPS, and HPPD modes of action, pose considerable risks to lettuce production due to their low safety margins. In contrast, herbicides that inhibit acetolactate synthase (ALS), namely flumetsulam, imazamox, imazapic, and imazethapyr, exhibited more variable and generally lower levels of injury. This variation suggests that certain ALS inhibitors may be selectively used in lettuce, depending on the genotype.

The PCA biplot revealed distinct genotype clustering patterns based on herbicide sensitivity profiles. Genotypes such as Floribibb, H1098, and 45060 were more sensitive to HPPD and PS II inhibitors, while genotypes like 10207, 10221, and 60182 showed greater susceptibility to EPSPS, GS, and ALS inhibitors. These clustering patterns aligned with the injury data and biomass responses observed in other analyses.

It is important to note that several genotypes consistently stood out as the best performers in all analytical methods. For example, Batavia Reine de Glaces showed only 1% injury and retained 77% relative biomass, while PI 491224 showed 4% injury with 79% biomass, and 10221 had only 3% injury and 86% biomass. These genotypes consistently showed high performance in Tukey mean comparisons, were part of the low injury and high relative biomass clusters.

In addition, 49017 and H1098 demonstrated strong tolerance potential. 49017 experienced only 11% injury with 83% biomass, and H1098, despite showing 14% injury, maintained 98% relative biomass, indicating a high recovery capacity. These results suggest a degree of broad-spectrum tolerance in these genotypes, which makes them valuable candidates for breeding programs aimed at improving herbicide tolerance in lettuce.

Among the herbicides tested, flumetsulam emerged as the most selective, consistently causing the least injury and supporting high biomass retention across multiple genotypes. Injury levels ranged from 1% to 4% in the most tolerant lines, which is well below the 10% injury threshold considered acceptable by the Pest Management Regulatory Agency (2016) and the Canadian Weed Science Society (2018). According to these guidelines, injury under 10% is generally outgrown and does not result in yield loss, reinforcing the potential of flumetsulam as a safer option for postemergence lettuce weed control.

Nonetheless, variability was still present within the ALS-inhibitor group. While flumetsulam was highly selective in most genotypes, it caused up to 29–30% injury in Cooper and a specific breeding line (45060), demonstrating that tolerance is still genotype-dependent. Furthermore, rimsulfuron, also an ALS inhibitor, was consistently phytotoxic, causing 72% to 98% injury and significantly reducing biomass across all genotypes evaluated. This indicates that rimsulfuron is not a suitable option for postemergence in lettuce, despite belonging to the same herbicide group as flumetsulam.

In summary, the observed diversity in genotype responses provides a valuable foundation for the development of herbicide-tolerant lettuce cultivars. Identifying genotype × herbicide combinations with low injury and high biomass retention can contribute to more flexible, targeted, and sustainable integrated weed management strategies. These results support the adoption of a more genotype-specific approach to herbicide selection in lettuce production, balancing effective weed control with crop safety.

Conclusions

The preliminary results of this study reveal differential responses among lettuce genotypes to herbicides with distinct modes of action, underscoring potential implications for crop safety and weed management. The observed patterns of genotype-specific sensitivity offer a promising foundation for the selection or development of cultivars with improved herbicide tolerance, warranting further investigation in more comprehensive trials.

Specific lettuce genotypes, including Batavia Reine de Glaces, PI 491224, 10221, 49017, and H1098, exhibit notable tolerance to herbicide applications, particularly to the ALS inhibiting herbicide flumetsulam, which showed high selectivity and minimal phytotoxicity. Their consistent performance characterized by low injury and high biomass retention combined with moderate responses to other herbicides, highlights their potential as candidates for breeding programs aimed at developing broadly herbicide-tolerant cultivars.

Recommendations

Explore alternative herbicides within the same mode of action: Evaluate other active ingredients in the same chemical family (ALS inhibitors) that have preliminarily shown low phytotoxicity to broaden options for safe weed control.

Expand research with the most promising accessions: Conduct additional trials using the lettuce genotypes that showed the best tolerance, validating their performance under different environmental conditions, in open field and considering interactions with biotic and abiotic stress factors.

Evaluate different application rates: Implement dose-response experiments with the least phytotoxic herbicides to determine optimum application rates that maximize weed control without compromising growth and yield of tolerant accessions.

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Appendices

Appendix A

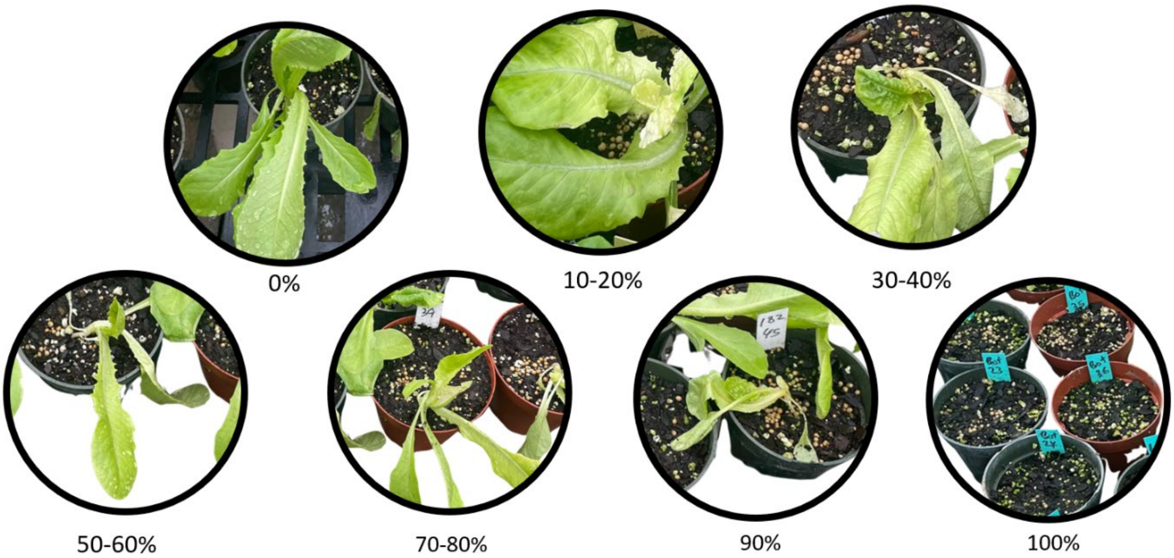
Experimental set-up of greenhouse pots containing UF/IFAS Lettuce Breeding Lines and Commercial Cultivars at EREC

Appendix B

Herbicide treatments applied using a CO_2-Pressurized Moving-Nozzle Spray Chamber

Appendix C

Visual rating scale for herbicide injury for lettuce accessions at the Everglades Research and Education Center (EREC) of the University of Florida, Belle Glade, Florida.



0%

10-20%

30-40%

50-60%

70-80%

90%

100%

Appendix D

Lettuce accessions harvest and biomass dry weight measurement