THESIS

UNRAVELLING THE RESISTANCE MECHANISM TO DICAMBA IN Palmer Amaranth (*Amaranthus palmeri*)

Submitted by

Dustin Abdiel Moreno Serrano

Department of Agricultural Biology

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Master's Committee:

Advisor: Franck E. Dayan

Todd A. Gaines
Meagan Schipanski

ABSTRACT

UNRAVELLING THE RESISTANCE MECHANISM TO DICAMBA IN PALMER AMARANTH (*Amaranthus palmeri*)

Auxin-mimic herbicides (AMHs) have been widely used for more than 70 years, primarily for control of pernicious broadleaf weeds and a few grassy weeds. So far 87 weeds have evolved resistance to AMHs, and it is expected to continue to increase over time. Resistance mechanisms to AMHs are not well understood and most of the reported cases have not been investigated to determine each individual resistance mechanism. Herbicide resistance mechanisms are classified into two main branches. Target-site-resistance (TSR) is when a mutation in herbicide binding site prevents the herbicide from interacting with the enzyme or when overexpression of the herbicide target site results in more enzyme than what the herbicide can inhibit in the plant cell. Non-target-resistance (NTSR) include enhanced herbicide metabolism, reduced absorption, altered translocation, and sequestration. In this current research, resistance to dicamba in a population of *Amaranthus palmeri* (Palmer amaranth) was investigated.

In 2020 a population of the troublesome weed *Amaranthus palmeri* (Palmer amaranth) from Lauderdale county in Tennessee, USA, with a 12-fold resistance to dicamba was identified. Metabolism of dicamba was evaluated and tested using inhibitors of important enzymes involved in herbicide detoxification (e.g., cytochrome P450 monooxygenase and glutathione *S*-transferases). There was no difference in dicamba metabolism between the resistant Lauderdale (R_PA) and susceptible Arizona (S_PA) populations. RNA-seq study was conducted to investigate potential mutations in AUX/IAAs, which are transcriptional repressors. They regulate

transcription factors like Auxin Response Factors (ARFs), and are also co-receptors of auxin-mimic herbicides, and are involved in the regulation of auxin response genes. A mutation in co-receptors can lead to auxin-mimic herbicide resistance; however, there were no mutations in 18 AUX/IAAs and also in other important proteins such as Transport Inhibitor Response 1 (TIR1), Auxin Binding Protein (APB), and Auxin Signaling F-box (AFB). In addition, auxin-response genes responded similarly or differently to dicamba in treated biotypes. Nevertheless, it is noteworthy that the expression of some AUX/IAAs genes changed after dicamba treatment in sensitive plants but not in resistant plants, especially AUX/IAA29. The results suggest that a physiological response is not primarily involved in the resistance mechanism to dicamba because no significant differences in dicamba metabolism were identified, suggesting that dicamba is broken down to less active metabolite, but at the same rate in both R_PA and S_PA. Additionally, no epinasty was observed in resistant plants, a common response when TSR is involved as a primary mechanism. PIF3/4, which is a key transcription factor involved in regulating plant development and response to light, responded to dicamba treatment in sensitive plants, while not responding in resistant plants after dicamba application. Also, expression of AUX/IAA29 did not respond in resistant plants, which is directly involved in the activation of PIF3/4, a transcription factor involved in auxin perception. We hypothesize that PIF3/4 may be involved in the resistance mechanism to dicamba through auxin signaling and/or regulation. However, this hypothesis should be validated by molecular techniques to confirm it. This dicamba-resistant Palmer amaranth biotype has a novel resistance mechanism that remains to be fully elucidated.

ACKNOWLEDGEMENTS

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INTRODUCTION

Effective management of weeds is crucial in cropping systems to optimize and obtain high crop yields. Weeds compete for important resources such as nutrients, light, space, and water and they can be more efficient in competing than desirable plants (Storkey & Neve, 2018). Historically, herbicides have been the primary tool for weed control and management above other control strategies. In addition, genetically modified crops with herbicide-resistant traits have also proven to be effective, facilitating post-emergence weed control (Oerke, 2006).

However, overreliance on herbicides with similar chemistries and modes of action has led to the exponential emergence of herbicide-resistant weed populations, presenting great challenges to global agriculture systems (Green & Siehl, 2021). Recurrent use of this technology has led to an increase in the number of weed resistance cases to individual herbicides, as well as multiple herbicides from different (crossed) or similar (multiple) chemical families (Rigon et al., 2020). Notably, herbicide resistance has become a complex problem in modern agriculture systems and will continue increasing over time. So far more than 500 cases of herbicide resistance have been reported in multiple cropping systems such as rice, sugar beet, corn, soybean, cotton, and sugar cane (Heap, 2024).

Generally, weeds can evolve resistance through two wide mechanisms: target site (TSR), which usually involves a mutation in or near target genes, conferring a change in the structure of the binding site of the herbicide in the protein without disabling the function itself. This resistance mechanism usually confers resistance to one particular herbicide chemistry, but it can confer resistance to other similar chemistry families, depending on whether herbicides interact with the same protein or target site (Gaines et al., 2020). The other herbicide resistance mechanism is known as non-target-site resistance (NTSR), and it involves different sub-mechanisms that are related to the physiological response of the plant after herbicide treatment, avoiding or limiting the amount of herbicide that can reach the target site. It usually involves reduced absorption, reduced translocation, vacuolar sequestration, and metabolic detoxification by the action of multiple enzymes that efficiently inactivate the molecule in a less phytotoxic compound for the plant (Gaines et al., 2020). Even though there is an increase in herbicide resistance cases, the auxin-mimic herbicides (AMHs) (Group 4) by HRAC (Herbicide Resistance Action Committee) have fewer cases reported compared to ACCase inhibitors (Group 1), glyphosate (Group 9), and/or ALS (Group 2) (HRAC, 2024).

Therefore, AMHs like dicamba have taken relevance from a couple of years ago, fundamentally in post-emergence broadleaf control, except for quinclorac, which is used in rice fields to control grassy and some broadleaf weeds (Rangani et al., 2022). This is due to the introduction of soybean and cotton with resistance traits tolerant to dicamba. However, the overreliance on dicamba-resistance crops has led to resistance in *Amaranthus palmeri* (Palmer amaranth), one of the most troublesome weeds to manage and control in the USA. So far Palmer amaranth has evolved resistance to 9 different herbicide modes of action that include groups 2, 3, 4, 5, 9, 14, 10, 15, and 27, limiting the options of control (Heap, 2024).

The most recent case of AMH resistance in Palmer amaranth was reported in Tennessee where a population from Lauderdale County was highly resistant to dicamba. In this study, we conducted several dose-response curve experiments, RNA-seq analysis, and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) to elucidate the resistance mechanism of this population of Palmer amaranth associated with soybean and cotton cultivation from Tennessee. We aim to provide a valuable understanding of the resistance mechanism to growers and the scientific community and therefore contribute to the potential management and control based on our findings.

REFERENCES

Gaines TA, Duke SO, Morran S *et al.* (2020) Mechanisms of evolved herbicide resistance. *Journal of Biological Chemistry* **295(30)**:10307-10330. doi:10.1074/jbc.REV120.013572

Green, J. M., & Siehl, D. L. (2021). History and outlook for glyphosate-resistant crops. In (pp. 67-91). Springer International Publishing. https://doi.org/10.1007/398_2020_54

Gulab, R., Christopher, R., Christopher, S., Rooksana, N., Vijay, S., Amy, L.-R., Isabel, W., & Nilda, R.-B. (2022). High resistance to quinclorac in multiple-resistant *Echinochloa colona* associated with elevated stress tolerance gene expression and enriched xenobiotic detoxification pathway *Genes*, *13*(3), 515. https://doi.org/https://doi.org/10.3390/genes13030515

Heap, I. (2024). *International herbicide-resistance weed database* Retrieved March from http://www.weedscience.org/Home.aspx

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *1*, 31-43. https://doi.org/https://doi.org/10.1017/S0021859605005708

Rigon, C. A. G., Gaines, T. A., Küpper, A., & Dayan, F. E. (2020). Metabolism-based herbicide resistance, the major threat among the non-target site resistance mechanisms. *Outlooks on Pest Management*, *34*(4), 7. https://doi.org/https://doi.org/10.1564/v31_aug_04

Storkey, J., & Neve, P. (2018). What good is weed diversity? *Weed Research*, *58*(4), 5. https://doi.org/DOI: 10.1111/wre.12310

CHAPTER 1: CURRENT STATUS OF AUXIN-MIMIC HERBICIDES

INTRODUCTION

Auxin-mimic herbicides (AMHs) (Herbicide Resistance Action Committee or HRAC-Group 4) encompass a group of molecules that mimic the action of the natural hormone (indole-3-acetic acid, IAA) in plants, affecting plant signaling, disrupting regulatory pathways, and ultimately inhibiting their growth and development. This group of herbicides has been used for more than 70 years, with 2,4-D being the first commercialized herbicide in this group. AMHs are principally used to control broadleaf weeds in monocot crops such as rice, wheat, and other cereals, with the exception of quinclorac, which is used to control certain grasses and broadleaf weeds in rice fields (Rangani et al., 2022). The specificity of AMHs against dicots relative to monocots is hypothesized to be due to combinations of reduced auxin translocation, enhanced metabolism, differences in the physiology of xylem and phloem cells between dicots and monocots, as well as different mechanisms of auxin perception (McSteen, 2010).

AMHs are currently among the top three most used herbicides globally just behind glyphosate (Group 9), and acetolactate synthase inhibitors (Group 2) (Busi et al., 2018). AMHs are commonly characterized by a phenyl ring attached to a carboxylic group (Figure 1.1). A new HRAC classification released in 2024 categorizes AMHs into six different chemical classes according to their structure: Phenoxycarboxylates, 6-chloropicolinates, 6-arylpicolinates, pyridyloxycarboxylates, benzoates, and quinolinecarboxylates acids (Table 1.1 & Figure 1.2). Phenoxycarboxylates (2,4-D) and benzoates (dicamba) are the most extensively used chemical class within group 4 in different important crops in the USA such as corn, wheat, soybean, and others. The first herbicide used within group 4 was 2,4-D and 2,4-D analogs starting in the late 1940s for selective weed management in agricultural settings (Busi et al., 2018). Both 2,4-D and

dicamba have been very efficacious in controlling difficult weeds like *Amaranthus palmeri* (Palmer amaranth) and *Amaranthus tuberculatus* (waterhemp), which have evolved resistance to multiple sites of action and are in the top 15 most troublesome weeds in North America (Heap, 2024). However, there have been recent reports of resistance to 2,4-D and/or dicamba in Palmer amaranth and waterhemp in North America (Heap, 2024).

AMHs are particularly relevant to modern agriculture due to their low number of resistance cases reported compared to other herbicide groups like ACCase (Group 1), ALS (Group 2), PSII (Group 5), and EPSPS (Group 9) inhibitors, even though they have commercially been used for more than 70 years in cropping systems. The increase in weed resistance is due to overreliance on glyphosate after the introduction of glyphosate-resistant (GR) soybeans (*Glycine max*) in 1996, and subsequent GR-crops, which revolutionized modern agriculture and weed management (Benbrook, 2016). Currently, glyphosate is the most used herbicide in the USA, with approximately over 1.6 million metric tons of active ingredient sprayed, which represents around 19% of the global use of glyphosate (Benbrook, 2016). Consequently, the overreliance on glyphosate has caused 59 weed species to evolve resistance to glyphosate by selection pressure, whereas monocot weeds have exhibited more resistance reports than dicot, with 31 and 28 in the world, respectively (Heap, 2024) .

Recently, a new era of herbicide-tolerant crop traits has been in the middle of not only a public debate but also scientific scrutiny, where AMHs (2,4-D and dicamba) have taken on relevance increased importance in weed management in the USA (Soltani et al., 2020). In 2016, dicamba (3,6-dichloro-2-methoxybenzoic acid) transgenic herbicide tolerant varieties traits were commercialized under the label of Xtendimax® with Vapor Grip® technology for use with Roundup Ready® Xtend in cropping systems as well as Enlist E3 soybean in 2019, which is tolerant to 3

different modes of action: 2,4-D choline (Group 4), glyphosate (Group 9), and glufosinate (Group 10). This technology has been used since 2016 as an alternative to mitigate and manage glyphosate-resistant weeds in both cotton and soybean fields, and thus it has caused a quantitative increase in both 2,4-D and dicamba, not only in amount used but also in agricultural land area treated in the United States (Dayan, 2022). However, there are some concerns about the potential problems associated with the increased use of dicamba and 2,4-D in weed management because of their high volatility potential, injury to wild vegetation, and off-target injury to other crops (Riter et al., 2021). Therefore, the objective of this study is to summarize the current status of AMHs in the USA as well as a comprehensive review of resistance mechanisms to AMHs.

AUXIN-MIMIC HERBICIDES RESISTANCE AND EVOLUTION

The first reported case of resistance to AMHs was spreading dayflower (*Commelina diffusa Burm. f.*) to 2,4-D in sugarcane (*Saccharum officinarum* L.) fields from Hawaii (Heap, 2024). Currently, there are 23 cases of resistance to AMHs (i.e., 10 to 2,4-D, 11 to dicamba, and 2 to quinclorac) in the USA. These 23 reports are grouped into 9 weed species, including 7 dicots and 2 monocot weed species (Figure 1.3) (Heap, 2024) . Even though most of the group 4 herbicides control dicot weeds, there are some exceptions such as quinclorac and quinmerac, which are extensively used in rice fields against monocots and some dicot weed species.

Globally, there have been 87 weed species evolving resistance to different AMHs, of which 23 have been reported in the USA since 1957. Notably, most resistance cases have been in dicots rather than monocots and involve primarily the phenoxy carboxylates and benzoic acids. New reports of auxin-mimic herbicide-resistant weeds have substantially increased in recent years (Todd et al., 2020).

One of the most troublesome weeds, Palmer amaranth evolved resistance to dicamba in

sorghum fields from Kansas in 2018 and soybean and cotton fields from Tennessee in 2022 (Foster & Steckel, 2022; Heap, 2024).

The introduction of dicamba and 2,4-D resistance traits in soybean and cotton in 2016 revolutionized weed management after GR crops. Therefore, the amount in metric tons (t) of these herbicides increased dramatically, driven by the need to control GR weeds (Figure 1.4). The regions with the greatest dicamba and 2,4-D use are in the Midwest where soybean, cotton, corn, and others are grown. Some states, such as Nebraska and surrounding states, as well as Texas in the mid-south area of the USA, make sense because they are well-known as productive areas for annual crops such as soybeans and cotton (Figure 1.5). While there are few reports of resistance to AMHs, relative to glyphosate (Group 9) or ALS inhibitors (Group 2), the existence of dicamba-resistant Palmer amaranth in Tennessee is concerning because resistance alleles from this dioecious species can be transferred to other sensitive populations, which may negatively impact its control and making it a difficult weed to manage in a near future (Sarangi et al., 2021; Sosnoskie et al., 2012).

AUXIN-MIMIC HERBICIDE MECHANISM OF ACTION

The mode of action of AMHs is very complex, so we will only provide a brief overview of this process in this review. Auxins are transported from cell to cell through influx and efflux transporters such as PIN-FORMED (PIN), ATP-Binding Cassette sub-family B (ABCB), and AUXIN1/LIKE AUX1 (AUX/LAX) (Todd et al., 2020). AMHs are organic acids that share some similarities to the structure of IAA (the natural auxin) and thus are transported within the cell. Therefore, the presence of AMHs inside a cell causes strong changes in gene expression of some important genes that are involved in the biosynthesis of abscisic acid (ABA), such as 9-cis-epoxycarotenoid dioxygenase (NCED), 1-aminocyclopropane-1 synthase (ACS), Gretchen Hagen

3 (GH3) as well as auxin response genes (McCauley et al., 2020). The substantial increase in the expression of these genes is due to a high level of AMHs in the plant. The presence of AMHs causes AUX/IAA protein degradation. AUX/IAA proteins are transcriptional repressors, so the degradation of AUX/IAA can increase in auxin response gene expression, mediated by the action of the ubiquitin-proteasome system (UPS) (Figure 1.6). This system covalently binds specific proteins to ubiquitin, a small protein, and this process targets those specific proteins for degradation by the 26S proteasome (Santner & Estelle, 2010; Todd et al., 2020). The marked or ubiquitinated proteins are then identified by SKP1-CULLIN1-F-BOX PROTEINS (SCF)-type E3 ligases, an enzymatic complex also called $SCF^{TIR1/AFB1-5}$, which contains multiple interactive substrate recognition units that facilitate ubiquitination in the SCF complex. It mediates the degradation of targeted proteins where the F-box protein, TRANSPORT INHIBITOR RESPONSE 1 (TIR1), and AUXIN SIGNALING F-BOX 1-5 are the substrate receptors for the SCF complex (Calderón-Villalobos et al., 2012; Smalle & Vierstra, 2004). Structurally, TIR1 has an 18-leucine-rich repeat (LRR) domain that contains a binding pocket, making it suitable for interacting with the hormone. The LRR allows hormones to interact as a molecular glue in a conserved motif "GWPPV" in the degron region of AUX/IAA proteins. Consequently, a mutation in the Aux/IAA degron region reduces AMH binding, reducing the ubiquitination rate and subsequent proteasome degradation of the Aux/IAA protein (Figueiredo et al., 2021).

AUXIN-MIMIC HERBICIDE RESISTANCE MECHANISMS

How weeds can evolve herbicide resistance is principally categorized into two main groups: target-site resistance (TSR) and non-target-site resistance (NTSR) (Gaines et al., 2020). TSR is often mediated by a single nucleotide substitution or mutation in the gene that encodes the

protein of the target site, resulting in a change of the amino acid in the functional part of the protein that can lead to less effective binding of the herbicide to the target protein. Therefore, the target protein can complete its role without any disruption even though the herbicide is still in the cell. Often, mutations that occur in nearby areas or somewhere else near the target site can lead to a change in the protein structure, which negatively reduces the affinity of the herbicide (Rigon et al., 2020). The level of resistance that every mutation causes is relative, some of them can cause high, medium, or even low resistance, depending on the location and how it impacts the protein structure and interactions (Tan et al., 2007). Furthermore, other types of variations can lead to TSR, such as whole codon deletion, which impacts the deletion of a complete amino acid, changing the structure of the protein but leaving the protein structurally functional. In addition, another interesting mechanism that can lead to TSR is mediated by gene amplification, which causes an over-transcription of the gene that encodes for the enzyme, producing more enzymes that the inhibitor can inhibit (Gaines et al., 2020). So far 44 weeds have evolved auxin-mimic herbicide resistance globally by either TSR or NTSR (Heap, 2024).

A population of a major weed in the USA, *Kochia scoparia* (kochia), from Nebraska evolved resistance to dicamba by TSR through a two-nucleotide base substitution (**GG**T → **AA**T) within the highly conserved degron region of the IAA16 coreceptor (KsIAA16), resulting in an amino acid substitution from glycine to asparagine (**G**WPPV → **N**WPPV). This substitution at Gly-127-Asn led to less binding or interaction between TIR1/F-box, dicamba, and AUX/IAA protein (LeClere et al., 2018). In another example of TSR, a deletion of 27 nucleotides, which completely removed 9 amino acids in the degron tail of the AUX/IAA2 in Indian hedge mustard (*Sisymbrium orientale* L.), confers resistance to 2,4-D (Figueiredo et al., 2021). This deletion conferred resistance to both 2,4-D and dicamba *Arabidopsis* transgenic lines. In addition, a

population of lambsquarters (*Chenopodium album* L.) from New Zealand was about 25-fold more resistant to dicamba than the sensitive population because of a novel mutation in the degron region of the AUX/IAA16. This novel mutation resulted in one change in the second nucleotide of the first codon from guanine to adenine (G**G**T → G**A**T), which caused an amino acid change from glycine to aspartic acid in the degron (**G**WPPV → **D**WPPV) that led to a high resistance population of *C. album*, making it the most recent report of TSR in AMHs (Ghanizadeh et al., 2024). These examples highlight that the interaction among AUX/IAA (degron), auxins, and TIR1 has a critical role in the regulation of the auxin pathway and AMH mode of action in planta; therefore, a mutation in AUX/IAA or TIR1 can potentially lead to AMH resistance, although no field-evolved examples of TSR mutations in TIR1 have yet been reported.

On the other hand, NTSR also plays an important role in herbicide resistance through the alteration of one or more metabolic processes, which include herbicide translocation, absorption, sequestration, and/or detoxification by metabolism (Gaines et al., 2020). As NTSR can involve different physiological processes, they are more complex and thus more difficult to decipher. NTSR can also impart cross-resistance within the same chemical class but also multiple herbicide resistance to other chemical herbicide groups even though these have not been sprayed before (Jugulam & Shyam, 2019). Reduced translocation has been well documented in several broadleaf species such as prickly lettuce (*Lactuca serriola* L.), resulting in a reduction of 2,4-D movement, and thus a limited amount of the herbicide achieves the target site (Riar et al., 2011). Resistance to dicamba in a kochia population from Nebraska in 2017 involved decreased herbicide translocation in the resistant biotype relative to the sensitive population (Pettinga et al., 2018). In addition, reduced absorption has also been implicated in a few resistance reports; however, foliar absorption of herbicides depends on different weed morphological aspects like leaf morphology,

cuticle structure, trichomes, and gland conformation (Gaines et al., 2020).

Enhanced metabolism is another major NTSR mechanism that has been reported in diverse broadleaf weeds. However, metabolic detoxification is one of the most troublesome and complex resistance mechanisms to manage because weedy plants contain a wide group of enzymes and possess the ability to degrade different herbicides from or within the same chemical family, which is called cross- or multiple-herbicide resistance. Enhanced metabolism involves three main phases that will not extensively be covered in this manuscript. Phase I is commonly mediated by P450 monooxygenases; a large group of enzymes that can recognize different herbicides as substrates, altering their structure by different reactions such as hydroxylation, deamination, and/or oxidation. Phase II, at this point, the conjugation of the herbicide or its previous product from the P450 reaction can be conjugated with other enzymes such as glutathione *S*-transferases (GST), *O*-glycosyl, and/or others, into a less phytotoxic metabolite. Phase III involves the incorporation and encapsulation of the non-phytotoxic metabolites into a vacuole or wall cell as a waste product (Rigon et al., 2020). An *Amaranthus tuberculatus* (waterhemp) population from Nebraska was resistant to 2,4-D by enhanced metabolism, where the half-life of 2,4-D in resistant plants was around 22 h, compared to 105 h in sensitive plants. Furthermore, the tested inhibitor, malathion, caused a substantial decrease in the rate to cause 50% GR. This suggests that the herbicide is converted into a less phytotoxic compound that is potentially hydroxylated by P450 in this 2,4-D-resistant population, losing its herbicide activity (Figueiredo et al., 2018). The following study used the same population from Nebraska to validate the hydroxylation of 2,4-D as previously hypothesized. Resistant plants detoxified 2,4-D by hydroxylation through P450 and also by conjugation into sugar and subsequently with malonyl in multiple reactions that led to the formation of 2,4-D-(6′-*O*-malonyl)-5-*O*-D-glucopyranoside, which was non-phytotoxic. The non-

reversible P450-mediated resulted in a high 2,4-D resistance level, while sensitive plants had a reversible conjugation of 2,4-D with aspartate. The aspartate conjugate lost some auxin activity, while the P450-mediated metabolite had a substantial reduction in auxin activity (Figueiredo et al., 2022). A similar case was reported in a fluroxypyr-resistant kochia population from Colorado where the overexpression of well-documented metabolism genes such as P450 monooxygenases, glutathione S -transferases, and glucosyl transferases, and transporters like ATP-binding cassette transporters (ABC transporters) can potentially be involved in the resistance mechanism to fluroxypyr through faster metabolism and transport of the inactive fluroxypyr to other places in the cell, such as the vacuole or cell wall, to store there as a waste product (Todd et al., 2024).

FIGURES

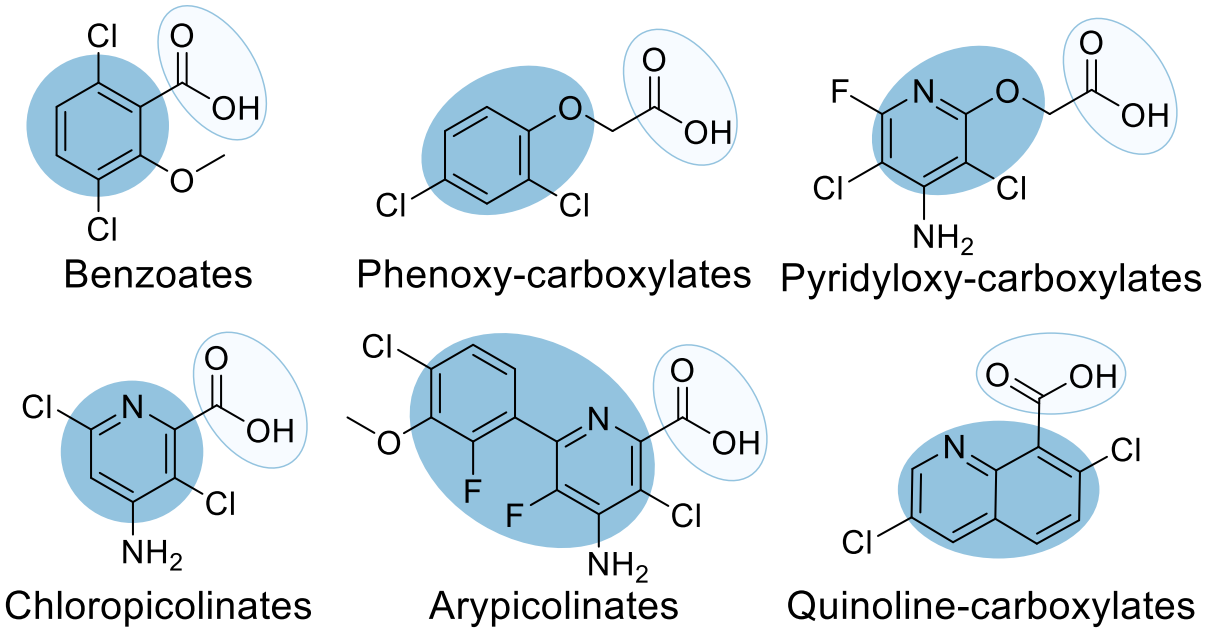


Figure 1.1. Structures of dicamba, 2,4-D, fluroxypyr, aminopyralid, florpyrauxifen and quinclorac are shown as examples of the benzoates, phenoxy-carboxylates, pyridyloxy-carboxylates, chloropicolinates, arylpicolinates and quinoline-carboxylates, respectively.

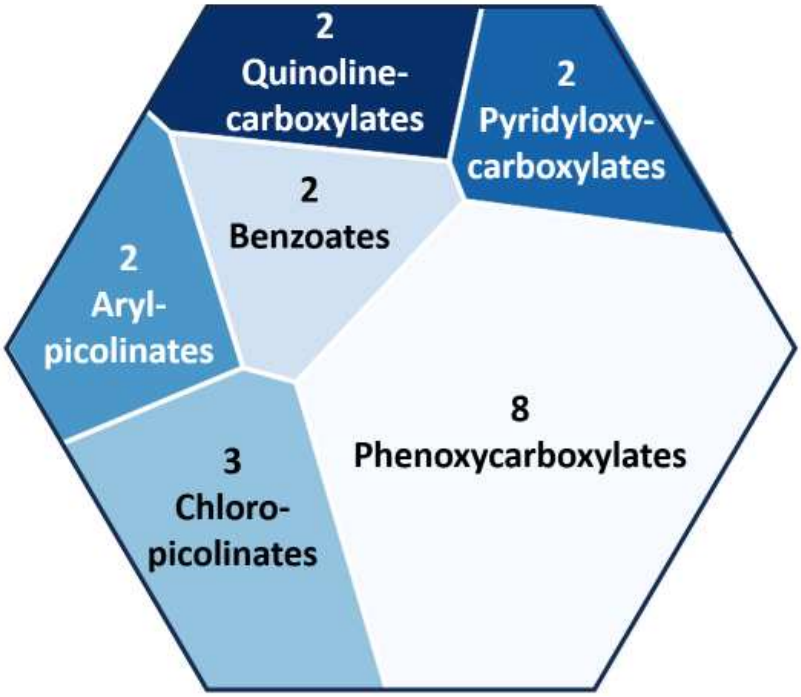


Figure 1.2. The relative size of each chemical class within HRAC Group 4 herbicides. Numbers and areas inside the shapes represent the number of active ingredients within each class.

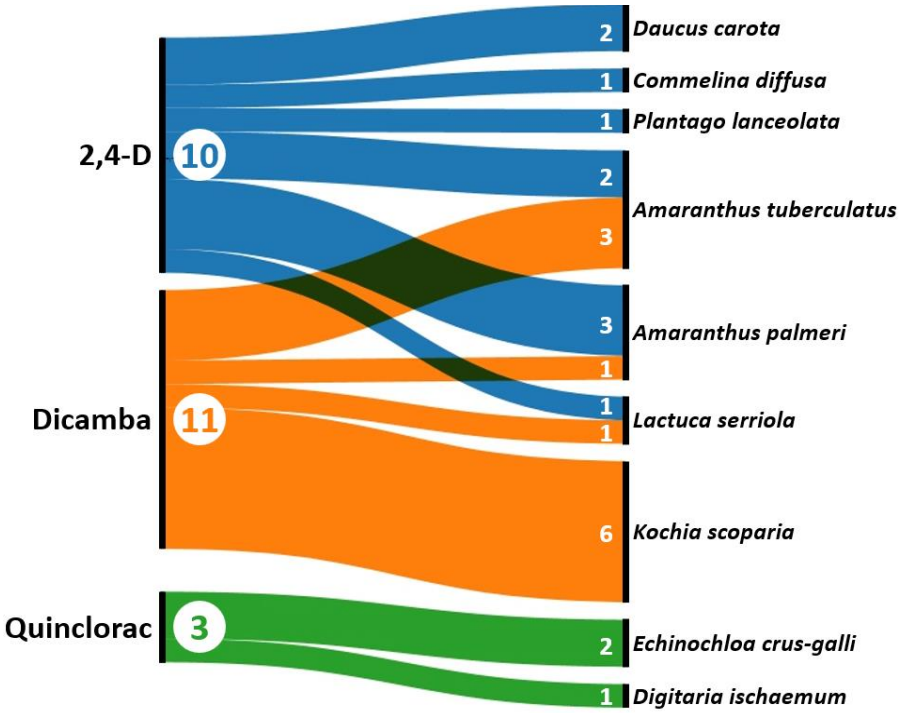


Figure 1.3. Reported weed species to evolve resistance to 2,4-D, dicamba, and quinclorac HRAC Group 4. Total numbers of unique cases are in circle on left side and details about specific cases in each species are shown on the right. Data from Heap, 2024.

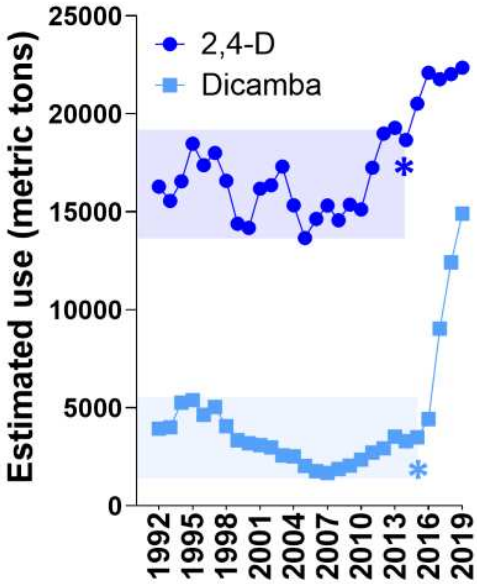


Figure 1.4. Estimated use of 2,4-D and dicamba from 1992 to 2019 in metric tons . The asterisk (*) represents the introduction of 2,4-D resistant and dicamba-resistant soybean and cotton. Most recent complete data available is for 2019, obtained from USGS Pesticide National Synthesis Project (USGS, 2024).

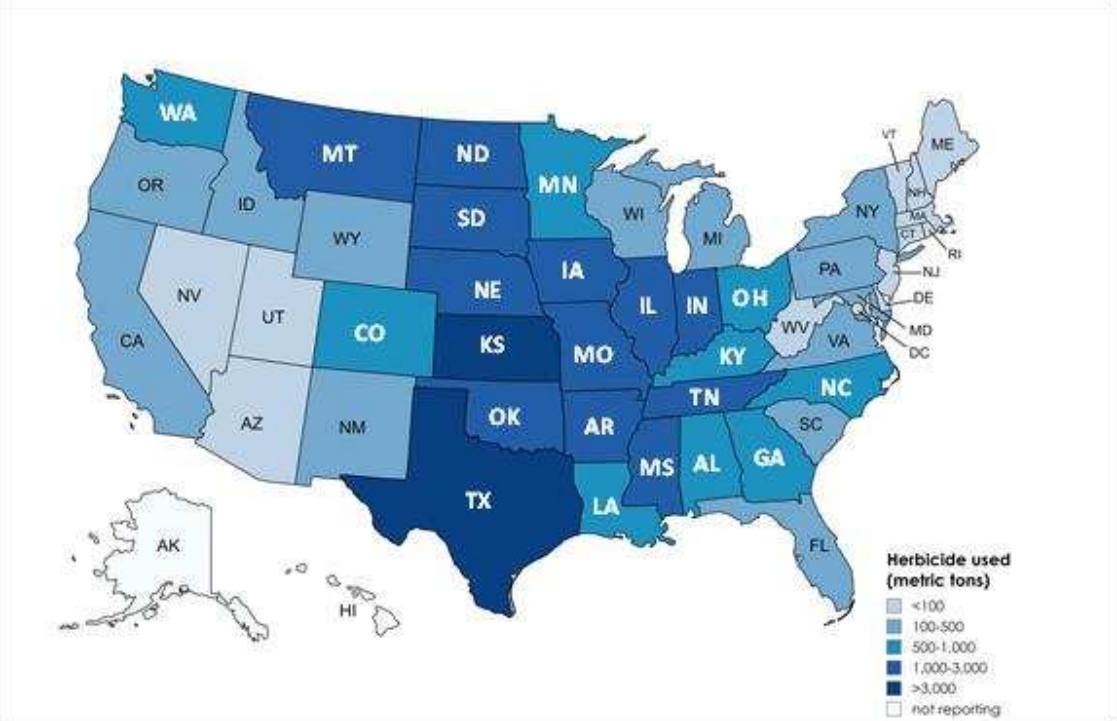


Figure 1.5. Estimated agricultural use of group 4 herbicides in 2018 in different states in the USA. The darkest blue means the amount used is more than 3,000 metric tons. Data from USGS Pesticide National Synthesis Project (USGS, 2024). The map was generated with MapChart (https://www.mapchart.net/usa.html).

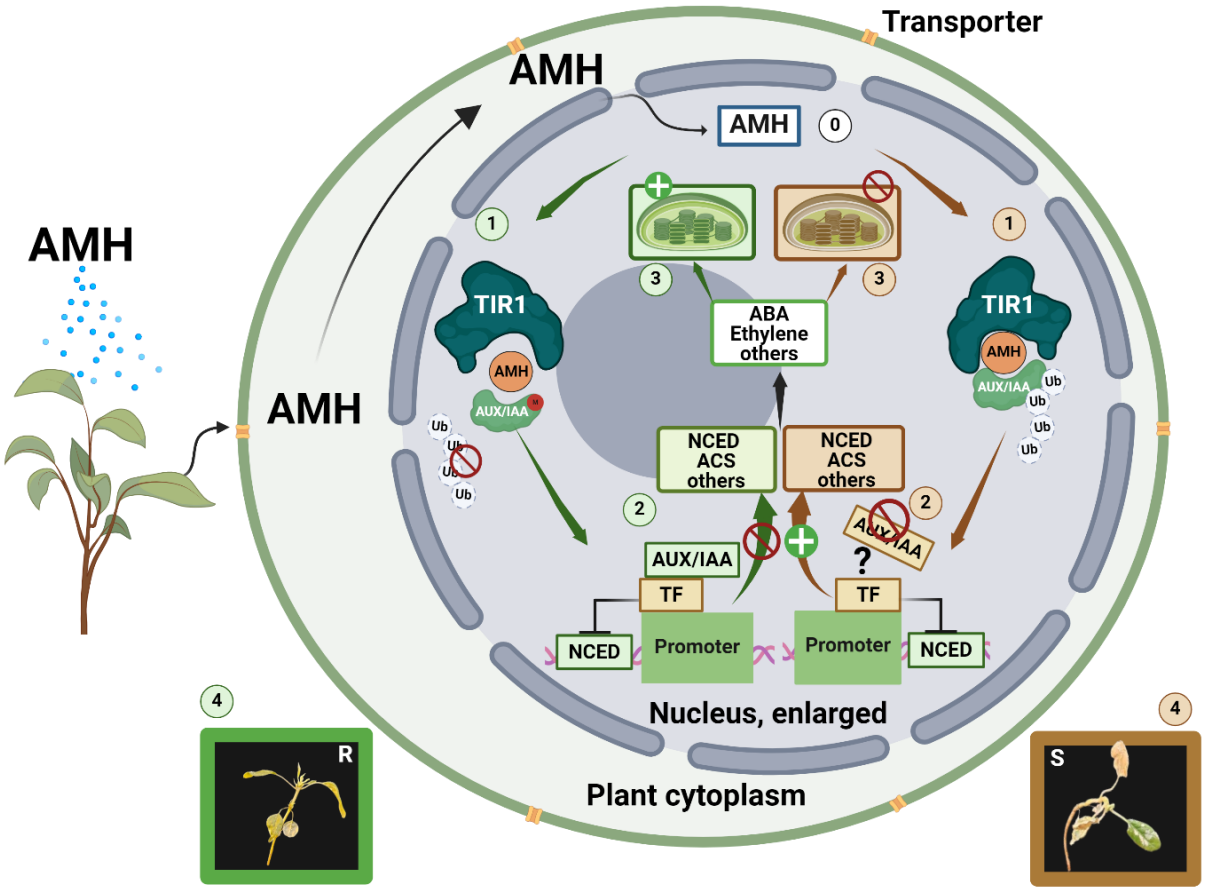


Figure 1.6. Proposed pathway of auxin-mimic herbicide (AMH) action and plant response between sensitive (denoted as numbers in brown circles) and resistant (denoted as numbers in green circles) plants (adapted from: (Gaines, 2020). In a sensitive plant, an elevated dose of AMHs promotes the interaction of (TIR1) and AUX/IAA ❶, where the herbicide acts as a molecular glue between them. This leads to the degradation of AUX/IAA (transcriptional repressor) through ubiquitination. Degradation of AUX/IAA leads to the rapid induction of auxin-responsive genes (ARG) like 9-cis-epoxy carotenoid dioxygenase (NCED), 1-aminocyclopropane-1-carboxylate synthase (ACS) and others ❷. The rapid transcription of NCED is part of the proposed herbicide mechanism because it is involved in the synthesis of abscisic acid (ABA); therefore, overexpression of ARG can increase and accumulation of ABA ❸, negatively impact photosynthesis, and lead to plant death ❹ (McCauley et al., 2020). In contrast, in resistant plants, mutations in AUX/IAAs prevent AMHs from acting as a molecular glue ❶. Therefore, the AUX/IAA proteins are not degraded, so the transcriptional repressor still carries out its normal functions, without deregulation of ARGs ❷, nor affecting ABA levels ❸, resulting in plant survival ❹. Figure created using BioRender.

TABLES

Table 1.1. Distribution of auxin-mimic herbicides (Group 4) by chemical class, herbicide, year of introduction, target plants, reported cases as well as first reported case and major use in the USA, respectively. *Pesticide Properties DataBase (PPDB) (Lewis et al., 2006); **(Heap, 2024); †(Choudhury et al., 2016).

Chemical class	Group-4 Herbicide	Year of introduction *	Target plants	Reported Resistance cases**	1st resistance report**	Major use
Phenoxycarboxylates	2,4-D	1950	Broadleaf	9	1957	Corn, wheat, soybean, barley, other crops
	2,4-DB	1944				
	2,4,5-T	1947				
	Clomeprop†	1989†				
	Dichlorprop	1961				
	MCPA	1950				
	MCPB	1960				
	Mecoprop	1956				
Benzoates	Chloramben	1958	Broadleaf	11	1994	Corn, wheat, barley, soybean, and sorghum
	Dicamba	1963				
Pyridyloxycarboxylates	Fluroxypyr	1983	Broadleaf	2	1994	Wheat, canola, barley, other crops
	Triclopyr	1979				
Quinolinecarboxylates	Quinmerac	1993	Broadleaf	3	1998	Rice
	Quinclorac	1989	Grasses			
6-Arylpicolinates	Florpyrauxifen	2016	Grass / Broadleaf	NA	NA	Wheat, and other cereals
	Halauxifen	2014	Broadleaf			
6-Chloropicolinates	Aminopyralid	2005	Broadleaf	3	1988	Small grains crops, pasture, and other crops
	Clopyralid	1977	Broadleaf			
	Picloram	1963	Broadleaf			

CHAPTER 2: UNRAVELLING THE RESISTANCE MECHANISM TO DICAMBA IN PALMER AMARANTH (*Amaranthus palmeri*)

INTRODUCTION

Herbicides have revolutionized global agriculture and weed control for the last eight decades, providing effective and economical tools for control of these pernicious plants that interfere and compete in all crop systems in the world, contributing to a large decrease in yield (Heap, 2014; Oerke, 2006). This tool is often regarded as the first option for weed control and has largely replaced laborious manual and mechanical control in cropping systems (Adamczewski et al., 2019). However, dependence and the overuse of herbicides with the same site of action (SOA) as the primary option for weed control has resulted in the evolution of resistance through pressure selection that occurs in these cropping systems (Jugulam & Shyam, 2019). Another reason, that has contributed dramatically to the increasing cases of resistance in weeds is the introduction of transgenic herbicide-resistance crops (THRC), of which bromoxynil, glufosinate, and glyphosate were the first introduced commercially in 1995 and 1996 in the USA. Introduction of new technologies entails new challenges, hence the frequent use of THRC and herbicides with the same SOA on fields have caused weeds to evolve resistance either by enhancing their metabolism nontarget site resistance (NTSR), increasing the degradation of the herbicide by different metabolic mechanisms, or by a mutation that alters the herbicide's bind site target site resistance (TSR), preventing the herbicide from inhibiting its target (Duke, 2014; Gaines et al., 2020).

A common example that weed resistance is playing an important role in food production is the number of resistance cases to herbicides belonging to groups 2 (acetolactate synthase), 5 (inhibition of photosystem II), 9 (inhibition of enolpyruvyl shikimate phosphate synthase), 1 (inhibition of acetyl-CoA carboxylase), 4 (auxin-mimic), and 22 (PSI electron diversion) according to the classification provided by the Herbicide Resistance Action Committee (HRAC) (HRAC, 2024). These groups of herbicides have the most number of resistance cases in the world, with 171, 87, 56, 50, 42, and 32 reported cases, respectively where dicotyledonous tends to present more resistance cases than monocotyledonous. The most problematic dicotyledonous weed families are Amaranthaceae and Brassicaceae, while monocotyledonous weeds are represented primarily by the Poaceae family (Heap, 2024).

During the past 60 years, auxin-mimic herbicides (AMH) have been an important tool for farmers, mainly in grain systems, because of their high selectivity and good control of pernicious

broadleaf weeds. They act in cell division and growth as a growth regulator due to their structural similarity to the natural plant hormone indole-3 acetic (IAA) acid, occasioning the same physiological and biochemistry reaction as the natural hormone IAA at low concentrations (Grossmann, 2009). Because of their structural similarity to IAA, AMHs compete for the same binding site and transport processes as IAA (Ghanizadeh et al., 2018; Grossmann, 2009).

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is considered one of the most important AMH (group 4), controlling more broadleaf species than 2,4-D (2,4-dichlorophenoxyacetic acid), which is the most used herbicide in this group (Moreno-Serrano et al., 2024). The recent increase of dicamba use in cropping systems is due to the many resistance cases to 2,4-D in multiple broadleaf weeds, with 43 cases reported globally so far (Chandrima et al., 2022; Heap, 2024). Resistance mechanisms are the result of either targe-site resistance (TSR), which usually involves a point mutation in genes that encode the target protein, decreasing the binding site of the herbicide and consequently reducing the affinity, however, in the case of 2,4-D, mutations have not been reported in the receptor (TIR1) but have been reported in the co-receptor AUX/IAA, which have led to 2,4-D resistance. In addition, other resistance mechanisms have also been reported conferring 2,4-D resistance, such as reduced absorption, altered translocation, and/or detoxification of the herbicide by the action of specific metabolic enzymes (cytochrome P450 monooxygenases, glutathione *S*-transferases, glucosyl-transferases, and other transferases) that convert the herbicide through chemical reactions into a less toxic or inactive molecule (Chandrima et al., 2022; Gaines et al., 2020; Rigon et al., 2020). In the case of AMHs, TSR resistance is a little different because the mutations are not in the receptor (TIR1 protein) but rather on the AUX-IAA protein. However, it is important to consider that despite the substantial recent increase in 2,4-D and other group 4 herbicide usage, the number of resistance reports are still lower compared to ALS, PSII, and glyphosate (Heap, 2024). Therefore, an alternative proposed to delay the development of 2,4-D resistance in broadleaf weeds has been the commencement of the use of dicamba.

Dicamba is also a group 4 herbicide which has been widely used and commercialized since 1960. It has high selectivity, low cost, persistence in soil, and low toxicity levels to humans and animals, allowing the release of dicamba-resistant soybean cultivars (Behrens et al., 2007; Bobadilla et al., 2022). The use of dicamba-resistant crops allowed the implementation of a mixture of herbicides, reducing the possible pernicious weed resistance cases on crop fields

(Behrens et al., 2007; Bobadilla et al., 2022). However, the repeated use of this technology, sub-lethal doses as a unique resource in weed management, and the known volatility, which contribute to the frequent exposure to sub-lethal doses due to drift have probably caused the substantial development of resistance in certain problematic weeds in the USA. Some notable examples are *Amaranthus palmeri* (Palmer amaranth), *A. tuberculatus* var = rudis (Waterhemp), and *Bassia scoparia* (Kochia) (Bobadilla et al., 2022; Foster & Steckel, 2022; Kumar et al., 2019). Palmer amaranth is considered the most troublesome weed, especially in the southwestern part of the USA, but its presence has increased in northern states recently (Ward et al., 2013).

Palmer amaranth has been a problem in crop systems across the United States, causing great yield losses and establishing Palmer amaranth as one of the most difficult weeds to control due to its ability to accumulate biomass and grow quickly compared to other Amaranthus species. Some authors have demonstrated that this weed can reduce yield as much as 91% in corn (*Zea mays*) (Massinga et al., 2001), 54% in cotton (*Gossypium hirsutum*) (Morgan et al., 2001), and from 68 to 79% in soybean (*Glycine max*) (Klingaman & Oliver, 1994). Also, Palmer amaranth is a dioecious plant, so it is an obligate out-crosser, showing high genetic diversity and allowing it to adapt to different biotic conditions. This weed can produce more than 200,000 seeds under moisture stress conditions, but can produce over 800,000 seeds in soybean fields (Chandrima et al., 2022). During 2021 and 2022, some dicamba-resistant Palmer amaranth populations have been identified in cotton and soybean fields in western Tennessee and Arkansas, causing serious problems in cotton and soybean agroecosystems (Foster & Steckel, 2022). Therefore, the objective of this study was to characterize the mechanism of dicamba resistance in the Palmer amaranth population from Lauderdale County, Tennessee, USA.

MATERIALS AND METHODS

Plant material

A Palmer amaranth population from Lauderdale County, Tennessee, USA (35.7123, -89.9175) characterized as resistant to dicamba (R-PA) was evaluated (Foster & Steckel, 2022). A population from Tucson, Arizona, USA (32.2500, -111.0000) (GRIN accession: RRC 686) was used as a dicamba sensitive (S_PA) population.

Dose-response curve

A dose-response experiment was conducted twice under greenhouse conditions in the fall of 2022 to determine the sensitivity to dicamba rates (Engenia, BASF, Research Triangle Park, NC) using S_PA and R_PA populations. Dicamba doses were 0, 18, 35, 70, 140, 280, 560, and 1120, and 2240 g a.e./ha with $n = 4$. Seeds of both S_PA and R_PA populations were grown in a 100-cell plug flat using Lambert LM-2® germination soil. In addition, because of the constant use of both dicamba and 2,4-D in Lauderdale County, another dose-response curve was also conducted using 2,4-D to determine the potential cross-resistance between AMHs.

After 10 d, seedlings from each biotype were transferred to an 18-cell plug flat using the previously described soil. At the 4 to 6-leaf stage, seedlings of both S_PA and R_PA populations were sprayed with dicamba at the doses described above. All treatments were sprayed with an overhead track sprayer (DeVries Manufacturing Hollandale, MN) equipped with a flat-fan nozzle tip (Teejet 8002EVS, Spraying System) calibrated to deliver 187 L/ha^{-1} of spray solution at 172 kPa. Plants treated were grown in the greenhouse, and the number of surviving plants was counted 21 d after treatment (DAT) (Supplemental File 2.2 and 2.3). To calculate the percentage (%) of injury and mortality, a scale from 0 to 100% was used, with 0 representing no injury and 100% representing death. Additionally, due to the subjective nature of the injury scale, the data were transformed into a binomial scale where 0 represents alive and 1 represents dead. This transformation enabled the use of a binomial distribution for performing non-linear regression analysis (Kniss & Streibig, 2021).

Metabolism of dicamba

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis was employed to test whether faster metabolism was involved in R_PA. To do it, dicamba was sprayed at 280 g a.e./ha on both the R_PA and S+PA biotypes, considering this rate as a discriminatory rate. The whole plant was considered a sample. Therefore, they were collected after 1, 2, 4, and 8 d after dicamba spraying, and each biotype had ($n = 4$) for each treatment, totaling 16 experimental units for each biotype. After collecting samples, they were immersed in a 50% solution of acetone for 30 seconds to remove any dicamba residue from the leaf surface and then disposed it in falcon tubes and stored at -20 C until analysis.

Dicamba quantification was made using a Shimadzu® LC-MS/MS system, which consisted

of a Nexera X2 UPLC with 2 LC-30AD pumps, a SIL-30AC MP autosampler, a DGU-20A5 prominence degasser, a CTO-30A column oven, and an SPD-M30A diode array detector coupled to an 8040-quadrupole mass spectrometer with ESI. In addition, the MS was in negative modes for dicamba detection, and the samples were chromatographed using Phenomenex Kinetex Omega 3 (C18) (cat 00D-4759-E0) maintained at 40°C. Solvent A consisted of water with 0.1% formic acid, and the solvent B was methanol with 0.1% formic acid (Sack et al., 2015). The solvent gradient started at 80% B, gradually rose to 100% in 3.5 min, and returned to 80% B after 4.5 min. The total run was 10 min and multiple reaction monitoring (MRM) was optimized for 218.7 > 174.75 and set for 100 ms dwell time with a Q1 pre-bias of 14V, a collision energy of 5V, and a Q3 pre-bias of 18V with a t_R dicamba 3.1 min (Supplemental File 2.1).

Effect of metabolic inhibitors

To assess the involvement of either P450 monooxygenases (P450) or GST in mediating the metabolism of dicamba, three dose-response experiments were conducted on both S_PA and R_PA populations at the 4-6 expanded leaf stage. Plants were treated with and without the P450 inhibitor malathion (Spectracide®) at 2,000 g a.e./ha. This experiment was replicated twice. Additionally, 4-chloro-7-nitrobenzofurazan (NBD-Cl; Sigma-Aldrich®) applied at 270 g a.e./ha, was used to explore the potential involvement of glutathione-S transferase inhibitor (GST) in the resistance mechanism. P450 and GST inhibitors were sprayed 24 and 48 h before dicamba spraying. Dicamba rates applied after either application of P450 or GST inhibitors were 0, 18, 35, 140, 280, 560, and 1120 g a.e./ha, in which each rate had five replicates ($n = 5$).

RNA-Sequencing (RNA-seq)

An RNA-seq experiment was performed on S_PA and R_PA populations of Palmer amaranth. The experimental design included 36 plants of each biotype, with two harvest times: 0 and 6 h. At time 0, 18 plants of each biotype were sprayed with water as a control treatment. The remaining plants were sprayed with dicamba (Engenia, BASF, Research Triangle Park, NC) at 280 g a.e./ha as a discriminatory rate. Meristematic tissue was collected from all plants at time 0 after water spraying for the control group, and at 6 h after dicamba spraying for the treatment group. The collected plant tissue was labeled in Eppendorf tubes, immediately frozen in liquid nitrogen, and stored at -20°C.

All individuals were kept in the greenhouse until 21 d after treatment (dat). At this point, the treatments were evaluated visually to identify the healthiest survivors among R_PA and the most sensitive individuals among S_PA. This allowed us to confirm which plants were truly resistant and which were sensitive. A total of 3 individuals for each biotype and treatment were chosen, for a total of 12 samples.

The total RNA was isolated from each sample using the Zymo Direct-zol RNA Miniprep Plus kit following the manufacturer's procedures (Zymo Research, Irvine, CA, USA), and the total RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were then submitted to Novogene (Sacramento, CA) for Illumina library generation and consequent short read (150 bp, paired-end) sequencing, achieving at least 31 million read pairs per sample. The quality of the raw reads was assessed using FastQC tools version (v0.12.1). Subsequently, the low-quality reads were removed, and the adaptors were trimmed using the same bioinformatic tool, ensuring the data was prepared appropriately for subsequent analysis.

The trimmed reads were aligned to the recently updated high-quality reference transcriptome of Palmer amaranth (available at: https://www.weedgenomics.org/) using Hisat2 (version 2.2.0). Alignments for AUX/indole-3-acetic acid (IAA) (AUX/IAA), Transport Inhibitor response 1 (TIR1), Auxin Binding Protein (ABP), and Auxin Signaling F-box (AFB) annotation were visually inspected looking for single nucleotide variants (SNVs) or gaps between S_PA and R_PA populations using the resulting alignment file (.bam) and Integrative Genomic Viewer (IGV) version 2.16.1. In addition, .bam files generated from the alignment were used to conduct variant calling using Samtools version (1.20) mpileup (Li & Durbin, 2009). The resulting variant call file and the high-quality reference transcriptome were used to generate a consensus sequence of every sample using BCFtools version (1.20) (Li & Durbin, 2009).

Differential gene expression was conducted using the raw read counts generated by featuresCounts version (2.0.5). Differential gene expression was determined with the DESeq2 package (Anders & Huber, 2010) using the statistical software R version (2024.04.2). Gene expression in each population was compared within and between treatments. As a result of the analysis, all genes with a log2 fold change (FC) >2 were considered differentially expressed. Read counts were analyzed to determine the number of reads for all the AUX/IAA genes to examine whether the expression levels of these genes were similar or different across all four treatments. In addition, other important genes involved in auxin perception (such as TIR1 and AFB), ethylene

biosynthesis (including aminocyclopropane-1-carboxylic acid synthase, oxidase, and deaminase), abscisic acid biosynthesis like 9-cis-epoxycarotenoid dioxygenase (NCED), and hormone regulation like Gretchen Hagen (GH3) (Luo et al., 2018).

To identify biological pathways that were induced by AMH application in S_PA and R_PA, we employed a gene ontology (GO) term enrichment technique. Here, we used blastp (Camacho et al., 2009) to align protein sequences of all genes to the Uniprot Swiss-Prot database (e-value cutoff of 5e-5). Alignments were filtered to retain the alignment for each gene with the highest bitscore using a custom Python script. Biological process GO terms associated with each up-regulated gene (adjusted p-value <0.05 and log2fold change >2 in the treated vs untreated contrast for each species) were then collected using the Uniprot Retrieve/ID mapping tool on the uniport website (https://www.uniprot.org/id-mapping). The DAVID web-server was used to determine enrichment across GO terms for each contrast using Arabidopsis as a background (Sherman et al., 2022). Results were plotted using ggplot2 (Villanueva & Chen, 2019) in R (Team, 2020).

Statistical analysis

The data from the dose-response experiments were fitted to a four-parameter log-logistic model (drm-function) of the drc-package in the software R version (2024.04.2). R software was used for the dose-response and metabolism inhibition analysis, as is shown in Equation 1 (Knezevic et al., 2017).

$$Y(x) = \frac{d - c}{1 + \exp\big(b(log(x) - e)\big)}$$

Equation 1. Four-parameter log-logistic model *b, c, d,* and *e,* where *Y* is the percent non-DT (non-dicamba treatment), *d* is the upper and c is the lower limit of *Y, and e* (inflection point) represents 50% *Y* reduction relative to *d*. The parameter *b* is the relative slope around *e*, which is ED50 and is the dose causing 50% inhibition (Knezevic et al., 2017).

To obtain the resistant to sensitive (R/S) ratio, data from the metabolism experiments were pooled (treatment without inhibitors) and then drc analysis was conducted with a $n = 15$ for each dose, which was described.

Herbicide degradation was analyzed as non-linear regression using the "drc" package version (2024.04.0) in R (Ritz et al., 2015). To estimate the dissipation rate, DT_{50} (the time required for the concentration to decline to half of its initial value) was used in the method and Equation 2 described by the EPA (2023):

$$DT_{50} = log(2) * k$$

Equation 2. DT_{50} is the time (d) when 50% of the initial herbicide concentration has been degraded, and k is considered the rate constant because dicamba was applied at specific concentrations in both populations.

Phylogenetic analysis

Protein sequences translated from the consensus gene sequences of Palmer amaranth IAA genes were aligned to all the IAA genes from Arabidopsis (sequences obtained from TAIR arabidopsis.org) Supplemental File 2.4). Alignment was performed using Used MAFFT-L-INS-I (v7.511) and tree was saved in Phylip format (Katoh et al., 2005). The circular phylogenetic tree was generated using Interactive Tree Of Life (iTOL version 6.9.1) (https://itol.embl.de/) (Ciccarelli et al., 2006).

Statistical significance of read count numbers was determined by Analysis of Variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test (Williams & Abdi, 2010) at the α=0.05 level using module agricolae in RStudio (2024.04.2 Build 764) and R version 4.3.1 (2023-06-16 ucrt) (Supplemental File 2.5).

RESULTS

Dose-response curves

Lauderdale accession was resistant to the AMH dicamba. All of the Lauderdale plants survived the highest rate of 1,120 g a.e./ha dicamba treatment, whereas all the S_PA plants were effectively controlled by 280 g a.e./ha (Figure 2.1). The calculated GR_{50} for the R_PA and S_PA populations based on the injury data were 390.0 and 69.9 g a.e./ha, respectively (Figure 2.4, and Table 1.2). The R/S ratio obtained was approximately 12-fold. In contrast, the obtained 2,4-D GR50 was approximately 104.1 and 25.5 g a.e./ha with a R/S ratio of approximately 4-fold (Figure 2.2).

Metabolism of dicamba and effect of metabolic inhibitors

The rate of dicamba metabolism was similar in S_PA and R_PA over the duration of the

experiment. The half-life (t1/2 or time to reduce half of the initial amount) of dicamba was also similar for both populations (S_PA = 6.1 ± 1.8 d and R_PA = 5.0 ± 1.3 d) (P = 0.65) (Figure 2.3). Hence, enhanced metabolism is not likely to contribute to the level of herbicide resistance observed in the R_PA population.

Nonetheless, the effect of the metabolic inhibitors of P450 and GST was also tested. As would be expected based on the initial metabolism experiment, neither malathion nor NBD-Cl strongly reversed resistance in the R_PA plants (Figures 2.4, 2.5, and 2.6; Table 2.1). This confirms that P450 or GST are not involved in resistance to dicamba.

RNA-Sequencing (RNA-seq)

During library preparation, the average alignment rate was 67%. All Palmer amaranth samples yielded between 31 and 57 million read pairs. After alignment to the high-quality reference transcriptome of Palmer amaranth, almost all the samples had >69% of their reads mapped to the Palmer amaranth reference transcriptome. The variance was visualized using principal component analysis (PCA), showing distinct groupings differentiating S_PA from R_PA as well as control from treated groups, all with small differences within each treatment (Figure 2.7).

A total of 18 AUX/IAA genes were visualized using the Integrative Genomic Viewer (IGV), and significant SNVs were not visually observed between R_PA and S_PA in AUX/IAA genes due to high protein similarities in high conserve regions like the degron that can lead to less interaction with TIR1/AFB proteins and consequently no AUX/IAA degradation (Luo et al., 2018). Regions surrounding all the AUX/IAA degrons were carefully inspected due to previous reports that SNVs either in or near can lead to a high auxin level of resistance (Figueiredo et al., 2021; LeClere et al., 2018). Furthermore, TIR1, AFB, and APB were also inspected to determine potential SNVs that may affect dicamba's interaction and cause resistance.

To confirm that potential SNVs were not involved, nucleotide sequences of 18 AUX/IAA, 1 TIR1, 4 AFB, and APB were obtained from the consensus of every sample. The consensus sequences were translated into protein and then aligned to inspect potential SNVs in the genes and regions previously mentioned. Protein sequences were blasted to determine their closest homology and functional annotation in *Arabidopsis thaliana* using the information resource (TAIR; http://arabidopsis.org). Phylogenetic analysis revealed that the protein sequences encoded by

different AUX/IAA proteins in Palmer amaranth are clustered in the same group with their closest homologs in *Arabidopsis thaliana* based on sequence similarities. Additionally, certain AUX/IAA proteins, such as IAA16 in Palmer amaranth, exhibited high homology with their counterparts in *A. thaliana*, indicating they belong to the same phylogenetic group (Figure 2.8).

Read counts obtained after alignment were analyzed to determine and compare the number of reads for AUX/IAA, TIR1, AFB, APB, NCED, and GH3 genes. AUX/IAA (1, 4, 12, and 16) responded similarly in treatments (treated versus non-treated) for R_PA and also for S_PA with no statistical significance (<0.05). While AUX/IAA (2(3), 6, 14, 17, 27(2), and 29) responded differently not only in R_PA treated versus non-treated but also in S_PA. Notably, S_PA treated S_PA plants overexpressed these AUX/IAA compared to treated R_PA plants (Figures 2.9 and 2.10). Isoforms of some AUX/IAA were noticed in the .GTF file. Therefore, they were named by their number in the annotation, followed by a number either 1, 2, or 3.

TIR1 did not respond to dicamba treatment in either biotype (Figure 2.11). On the other hand, the expression of AFB2/3 was lower in dicamba-treated S_PA plants, compared to R_PA plants. The impact of this decrease in gene expression is difficult to interpret. (Figure 2.11). In addition, AFB4/5 expression was the same in both biotypes regardless of treatment. The protein sequences of 5 AFB from the *A. thaliana* genome were blasted in Palmer amaranth genome. The *A. thaliana* AFB 2 and 3 returned the same Palmer amaranth gene, and *A. thaliana* AFB 4 and 5 returned a different Palmer amaranth gene, therefore, they were labeled as AFB2/3 and AFB4/5, respectively. Conversely, ABP was a little overexpressed in untreated R_PA plants compared to treated plants (Figure 2.11).

Key enzymes involved in ethylene biosynthesis (e.g. ACC synthase, ACC oxidase and ACC deaminase) were also analyzed. While the baseline expression of ACC synthase and ACC oxidase was the same in both biotypes, the expression pattern changed in response to dicamba treatment in treated R_PA and S_PA (Figure 2.12). In particular, the expression of ACC oxidase was dramatically greater in treated S_PA plants relative to R_PA plants. On the other hand, the baseline expression of ACC oxidase was much lower in untreated R_PA than S_PA plants, whereas there was no difference in treated plants. (Figure 2.12).

The expression of other genes known to response to AMH, such as NCED and GH3 genes, were also analyzed. The 2 NCED genes identified in the Palmer amaranth genome were named NCED1 and 2. NCED1 was more overexpressed in treated S_PA plants than in treated R_PA

plants. In contrast, there was no difference in NCED2 expression. There was no difference in expression in both untreated populations for NCED1 and NCED2 (Figure 2.13). In the case of the GH3 genes, 5 GH3 were identified in the Palmer amaranth genome. However, two of them had 0 reads after the alignment and were thus removed from the analysis. The other three were named GH3 (1, 2, and 5). All GH3 responded to dicamba treatment in comparison to untreated populations with the exception of GH3(1), which only responded in S_PA and not in R_PA plants (Figure 2.14).

Finally, the expression of phytochrome-interacting factor 4 (PIF3/4) was also analyzed since it participates in auxin regulation, biosynthesis, and environmental response to stress (Franklin et al., 2011). While PIF3/4 expression was the same in untreated plants, it responded to dicamba treatment in the S_PA, whereas there was no change in the R_PA. (Figure 2.15).

GO-term enrichment analysis indicated that DEGs were principally enriched for terms related to transport activity and regulation of metabolic processes. Major candidates were glycosyl transferases, UDP-glycosyltransferases, amino acid transport, as well as regulation of meristem development (Figure 2.16). All genes with increased read counts were included in a table together with their closest homolog in *Arabidopsis thaliana,* based on protein similarity (Table 2.2). In addition, all protein sequences of the previously mentioned genes and their close homologs were validated using TAIR arabidopsis.org (Supplemental Figure 2.6).

DISCUSSION AND CONCLUSION

Our initial hypothesis was that resistance to dicamba in Palmer amaranth would provide cross-resistance to other group 4 herbicides. The R_PA population was resistant to dicamba, with an R/S ±12, which is consistent with that reported in a similar population from Tennessee, with a R/S ratio of 14 (Foster & Steckel, 2022). None of the R_PA plants died at the recommended field rate of dicamba (560 g a.e./ha), while all S_PA plants were controlled at 280 g a.e./ha. The resistance trait present in R_PA is likely due to the increased selection pressure imparted by the recurrent use of Xtend® technology in cotton and soybean fields in Tennessee. The R_PA population also had a low resistance level to 2,4-D (4-fold). This result supports our initial hypothesis about cross-resistance, but the level of 2,4-D resistance is still relatively small, where the R_PA population is still controlled by the field rate of 2,4-D (1120 g a.e/ha) in the greenhouse. However, it is likely that the level of 2,4-D resistance may be greater in the field, which may be a

problem for farmers. Furthermore, the recurrent use of 2,4-D in Enlist-ready crops may lead to increased resistance and low control of Palmer amaranth in that or surrounding areas.

Similar findings were observed in an Arkansas population where after three years of recurrent selection, Palmer amaranth evolved resistance to dicamba (3-fold), suggesting that either sub-lethal or high doses can lead to different herbicide resistance levels (Tehranchian et al., 2017). Other interesting results were reported in populations from Montana, where a population of *Bassia scoparia* had a 5-fold resistance to dicamba with after several years of dicamba use in a crop/fallow small-grain system (Cranston et al., 2001). By contrast, higher resistance levels have been reported in a *B. scoparia* population from Nebraska, with an R/S ratio of ±30-fold. Differences in resistance level may be attributed to pressure selection and due to the recurrent use of dicamba as well as the presence of different resistance mechanisms (Ghanizadeh et al., 2024).

The rate of dicamba metabolism was similar between the biotypes, with half of the active ingredient metabolized (t1/2) within 5 and 6 days. While exposing plants to P450 or GST inhibitors slightly slowed dicamba metabolism, they did not reverse resistance in the R_PA, suggesting that enhanced metabolism is not involved in resistance to dicamba. A similar study was conducted using different biotypes of Palmer amaranth from Tennessee and Texas and also cotton (*Gossypium arboreum*) where the application of malathion in combination with dicamba did not improve control of the R_PA. On the other hand, cotton plants were severely injured, indicating that dicamba was not rapidly metabolized by Palmer amaranth (Foster et al., 2024). This suggests that resistance may have a target-site basis rather than metabolic basis (Foster et al., 2024; Gaines et al., 2020).

Similar findings were reported for dicamba in wild mustard (*Sinapis arvensis*) from Montana where dicamba metabolism was measured, but authors concluded that enhanced metabolism was not the main resistance mechanism due to no differences in the amount of dicamba between resistant and sensitive (Peniuk et al., 1993). In addition, an interesting study was conducted in a dicamba-resistant *Chenopodium album* population from New Zealand, where the metabolism rate did not differ enough to consider enhanced metabolism as the main cause of dicamba resistance (Ghanizadeh et al., 2018). Nonetheless, enhanced metabolism and other related NTSR mechanisms have been reported in AMH resistance in other weed-related species such as *A. tuberculatus* and *K. scoparia* (Chandrima et al., 2022; Figueiredo et al., 2018; Todd et al., 2024).

We also investigated the molecular basis of dicamba resistance using RNA-seq to identify potential SNVs or differences in expression patterns of specific genes such as AUX/IAA or the co-receptors TIR1, AFB, and APB as potential candidates. Our main focus was on AUX/IAA genes due to previous reports that certain SNVs in these genes can impart herbicide resistance. In the related species *B. scoparia* from Nebraska, a point mutation that led to an amino acid change from glycine to asparagine in the highly conserved region (degron) of the AUX/IAA16 (**G**WPPV → **N**WPPV) conferred dicamba, 2,4-D, and fluroxypyr resistance (LeClere et al., 2018). In addition, a similar finding was documented in a *C. album* biotype from New Zealand that amino acid change from glycine to aspartic acid (**G**WPPV → **D**WPPV) in the same highly conserved region of the AUX/IAA16 caused R/S ratio of ±25 folds to dicamba (Ghanizadeh et al., 2024).

However, consensus sequences and protein alignment revealed no SNVs in the degron region of the different AUX/IAA in R_PA. Even though this region is essential for AMH binding, we also inspected for gaps in the degron region since that can also lead to AMH resistance (Figueiredo et al., 2021), but none was found in R_PA. There were no SNVs and gaps in other co-receptors like AFB and APB.

Because there were no SNVs, expression of AUX/IAA, TIR1, AFB, and APB, as well as genes involved in ethylene biosynthesis and auxin response genes such as NCED and GH3 were analyzed. AUX/IAA expression was variable between treatments (treated and non-treated), so these genes were grouped based on their expression pattern between R_PA and S_PA. AUX/IAA 1, 4, 12, and 16 were overexpressed in response to dicamba in both treated R_PA and S_PA biotypes. This suggests that since they respond to dicamba, the variation in their expression might not be the primary factor differentiating R_PA and S_PA (Figure 2.2).

In contrast, other AUX/IAA genes (i.e. AUX/IAA 2(3), 6, 14, 17, 27(2), and 29) responded only in S_PA in response to dicamba treatment. These variations highlight the complex role these genes play in mediating herbicide response. Differences in gene expression can lead to various physiological outcomes, contributing to the survival of R_PA even when exposed to high amounts of herbicide (Figure 2.9). Therefore, gene expression variation in AUX/IAA can influence the ability of R_PA plants to mitigate the herbicide's effects through stress-responsive pathways or altering hormone signaling (Gleason et al., 2011). TIR1 and the receptors AFB2/3, AFB4/5, and APB are key components of the auxin signaling pathway (Parry & Estelle, 2006). However, there were no clear differences in the expression of these genes that could account for resistance (Figure

2.10), suggesting that these receptors may not play a important role in the resistance mechanism to dicamba in R_PA.

Genes related to ethylene biosynthesis had an interesting expression pattern. Expression of ACC synthase, the enzyme producing 1-aminocyclopropane-1-carboxylate (ACC) and the rate-limiting step in ethylene biosynthesis, was slightly higher in R_PA than S_PA. , and a little increase suggests that ethylene production may be differently modulated in response to dicamba in R_PA. On the other hand, expression of ACC oxidase, the enzyme convering ACC to ethylene, was significantly overexpressed in the S_PA plants compared to the R_PA plants under dicamba treatment ($p=0.00311$) (Figure 2.11and supplemental File 2.5). One potential hypothesis is that R_PA plants may have evolved an adaptation mechanism to minimize the ethylene-mediated stress response or a change in the ethylene signaling. This means how the ACC oxidase is regulated or controlled in R_PA but not in S_PA. This is important because ethylene is known to play a crucial role in stress response and herbicide action, so modulating the production of ACC oxidase might reduce the amount of ethylene in the plant, leading to surviving (Grossmann, 2003).

NCED1, a gene involved in abscisic acid (ABA) biosynthesis, was expressed at slightly higher levels in S_AP-treated plants compared to R_AP-treated plants. This suggests that S_AP plants exhibit a more pronounced response to dicamba through the modulation of NCED1, which could be part of a broader stress response mechanism involving ABA (McCauley et al., 2020). In contrast, there was no difference between treated and untreated plants for NCED2. This lack of variation suggests that NCED2 may not play a critical role in the immediate response to dicamba or that its regulation is not as sensitive to herbicide treatment as NCED1. It is potentially possible that NCED2 in stress response is less pronounced than NCED1.

While the baseline expression of PIF3/4 expression were the same between S_PA and R_PA, there was a great difference in expression in treated plants. This is intriguing since PIF3/4 directly activates the expression of IAA29, which was also overexpressed in S_PA, but not in R_PA ($p<0.0001$). The lack of response to dicamba in R_PA might indicate a deregulation of auxin signaling, which can result in auxin insensitivity or dysfunctional signaling (Sun et al., 2013). In addition, because IAA14 is also responding in S_PA and not in R_PA, it may mean that the deregulation of auxin response could be due to alteration of the function of transcription factors like ARF7 or ARF19, which are also called PA IAA22 and AT IAA22. Another finding is that IAA17, also overexpressed in S_PA, is also known as AXR3, an auxin co-receptor that controls

cell elongation, a symptom that resistant plants did not exhibit (Figure 2.7). Therefore, PIF3/4 and AXR3 no longer respond to dicamba in resistant plants. To our knowledge, PIF3/4 or AXR3 have not been reported to confer resistance to dicamba, it might be a potential candidate involved in the resistance mechanism of Palmer amaranth from Tennessee through auxin regulation or signaling; however, further research and validation should be done to elucidate and decipher the resistance mechanism to dicamba, which looks like a novel mechanism. Finally, all GH3 responded to dicamba in both S_AP and R_AP treated plants. This may indicate that both biotypes actively respond to the stress caused by dicamba. Interestingly, GH3_2 showed a little more response in R_PA than S_PA. This might potentially be a result of auxin response to maintain auxin homeostasis in R_PA plants. In contrast, GH3_1 responded significantly in treated S_PA and not in R_PA, so we may assume that dicamba can induce GH3 auxin response more in specific GH3 than others.

In conclusion, the rate of metabolism of dicamba was the same in S_PA and R_PA and resistance was not reversed by metabolic inhibitors of P450 or GST. Therefore, metabolic detoxification is not involved in the resistance to dicamba in the R_PA. Receptors involved in auxin perception do not appear to be involved in the resistance mechanism. Gene expression in key enzymes like ACC-synthase, deaminase, and oxidase involved in ethylene and ABA biosynthesis could be involved in the resistance. However, further research on this key enzyme can be explored as a potential physiological response to dicamba stress. Furthermore, PIF3/4 and AXR3 appear to be related to auxin signaling or regulation in resistant plants, so they can also be potential candidates involved in this particular dicamba resistance case. The resistance mechanism appears to be a novel one not previously reported for AMHs.

FIGURES

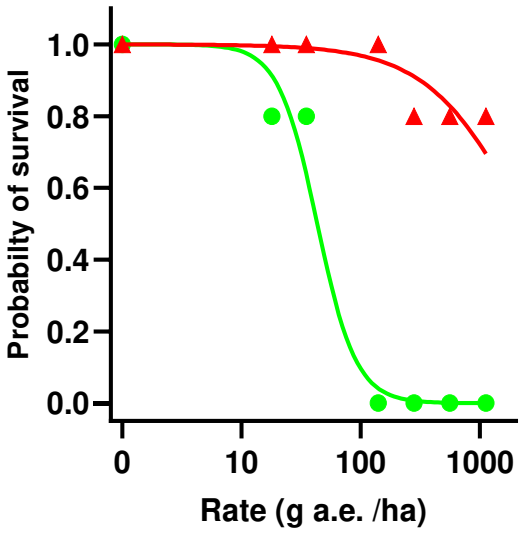


Figure 2.1. Dose-response curves of dicamba on susceptible and resistant Palmer amaranth populations (S_PA (●) and R_PA (▲), respectively). Data represents mean with n = 5 plants for each dose and 7 doses were tested.

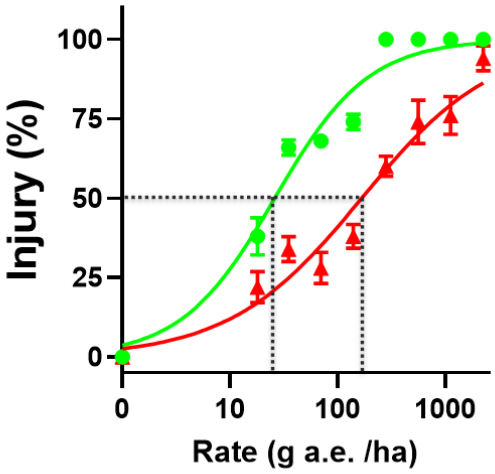


Figure 2.2. Dose-response curves of 2,4-D on susceptible and resistant Palmer amaranth populations (S_PA (●) and R_PA (▲), respectively. Data represents mean with n = 5 plants for each dose and 8 doses were tested.

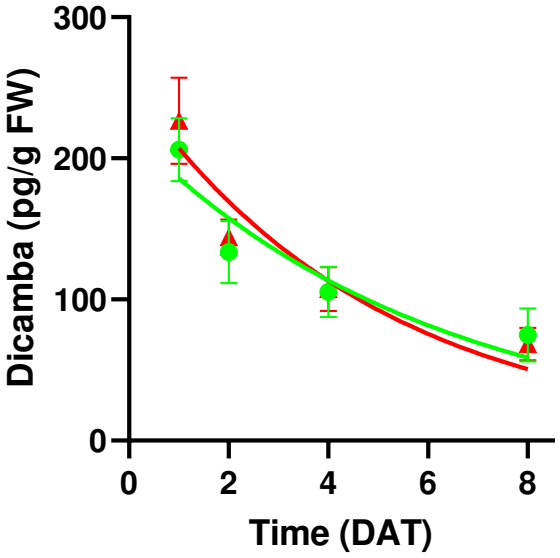


Figure 2.3. Metabolism of dicamba on susceptible and resistant Palmer amaranth populations at 1, 2, 4, and 8 DAT (S_PA (●) and R_PA (▲), respectively. Data represents mean and standard deviation with n = 4 plants for each treatment.

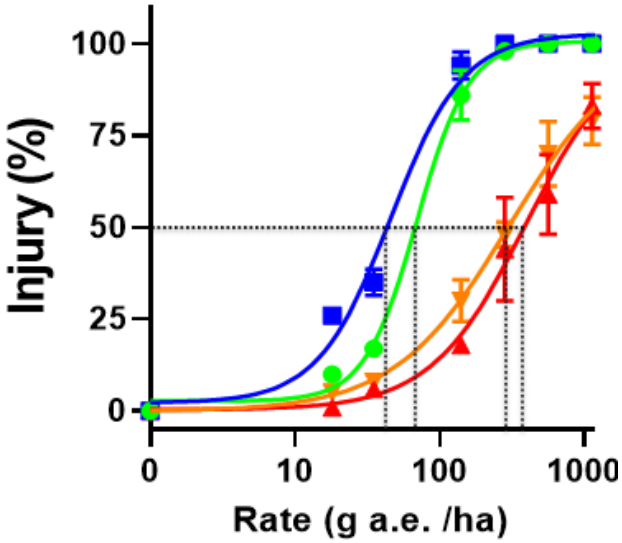


Figure 2.4. Dose-response curves of dicamba with or without malathion on susceptible and resistant Palmer amaranth populations (■=S_PA + malathion; ●=S_PA; ▼=R_PA + malathion; ▲=R_PA), respectively. Data represents mean and standard error with n = 4 plants for each dose.

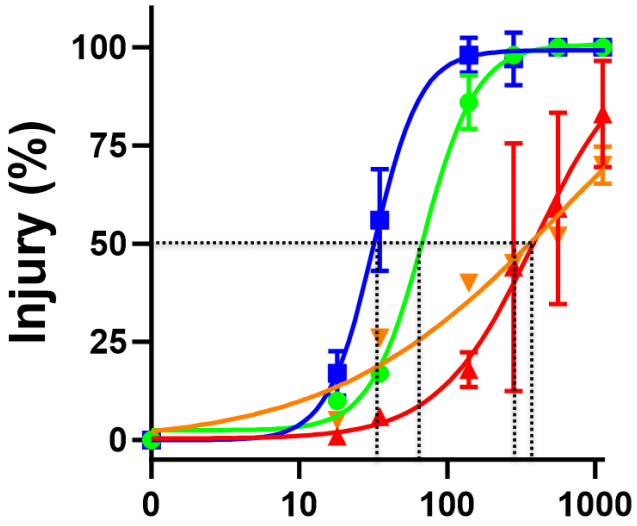


Figure 2.5. Dose-response curves of dicamba with or without NDB on S_PA and R_PA Palmer amaranth populations (■= S_PA + NBD-Cl; ●= S_PA; ▼=R_PA + NBD-Cl; ▲=R_PA), respectively. Data represents mean and standard error with n = 4 plants for each dose.

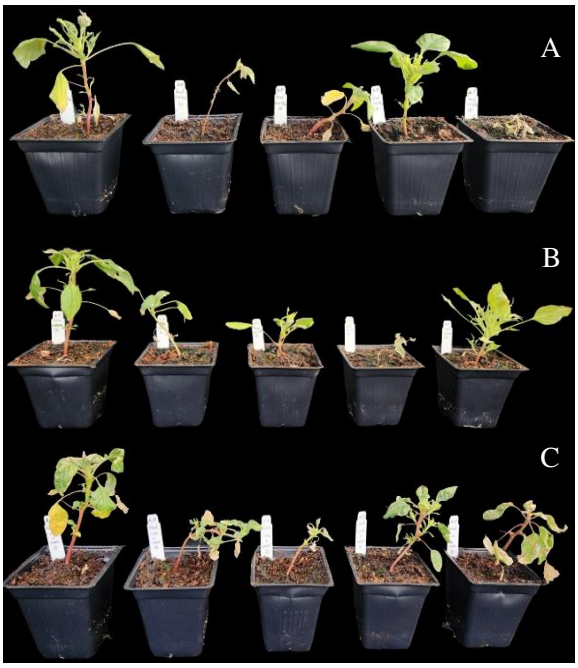


Figure 2.6. The phenotype of Lauderdale Palmer amaranth plants treated with 1,120 g a.e./ha dicamba (2× recommended rate) with and without metabolic inhibitors. A) R_PA at 1,120 g a.e./ha, B) R_PA at 1,120 g a.e./ha + malathion. And C) R_PA at 1,120 g a.e./ha + NBD-Cl.

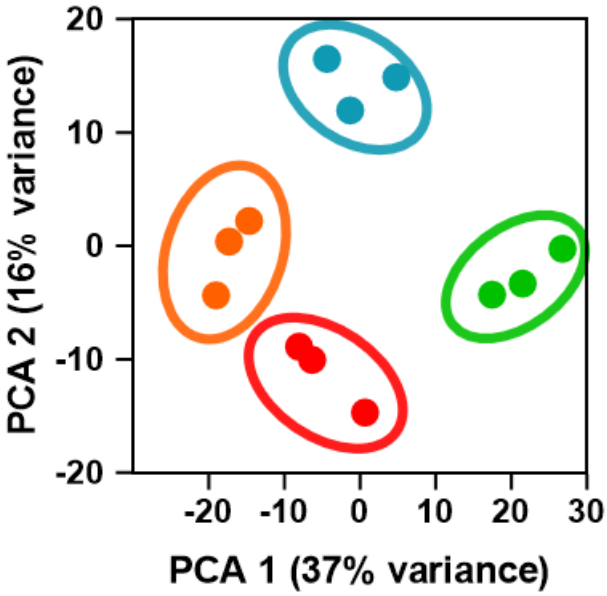


Figure 2.7. Principal component analysis plot of S_PA and R_PA populations of Palmer amaranth before and 6 h after treatment with dicamba. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●.

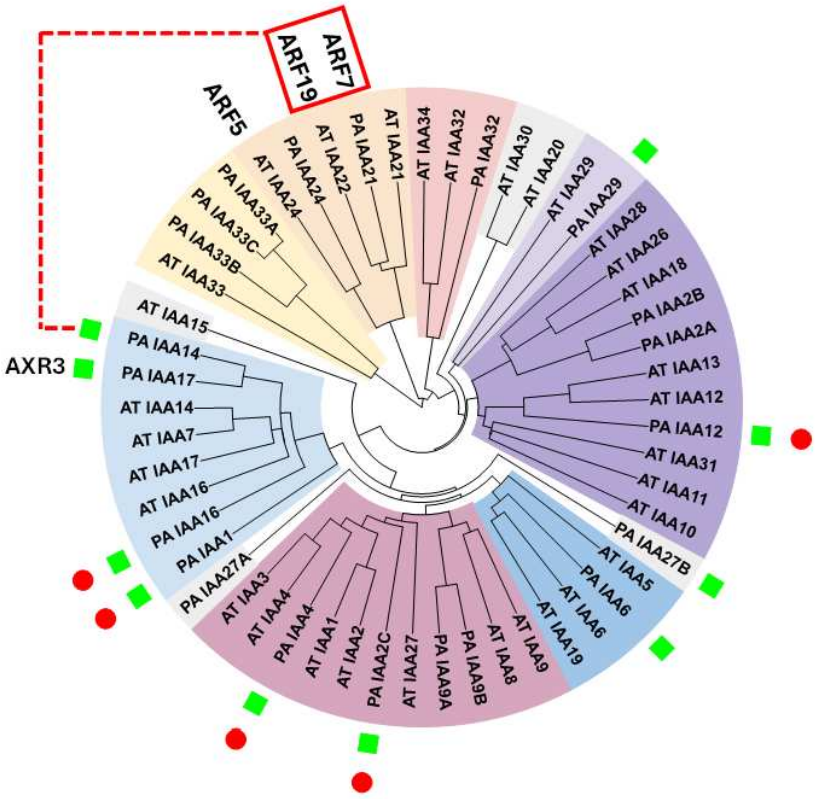


Figure 2.8. Phylogenetic tree analysis of Palmer amaranth AUX/IAA genes in relationship with AUX/IAA genes from *Arabidopsis thaliana.* ■ = upregulated AUX/IAA in S_PA plants, ● = upregulated AUX/IAA in R_PA plants. The dotted line represents the negative regulation of ARF7 (IAA21) and ARF19 (IAA22) by IAA14. IAA17 also known as AXR3 is an auxin co-receptor that controls cell elongation.

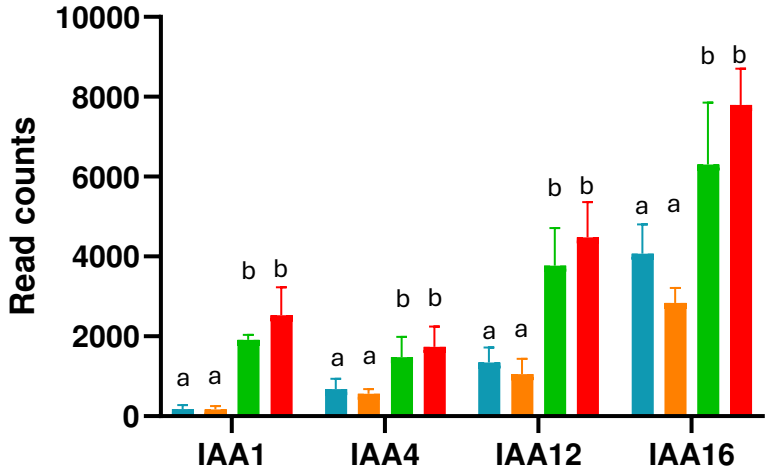


Figure 2.9. Gene expression of four AUX/IAA that responded similarly in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and AUX/IAA genes evaluated ($p < 0.05$).

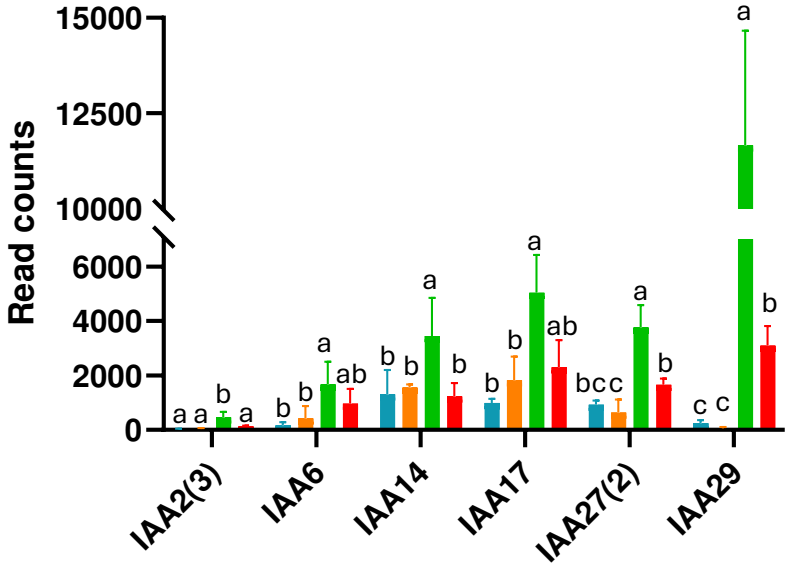


Figure 2.10. Gene expression of six AUX/IAA that responded differently in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and AUX/IAA genes evaluated ($p < 0.05$).

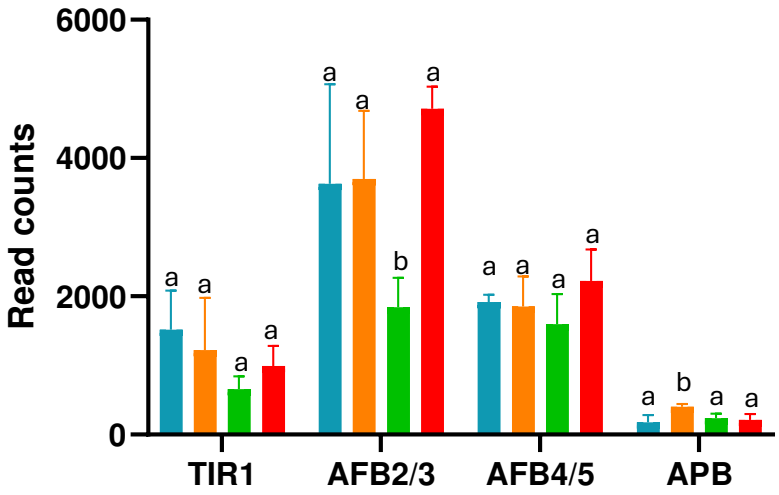


Figure 2.11. Gene expression of one TIR1, two AUXIN-SIGNALING F-BOX (AFB), and one Auxin Binding Protein (ABP) that responded similarly in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and (TIR1, AFB, and APB) genes evaluated ($p < 0.05$).

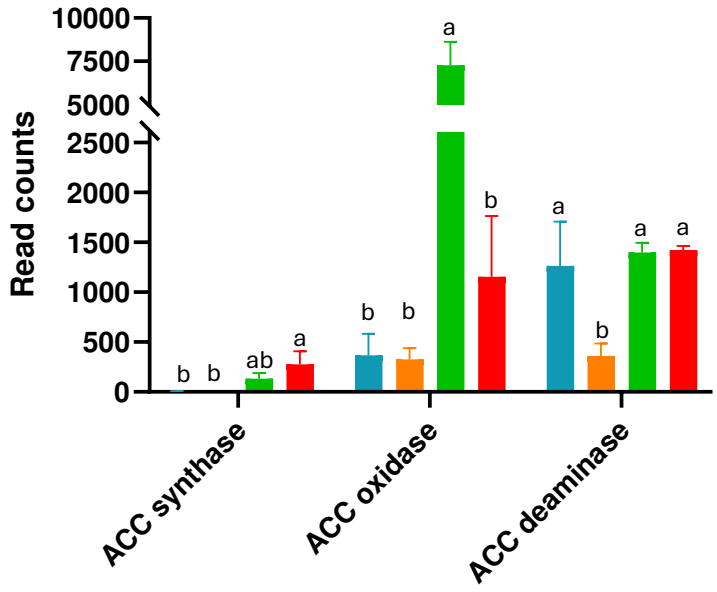


Figure 2.12. Gene expression of the three main enzymes involved in the ethylene biosynthesis pathway in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and ACC genes evaluated ($p < 0.05$).

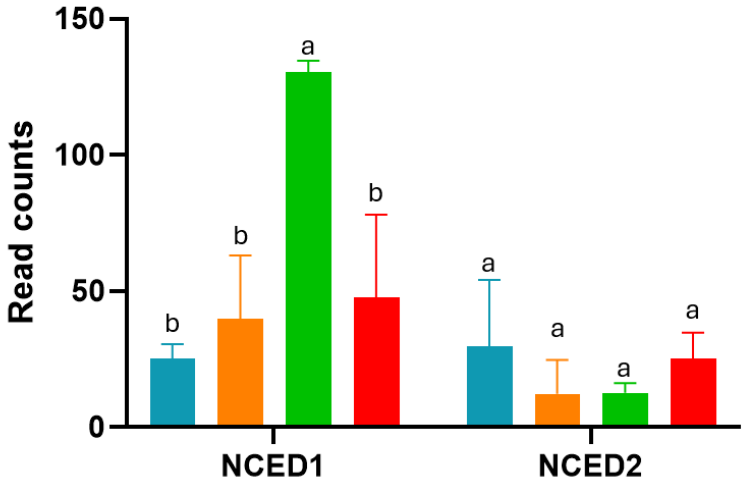


Figure I2.13. Gene expression of the auxin response NCED in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and NCED genes evaluated ($p < 0.05$).

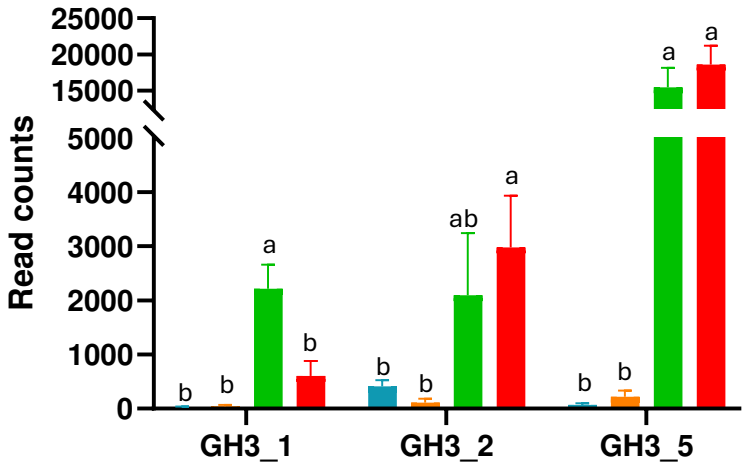


Figure 2.14. Gene expression of the auxin response gene GH3 in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and GH3 genes evaluated ($p < 0.05$).

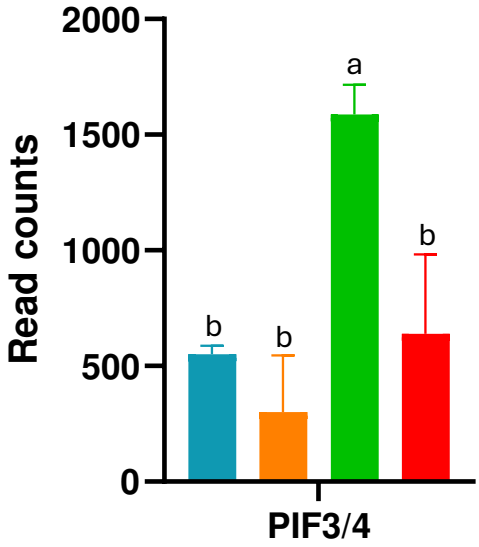


Figure 2.15. Gene expression of PIF 3/4 in treated and untreated R_PA and S_PA populations. R_PA untreated = ●, R_PA treated = ●, S_PA untreated = ●, and S_PA treated = ●. Different letters represent significant differences in read counts within each treatment and PIF3/4 gene evaluated ($p < 0.05$).

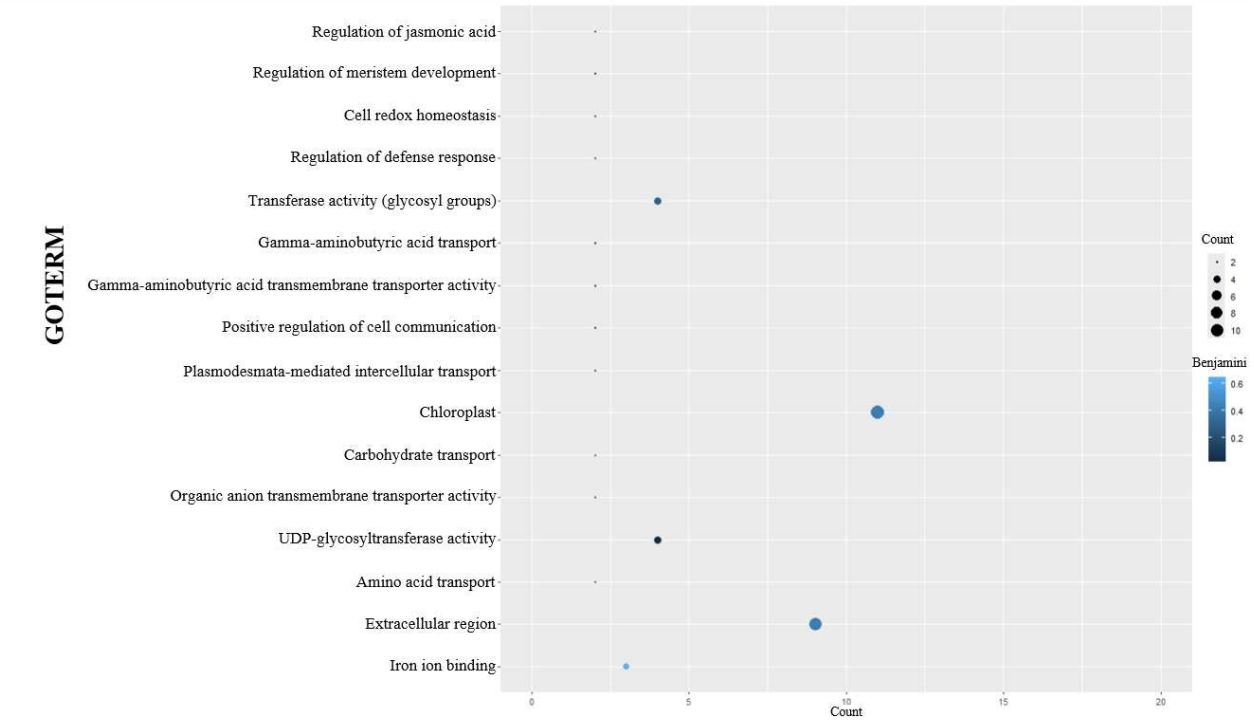


Figure 2.16. Gene ontology (GO-term) enrichment analysis. Circle size represents the read counts of overrepresented enrichment. The color gradient represents the significance based on the Benjamin-Hochberg correction. The x-axis represents the number of genes annotated with each GO-term in the Y-axis.

TABLES

Table 2.1. Effect of cytochrome P450 monooxygenase (malathion) and glutathione *S*-transferase (NBD-Cl) inhibitors efficacy of dicamba on visual injury on R_PA (Lauderdale County, TN) and S_PA (Tucson, AZ) populations.

Populations	**Treatments**	**VI 50 (g a.e./ha)**	**Reduction (%)**
S_PA	Dicamba	69±9	33
	Dicamba + malathion	46±6	
R_PA	Dicamba	390±44	22
	Dicamba + malathion	303±42	
S_PA	Dicamba	69±9	54
	Dicamba + NBD-Cl	32±2	
R_PA	Dicamba	390±41	22
	Dicamba + NBD-Cl	304±436	

REFERENCES

Adamczewski, K., Matysiak, K., Kierzek, R., & Kaczmarek, S. (2019). Significant increase of weed resistance to herbicides in Poland. *Journal of Plant Protection Research*, *59* (2), 139-150. https://doi.org/10.24425/jppr.2019.129293

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Nature Precedings*, *11*, R106. https://doi.org/https://doi.org/10.1186/gb-2010-11-10-r106

Behrens, M. R., Mutlu, N., Chakraborty, S., Dumitru, R., Jiang, W. Z., LaVallee, B. J., Herman, P. L., Clemente, T. E., & Weeks, D. P. (2007). Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. *Science*, *316*(5828), 1185-1188. https://doi.org/10.1126/science.1141596

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*, 1-15. https://doi.org/10.1186/s12302-016-0070-0

Bobadilla, L. K., Giacomini, D. A., Hager, A. G., & Tranel, P. J. (2022). Characterization and inheritance of dicamba resistance in a multiple-resistant waterhemp (*Amaranthus tuberculatus*) population from Illinois. *Weed Science*, *70*, 4-13. https://doi.org/10.1017/wsc.2021.76

Busi, R., Goggin, D. E., Heap, I. M., Horak, M. J., Jugulam, M., Masters, R. A., Napier, R. M., Riar, D. S., Satchivi, N. M., Torra, J., Westra, P., & Wright, T. R. (2018). Weed resistance to synthetic auxin herbicides. *Pest Management Science*, *74*(10), 2265-2276. https://doi.org/https://doi.org/10.1002/ps.4823

Calderón-Villalobos, L., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., Sheard, L. B., Tan, X., Parry, G., Mao, H., Zheng, N., Napier, R., Kepinski, S., & Estelle, M. (2012). A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nature Chemical Biology*, *8*(5), 477–485. https://doi.org/https://doi.org/10.1038/nchembio.926

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC bioinformatics*, *10*, 1-9. https://doi.org/doi:10.1186/1471-2105-10-421

Chandrima, S., E., P. D., & Jugulam, M. (2022). Resistance to 2,4-D in Palmer amaranth (*Amaranthus palmeri*) from Kansas is mediated by enhanced metabolism. *Weed Science*, *70*(4), 390-400. https://doi.org/10.1017/wsc.2022.29.full

Choudhury, P. P., Singh, R., Ghosh, D., & Sharma, A. R. (2016). *Herbicide use in Indian agriculture (information bulletin No. 22)*. Jabalpur, Madhya Pradesh, ICAR - Directorate of Weed Research. https://doi.org/https://dwr.icar.gov.in/Downloads/Information_Bulletin/Information%20Bulletin%20No%20-%2022%20-%20Herbicide%20Use%20in%20Indian%20Agriculture.pdf

Ciccarelli, F. D., Doerks, T., von Mering, C., Creevey, C. J., Snel, B., & Peer, B. (2006). Toward automatic reconstruction of a highly resolved tree of life. *Science*, *311*(5765), 1283-1287. https://doi.org/https://doi.org/10.1126/science.1123061

Cranston, H. J., Kern, A. J., Hackett, J. L., & Miller, E. K. (2001). Dicamba resistance in Kochia. *Weed Science*, *49*(2), 164-170. https://doi.org/doi:10.1614/0043-1745(2001)049[0164:DRIK]2.0.CO;2

Dayan, F. E. (2022). Dicamba-resistant crops - stumbling over the starting block. *Outlooks on Pest Management*, *33*(2). https://doi.org/https://doi.org/10.1564/v33_apr_09

Duke, S. O. (2014). Biotechnology: Herbicide-Resistant Crops. In N. K. Van Alfen (Ed.), *Encyclopedia of Agriculture and Food Systems* (pp. 94-116). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-444-52512-3.00218-7

Figueiredo, M. R. A., Barnes, H., Boot, C. M., Figueiredo, A. B. T. B., Nissen, S. J., Dayan, F. E., & Gaines, T. A. (2022). Identification of a novel 2,4-D metabolic detoxification pathway in 2,4-D-resistant waterhemp (*Amaranthus tuberculatus*). *Journal of Agricultural and Food Chemistry*, *79*(49), 15380-15389. https://doi.org/https://doi.org/10.1021/acs.jafc.2c05908

Figueiredo, M. R. A., Küpper, A., Malone, J. M., Petrovic, T., Figueiredo, A. B. T. B., Campagnola, G., Peersen, O. B., Prasad, K. V. S. K., Patterson, E. L., Reddy, A. S. N., Kubeš, M. F., Napier, R., Dayan, F. E., Preston, C., & Gaines, T. A. (2021). An in-frame deletion mutation in the degron tail of auxin coreceptor *IAA2* confers resistance to the herbicide 2,4-D in *Sisymbrium orientale*. *Proceedings of the National Academy of Sciences*, *119*(9), e2105819119. https://doi.org/10.1073/pnas.2105819119

Figueiredo, M. R. A., Leibhart, L. J., Reicher, Z. J., Tranel, P. J., Nissen, S. J., Westra, P., Bernards, M. L., Kruger, G. R., Gaines, T. A., & Jugulam, M. (2018). Metabolism of 2,4-dichlorophenoxyacetic acid contributes to resistance in a common waterhemp (*Amaranthus tuberculatus*) population. *Pest Management Science*, *10*, 2356-2362. https://doi.org/https://doi.org/10.1002/ps.4811

Foster, D. C., Dotray, P. A., Culpepper , S., & Steckel, L. E. (2024). Response of dicamba-resistant Palmer amaranth and cotton to malathion applied in conjunction with dicamba. *Weed Technology*, *38*(e9), 1-9. https://doi.org/https://doi.org/10.1017/wet.2023.62

Foster, D. C., & Steckel, L. E. (2022). Confirmation of dicamba-resistant Palmer amaranth in Tennessee. *Weed Technology*, *36*(6), 777-780. https://doi.org/10.1017/wet.2022.87

Franklin, K. A., Lee, S. H., Patel, D., Kumar, V. S., Spartz, A. K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J. D., Wigge, P. A., & Gray, W. M. (2011). Phytochrome-Interacting Factor 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proceedings of the National Academy of Sciences*, *108*(50), 20231-20235. https://doi.org/https://doi.org/10.1073/pnas.1110682108

Gaines, T. A. (2020). The quick and the dead: a new model for the essential role of ABA accumulation in synthetic auxin herbicide mode of action. *Journal of Experimental Botany*, *71*(12), 3383-3385. https://doi.org/https://doi.org/10.1093/jxb/eraa178

Gaines, T. A., Duke, S. O., Morran, S., Rigon, C. A. G., Tranel, P. J., Küpper, A., & Dayan, F. E. (2020). Mechanisms of evolved herbicide resistance. *Journal of Biological Chemistry*, *295*(30), 10307-10330. https://doi.org/10.1074/jbc.REV120.013572

Ghanizadeh, H., Harrington, K. C., & James, T. K. (2018). A comparison of dicamba absorption, translocation and metabolism in *Chenopodium album* populations resistant and susceptible to dicamba. *Crop Protection*, *110*, 112-116. https://doi.org/https://doi.org/10.1016/j.cropro.2018.04.007

Ghanizadeh, H., He, L., Griffiths, A. G., Harrington , K. C., Carbone, V., Wu, H., Tian, K., Boe, H., & Xinhuie, D. (2024). A novel mutation in IAA16 is associated with dicamba resistance in *Chenopodium album*. *Pest Management Science*, *80*(7), 3675-3683. https://doi.org/https://doi.org/10.1002/ps.8071

Gleason, C., Foley, R. C., & Singh, K. B. (2011). Mutant analysis in Arabidopsis provides insight into the molecular mode of action of the auxinic herbicide dicamba. *PLOS ONE*, *6*(3), e17245. https://doi.org/https://doi.org/10.1371/journal.pone.0017245

Green, J. M., & Siehl, D. L. (2021). History and outlook for glyphosate-resistant crops. In (pp. 24). Springer International Publishing. https://doi.org/10.1007/398_2020_54
Grossmann, K. (2003). Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation*, *22*, 109-112. https://doi.org/https://doi.org/10.1007/s00344-003-0020-0
Grossmann, K. (2009). Auxin herbicides: current status of mechanism and mode of action. *Pest Management Science*, *66*(2), 113-120. https://doi.org/10.1002/ps.1860
Heap, I. (2014). Global perspective of herbicide-resistant weeds. *Pest Management Science*, *70*(9), 1306-1315. https://doi.org/10.1002/ps.3696
Heap, I. (2024). *The international survey of herbicide resistant weeds*. Retrieved October from https://weedscience.org/Home.aspx
HRAC. (2024). *Global herbicide resistance action commitee* Retrieved 10/10/2024 from https://hracglobal.com/
Jugulam, M., & Shyam, C. (2019). Non-target-site resistance to herbicides: recent developments. *Plants*, *8*(10), 417. https://doi.org/10.3390/plants8100417
Katoh, K., Kuma, K.-i., Toh, H., & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic acids research*, *33*(2), 511-518. https://doi.org/https://doi.org/10.1093/nar/gki198
Klingaman, T. E., & Oliver, L. R. (1994). Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). *Weed Science*, *42*(4), 523-527. https://www.jstor.org/stable/4045448
Knezevic, S. Z., Streibig, J. C., & Ritz, C. (2017). Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology*, *21*(3), 840-848.
Kniss, A., & Streibig, J. (2021). *Statistical analysis of agricultural experiments using R*.
Kumar, V., Currie, R. S., Jha, P., & Stahlman, P. W. (2019). First report of Kochia (*Bassia scoparia*) with cross-resistance to dicamba and fluroxypyr in western Kansas. *Weed Technology*, *33*(2), 335-341. https://doi.org/https://doi.org/10.1017/wet.2018.113
LeClere, S., Wu, C., Westra, P., & Sammons, D. R. (2018). Cross-resistance to dicamba, 2,4-D, and fluroxypyr in *Kochia scoparia* is endowed by a mutation in an AUX/IAA gene. *Proceedings of the National Academy of Sciences*, *115*(13), E2911-E2920. https://doi.org/https://doi.org/10.1073/pnas.1712372115
Lewis, K. A., Tzilivakis, J., Warner, D., & Green, A. (2006). *Pesticide Properties DataBase-University of Hertfordshire*. Retrieved October from https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm
Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754-1760. https://doi.org/doi:10.1093/bioinformatics/btp324
Luo, J., Zhou, J.-J., & Zhang, J.-Z. (2018). Aux/IAA gene family in plants: molecular structure, regulation, and function. *International Journal of Molecular Sciences*, *19*(1), 259. https://doi.org/doi:10.3390/ijms19010259
Massinga, R. A., Currie, R. S., Horak, M. J., & Boyer, J. (2001). Interference of Palmer amaranth in corn. *Weed Science*, *49*(2), 202-208. https://doi.org/https://doi.org/10.1614/0043-1745(2001)049[0202:IOPAIC]2.0.CO;2
McCauley, C. L., McAdam, S. A. M., Bhide, K., Thimmapuram, J., Banks, J. A., & Young , B. G. (2020). Transcriptomics in *Erigeron canadensis* reveals rapid photosynthetic and

hormonal responses to auxin herbicide application. *Journal of Experimental Botany*, *71*(12), 3701-3709. https://doi.org/doi:10.1093/jxb/eraa124
McSteen, P. (2010). Auxin and monocot development. *Cold Spring Harbor perspectives in biology*, *2*(3), a001479. https://doi.org/10.1101/cshperspect.a001479
Moreno-Serrano, D., Gaines, T. A., & Dayan, F. E. (2024). Current status of auxin-mimic herbicides. *Outlooks on Pest Management*, *35*(3), 105-112. https://doi.org/https://doi.org/10.1564/v35_jun_04
Morgan, G. D., Baumann , P. A., & Chandler, J. M. (2001). Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. *Weed Technology*, *15*(3), 408-412. https://doi.org/https://doi.org/10.1614/0890-037X(2001)015[0408:CIOPAA]2.0.CO;2
Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *144*(1), 31-43. https://doi.org/https://doi.org/10.1017/S0021859605005708
Parry, G., & Estelle, M. (2006). Auxin receptors: a new role for F-box proteins. *Current opinion in cell biology*, *18*(2), 152-156. https://doi.org/https://doi.org/10.1016/j.ceb.2006.02.001
Peniuk, M. G., Romano, M. L., & Hall, J. C. (1993). Physiological investigations into the resistance of a wild mustard (*Sinapis arvensis* L.) biotype to auxinic herbicides. *Weed Research*, *33*(6), 431-440. https://doi.org/https://doi.org/10.1111/j.1365-3180.1993.tb01959.x
Pettinga, D. J., Ou, J., Patterson, E. L., Jugulam, M., Westra, P., & Gaines, T. A. (2018). Increased chalcone synthase (CHS) expression is associated with dicamba resistance in *Kochia scoparia*. *Pest Management Science*, *74*, 2306-2315. https://doi.org/https://doi.org/10.1002/ps.4778
Rangani, G., Rouse, C. E., Saski, C., Noorai, R. E., Shankar, V., Lawton-Rauh, A. L., Werle, I. S., & Roma-Burgos, N. (2022). High resistance to quinclorac in multiple-resistant *Echinochloa colona* associated with elevated stress tolerance gene expression and enriched xenobiotic detoxification pathway. *Genes*, *13*(515), 1-19. https://doi.org/https://doi.org/10.3390/genes13030515
Riar, D. S., Burke, I. C., Yenish, J. P., Bell, J., & Gill, K. (2011). Inheritance and physiological basis for 2,4-D resistance in Prickly lettuce (*Lactuca serriola* L.). *Journal of Agricultural and Food Chemistry*, *59*(17), 9417-9423. https://doi.org/dx.doi.org/10.1021/jf2019616
Rigon, C. A. G., Gaines, T. A., Küpper, A., & Dayan, F. E. (2020). Metabolism-based herbicide resistance, the major threat among the non-target site resistance mechanisms. *Outlooks on Pest Management*, *31*(4), 162-168. https://doi.org/https://doi.org/10.1564/v31_aug_04
Riter, L. S., Pai, N., Vieira, B. C., MacInnes, A., Reiss, R., Hapeman, C. J., & Kruger, G. (2021). Conversations about the future of dicamba: The science behind off-target movement. *Journal of Agricultural and Food Chemistry*, *69*(48), 14435-14444. https://doi.org/10.1021/acs.jafc.1c05589
Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *Plos One 10*(12), e0146021. https://doi.org/10.1371/journal.pone.0146021
Sack, C., Vonderbrink, J., Smoker, M., & Smith, R. E. (2015). Determination of acid herbicides using modified QuEChERS withFast switching ESI+/ESI−LC-MS/MS. *Journal of Agricultural and Food Chemistry*, *63*(43), 9657-9665. https://doi.org/10.1021/acs.jafc.5b04093

Santner, A., & Estelle, M. (2010). The ubiquitin-proteasome system regulates plant hormone signaling. *The plant journal*, *61*, 1029-1040. https://doi.org/https://doi.org/10.1111/j.1365-313X.2010.04112.x

Sarangi, D., Jhala, A. J., Govindasamy, P., & Brusa, A. (2021). *Amaranthus spp.* In B. Chauhan (Ed.), *Biology and Management of Problematic Crop Weed Species* (pp. 21-43). Elsevier, Inc. https://doi.org/https://doi.org/10.1016/C2019-0-04831-5

Sherman, B. T., Hao, M., Qiu, J., Jiao, X., Baseler, M. W., Lane, H. C., Imamichi, T., & Chang, W. (2022). DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic acids research*, *50*, 216 - 221.

Smalle, J., & Vierstra, R. D. (2004). The ubiquitin 26S proteasome proteolytic pathway. *Annual Review of Plant Biology*, *55*, 550-590. https://doi.org/https://doi.org/10.1146/annurev.arplant.55.031903.141801

Soltani, N., Oliveira, M. C., Alves, G. S., Werle, R., Norsworthy, J. K., Sprague, C. L., Young, B. G., Reynolds, D. B., Brown, A., & Sikkema, P. H. (2020). Off-target movement assessment of dicamba in North America. *Weed Technology*, *34*(3), 318-330. https://doi.org/10.1017/wet.2020.17.full

Sosnoskie, L. M., Webster, T. M., Kichler, J. M., MacRae, A. W., Grey, T. L., & Culpepper, S. A. (2012). Pollen-mediated dispersal of glyphosate-resistance in Palmer amaranth under field conditions. *Weed Science*, *60*(3), 366-373. https://doi.org/doi:10.1614/WS-D-11-00151.1

Steckel, L. (2020). *Dicamba-resistant Palmer amaranth in Tennessee: stewardship even more important*. Retrieved 10/3/2024 from https://news.utcrops.com/2020/07/dicamba-resistant-palmer-amaranth-in-tennessee-stewardship-even-more-important/

Storkey, J., & Neve, P. (2018). What good is weed diversity? *Weed Research*, *58*(4), 239-243. https://doi.org/10.1111/wre.12310

Sun, J., Qi, L., Li, Y., Zhai, Q., & Li, C. (2013). PIF4 and PIF5 transcription factors link blue Light and auxin to regulate the phototropic response in Arabidopsis. *The Plant Cell*, *25*(6), 2102-2114. https://doi.org/https://doi.org/10.1105/tpc.113.112417

Tan, X., Calderon-Villalobos, L. I. A., Sharon, M., Zheng, C., Robinson, C. V., Estelle, M., & Zhen, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*, *446*(7136), 640-645. https://doi.org/doi:10.1038/nature05731

Team, R. C. (2020). R: A language and environment for statistical computing, R foundation for statistical. *Computing*. https://www.R-project.org/

Tehranchian, P., Norsworthy, J. K., Powles, S., Bararpour, M. T., Bagavathiannan, M. V., Barber, T., & Scott, R. C. (2017). Recurrent sublethal-dose selection for reduced susceptibility of Palmer amaranth (*Amaranthus palmeri*) to dicamba. *Weed Science*, *65*(2), 206-212. https://doi.org/DOI: 10.1017/wsc.2016.27

Todd, O. E., Figueiredo, M. R. A., Morran, S., Soni, N., Preston, C., Kubeš, M. F., Napier, R., & Gaines, T. A. (2020). Synthetic auxin herbicides: finding the lock and key to weed resistance. *Plant Science*, *300*, 110631. https://doi.org/https://doi.org/10.1016/j.plantsci.2020.110631

Todd, O. E., Patterson, E. L., Westra, E. P., Nissen, S. J., Simões Araujo, A. L., Kramer, W. B., Dayan, F. E., & Gaines, T. A. (2024). Enhanced metabolic detoxification is associated with fluroxypyr resistance in *Bassia scoparia*. *Plant direct*, *8*(1), e560. https://doi.org/https://doi.org/10.1002/pld3.560

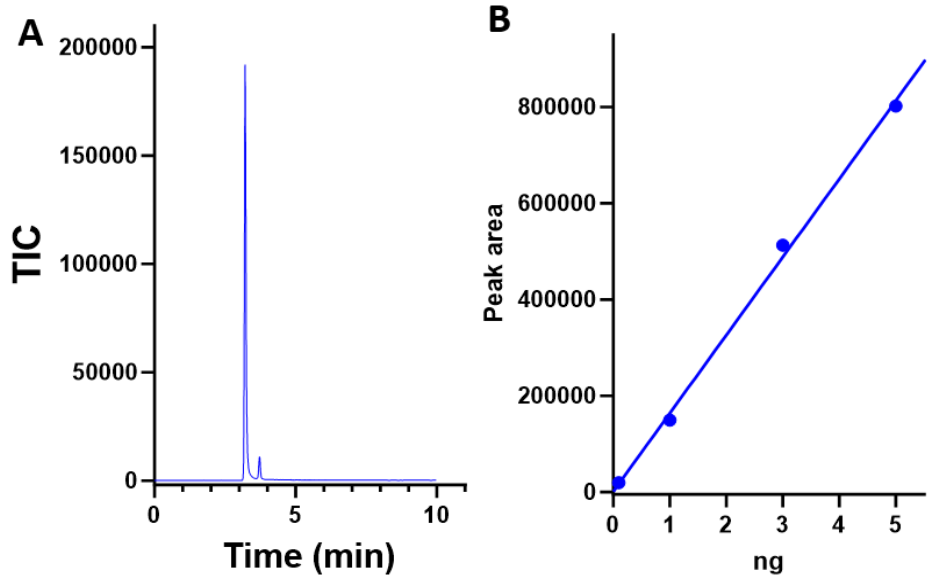
USGS. (2024). *Estimated annual agricultural pesticide use*. U.S. geological survey. Retrieved 10/2024 from https://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php

Villanueva, R. A. M., & Chen, Z. J. (2019). *Ggplot2: Elegant graphics for data analysis*. https://doi.org/https://doi.org/10.1007/978-3-319-24277-4_9

Ward, S. M., Webster, T. M., & Steckel, L. E. (2013). Palmer amaranth (*Amaranthus palmeri*): a review. *Weed Technology*, *27*(1), 12-27. https://doi.org/10.1614/wt-d-12-00113.1

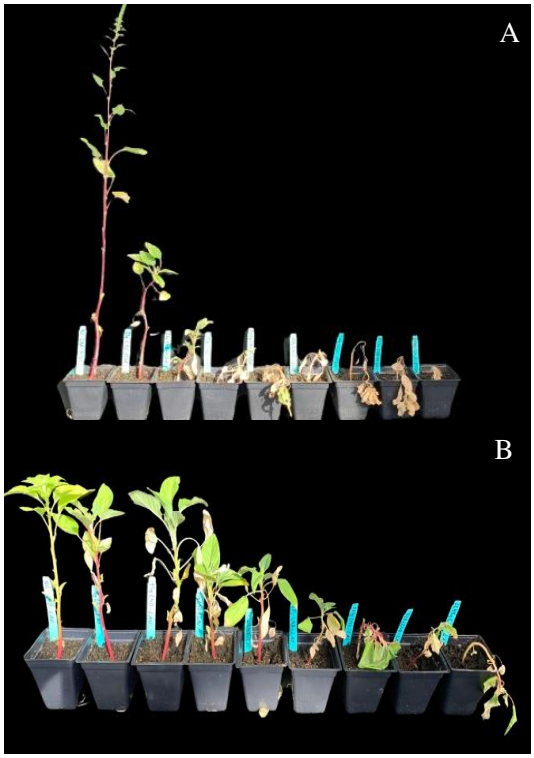
Williams, L. J., & Abdi, H. (2010). Fisher's least significant difference (LSD) test. *Encyclopedia of research design*, *218*(4), 840-853.

APPENDICES



Supplemental File 2.1: LC-MS/MS analysis of dicamba. A) Chromatographic separation of the dicamba technical grade. B) Calibration curve of dicamba.

Supplemental File 2.2: Visual injury caused by dicamba. A) sensitive B) resistant Palmer amaranth biotypes 21 DAT. Doses are expressed in g a.e./ha, and the lowest to highest doses start from left to right.



Supplemental File 2.3: Visual injury caused by 2,4-D. A) sensitive B) resistant Palmer amaranth biotypes 21 DAT. Doses are expressed in g a.e./ha, and the lowest to highest doses start from left to right.

Supplemental File 2.4. Sequences use for phylogenetic analysis

Palmer amaranth IAA sequences

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Arabidopsis IAA sequences

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>AT02 AT3G23030
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>AT11 AT4G28640
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IFLLVSYMFSL
>AT12 AT1G04550
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>AT14 AT4G14550 Functions as a negative regulator of ARF7/19
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>AT15 AT1G80390
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>AT18 AT1G51950
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>AT20 AT2G46990
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>AT31 AT3G17600
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Supplemental File 2.5.
ANOVA and Fisher's protected LSD analyses

1. IAA read counts

IAA1

```
            Df   Sum Sq Mean Sq F value   Pr(>F)
sample       3 13096871 4365624   33.65 6.94e-05 ***
Residuals    8  1037975  129747
          rc groups
LT 2529.0000      a
AT 1912.0000      a
AC  183.0000      b
LC  171.3333      b
```

IAA2_1

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 2534622  844874   3.544 0.0677 .
Residuals    8 1906959  238370
          rc groups
LT 1745.6667      a
AT  911.3333     ab
LC  755.6667      b
AC  528.6667      b
```

IAA2_2

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 1414865  471622   6.982 0.0127 *
Residuals    8  540407   67551
T 1763.000       a
AT 1621.000      ab
AC 1262.333      bc
LC  877.000       c
```

IAA2_3

```
            Df Sum Sq Mean Sq F value  Pr(>F)
sample       3 367487  122496   11.46 0.00288 **
Residuals    8  85482   10685
          rc groups
AT 456.66667      a
LT 128.33333      b
LC  35.00000      b
AC  26.33333      b
```

IAA4

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 3034313 1011438   6.766 0.0138 *
Residuals    8 1195926  149491
          rc groups
LT 1738.0000      a
AT 1480.6667      a
AC  680.3333      b
LC  566.6667      b
```

IAA6

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 3960921 1320307   4.409 0.0415 *
Residuals    8 2395535  299442
          rc groups
AT 1669.0000      a
LT  969.0000     ab
LC  428.6667      b
AC  168.3333      b
```

IAA9_1

```
            Df   Sum Sq  Mean Sq F value Pr(>F)
sample       3 63403670 21134557   7.183 0.0117 *
Residuals    8 23536789  2942099
         rc groups
```

```
LT 9663.000      a
AT 7248.333     ab
LC 4697.333     bc
AC 3777.000      c
```
IAA9_2
```
            Df   Sum Sq Mean Sq F value Pr(>F)
sample       3 10524751 3508250     3.5 0.0695 .
Residuals    8  8018885 1002361
         rc groups
LT 4059.333      a
AT 3302.000     ab
AC 1890.667      b
LC 1881.000      b
```
IAA12
```
            Df   Sum Sq Mean Sq F value   Pr(>F)
sample       3 26574733 8858244    18.2 0.000622 ***
Residuals    8  3893995  486749
         rc groups
LT 4478.333      a
AT 3772.000      a
AC 1344.000      b
LC 1052.667      b
```
IAA14
```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 9985482 3328494   4.451 0.0405 *
Residuals    8 5982212  747776
AT 3452.333      a
LC 1552.667      b
AC 1308.000      b
LT 1230.333      b
```
IAA16
```
            Df   Sum Sq  Mean Sq F value  Pr(>F)
sample       3 44371333 14790444    15.1 0.00117 **
Residuals    8  7835864   979483
         rc groups
LT 7792.000      a
AT 6305.667      a
AC 4071.667      b
LC 2836.333      b
```
IAA17
```
            Df   Sum Sq Mean Sq F value Pr(>F)
sample       3 22925119 7641706   4.658 0.0364 *
Residuals    8 13123458 1640432
          rc groups
AT 4709.0000      a
LT 2633.6667     ab
LC 1826.3333      b
AC  984.6667      b
```
IAA27_1
```
            Df Sum Sq Mean Sq F value Pr(>F)
sample       3 134210   44737   1.747  0.235
Residuals    8 204878   25610
         rc groups
LT 514.3333      a
LC 322.3333      a
AC 258.0000      a
AT 254.3333      a
```
IAA27_2
```
            Df   Sum Sq Mean Sq F value   Pr(>F)
sample       3 18033216 6011072   24.92 0.000206 ***
Residuals    8  1929593  241199
AT 3770.0000      a
LT 1658.0000      b
```

```
AC  927.3333     bc
LC  634.0000      c
```
IAA29
```
            Df    Sum Sq  Mean Sq F value   Pr(>F)
sample       3 266430133 88810044   36.95 4.92e-05 ***
Residuals    8  19230482  2403810
            rc groups
AT 11650.66667      a
LT  3095.00000      b
AC   240.33333      c
LC    56.33333      c
```

2. TIR1 and f-box read counts

TIR1
```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 1194145  398048   1.585  0.267
Residuals    8 2009031  251129
          rc groups
AC 1517.6667      a
LC 1223.0000      a
LT  992.0000      a
AT  656.3333      a
```
AFB2/3
```
            Df   Sum Sq Mean Sq F value Pr(>F)
sample       3 12808018 4269339    5.14 0.0285 *
Residuals    8  6644353  830544
         rc groups
LT 4711.000      a
LC 3697.000      a
AC 3628.667      a
AT 1841.333      b
```
AFB4/5
```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3  592145  197382    1.32  0.334
Residuals    8 1196048  149506
         rc groups
LT 2222.000      a
AC 1915.333      a
LC 1854.333      a
AT 1597.667      a
```
APB
```
            Df Sum Sq Mean Sq F value Pr(>F)
sample       3  90758   30253   5.043 0.0299 *
Residuals    8  47995    5999
LC 403.3333      a
AT 239.0000      b
LT 214.0000      b
AC 175.3333      b
```

3. Ethylene biosynthetic read counts

ACC synthase
```
            Df Sum Sq Mean Sq F value Pr(>F)
sample       3 154642   51547    3.31 0.0781 .
Residuals    8 124573   15572
           rc groups
LT 276.333333      a
AT 134.000000     ab
AC   3.333333      b
LC   0.000000      b
```
ACC oxidase
```
            Df    Sum Sq  Mean Sq F value  Pr(>F)
sample       3 133520321 44506774   11.19 0.00311 **
```

```
Residuals    8  31830556  3978820
          rc groups
AT 8281.3333      a
LT 1153.6667      b
AC  366.3333      b
LC  327.0000      b
```
ACC deaminase
```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 2296893  765631   4.558 0.0383 *
Residuals    8 1343900  167988
          rc groups
LT 1422.3333      a
AT 1397.3333      a
AC 1265.0000      a
LC  360.6667      b
```

4. NCED read counts

NCED1

AmaPaChr08Ag141780
```
            Df Sum Sq Mean Sq F value Pr(>F)
sample       3  20136    6712   5.916 0.0199 *
Residuals    8   9077    1135
          rc groups
AT 130.33333      a
LT  47.66667      b
LC  39.66667      b
AC  25.33333      b
```
NCED2

AmaPaChr16Ag245310
```
            Df Sum Sq Mean Sq F value Pr(>F)
sample       3    703   234.4    0.37  0.777
Residuals    8   5075   634.3
         rc groups
AC 29.66667      a
LT 25.33333      a
AT 12.66667      a
LC 12.33333      a
```

5. GH3 read counts

AmaPaChr01Ag019120

GH3_1
```
            Df  Sum Sq Mean Sq F value  Pr(>F)
sample       3 9569777 3189926   15.68 0.00103 **
Residuals    8 1627542  203443
          rc groups
AT 2217.33333      a
LT  605.66667      b
LC   43.66667      c
AC   29.00000      c
```
AmaPaChr03Ag050530

GH3_2
```
            Df   Sum Sq Mean Sq F value Pr(>F)
sample       3 16877262 5625754   3.348 0.0763 .
Residuals    8 13441613 1680202
          rc groups
LT 2979.0000      a
AT 2096.3333     ab
AC  410.0000      b
LC  108.6667      b
```
AmaPaChr05Ag087400

GH3_3
```
            Df Sum Sq Mean Sq F value Pr(>F)
```

```
sample       3  13296    4432   2.476  0.136
Residuals    8  14319    1790
AT 99.66667      a
LT 62.33333     ab
LC 22.66667     ab
AC 17.33333      b
```

AmaPaChr05Ag093880
GH3_4

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 1814210  604737   0.673  0.592
Residuals    8 7186752  898344
     rc groups
LT 1776      a
LC 1070      a
AT 1068      a
AC  703      a
```

AmaPaChr10Ag169160
GH3_5

```
            Df    Sum Sq   Mean Sq F value  Pr(>F)
sample       3 867918898 289306299   27.23 0.00015 ***
Residuals    8  84982628  10622828
            rc groups
LT 18580.33333      a
AT 15432.00000      a
LC   221.33333      b
AC    66.33333      b
```

AmaPaChr16Ag247120
GH3_6

```
            Df  Sum Sq Mean Sq F value Pr(>F)
sample       3 1254317  418106    2.12  0.176
Residuals    8 1577622  197203
         rc groups
AT 990.6667      a
LT 522.3333      a
AC 212.0000      a
LC 190.6667      a
```

6. PIF 3/4 read counts

AmaPaChr16Ag238940
PIF3/4

```
            Df  Sum Sq Mean Sq F value   Pr(>F)
sample       3 2860025  953342   19.45 0.000494 ***
Residuals    8  392065   49008

          rc groups
AT 1587.0000      a
LT  639.0000      b
AC  550.6667      b
LC  300.3333      b
```

Supplemental File 2.6.

Gene list of important auxin response genes in Palmer amaranth relative to *Arabidopsis thaliana*.

Gene family	*Amaranthus palmeri* genes	*Amaranthus palmeri* annotation	Homologue in *Arabidopsis thaliana*	*Arabidopsis thaliana* annotation
AUX/IAA	AmaPaChr10Ag173290	auxin-responsive protein IAA9_1	AT5G65670	IAA9
	AmaPaChr02Ag044990	auxin-responsive protein IAA9_2	AT2G22670	IAA8
	AmaPaChr05Ag088380	auxin-responsive protein IAA12	AT1G04550 AT2G33310	IAA12 IAA13
	AmaPaChr09Ag160520	auxin-responsive protein IAA14	AT3G23050 AT4G14550	IAA7 IAA14
	AmaPaChr03Ag060180	auxin-responsive protein IAA16	AT3G04730	IAA16
	AmaPaChr14Ag215810	auxin-responsive protein IAA27_1	AT4G29080	IAA27
	AmaPaChr01Ag005070	auxin-responsive protein IAA27_2	AT4G29080	IAA27
	AmaPaChr08Ag133280	auxin-responsive protein IAA29	AT4G32280	IAA29
	AmaPaChr02Ag039830	auxin-responsive protein IAA32	AT2G01200	IAA32
	AmaPaChr05Ag098480	auxin-responsive protein IAA33_1	AT5G57420	IAA33
	AmaPaChr08Ag138410	auxin-responsive protein IAA33_2	AT5G57420	IAA33
	AmaPaChr12Ag196030	auxin-responsive protein IAA33_3	AT5G57420	IAA33
	AmaPaChr01Ag022970	auxin-responsive protein IAA1	AT4G14560	IAA1
	AmaPaChr01Ag005320	auxin-responsive protein IAA2_1	AT3G16500	IAA26
	AmaPaChr01Ag017820	auxin-responsive protein IAA2_2	AT5G25890	IAA28
	AmaPaChr03Ag060140	auxin-responsive protein IAA2_3	AT5G43700	IAA4
	AmaPaChr03Ag060220	auxin-responsive protein IAA4	AT5G43700	IAA4
	AmaPaChr01Ag022960	auxin-induced protein IAA6	AT3G15540	IAA19
APB	AmaPaChr05Ag087460	APB	AT4G02980	Auxin binding protein 1
ACC-Synthase	AmaPaChr10Ag168620	ACC-Synthase	AT4G37770	ACC-synthase 8
ACC-Oxidase	AmaPaChr07Ag127620	ACC-Oxidase	AT1G77330	ACC-oxidase
ACC-Deaminase	AmaPaChr08Ag141530	ACC-deaminase	AT1G48420	ACC-deaminase
NCED	AmaPaChr08Ag141780	NCED (1)	AT1G30100	NCED 5
	AmaPaChr16Ag245310	NCED (2)	AT3G14440	NCED3
GH3	AmaPaChr01Ag019120	GH3_1	AT2G14960	GH3.1
	AmaPaChr03Ag050530	GH3_2	AT5G54510	GH3.6
	AmaPaChr10Ag169160	GH3_5	AT4G37390	GH3.2

PIF (T. Factor)	AmaPaChr16Ag238940	PIF3	AT1G09530	PIF4

Gene family	*Arabidopsis thaliana* ID	*Arabidopsis thaliana* annotation	*Amaranthus palmeri* homolog	*Amaranthus palmeri* annotation
Transport Inhibitor Response 1 (TIR1)	AT4G03190	AFB1	AmaPaChr03Ag060700	TIR1
Auxin Signaling F-BOX 2 (AFB)	AT3G26810	AFB2	AmaPaChr02Ag042540	AFB2/3
	AT1G12820	AFB3		
	AT4G24390	AFB4	AmaPaChr07Ag125910	AFB4/5
	AT5G49980	AFB5		