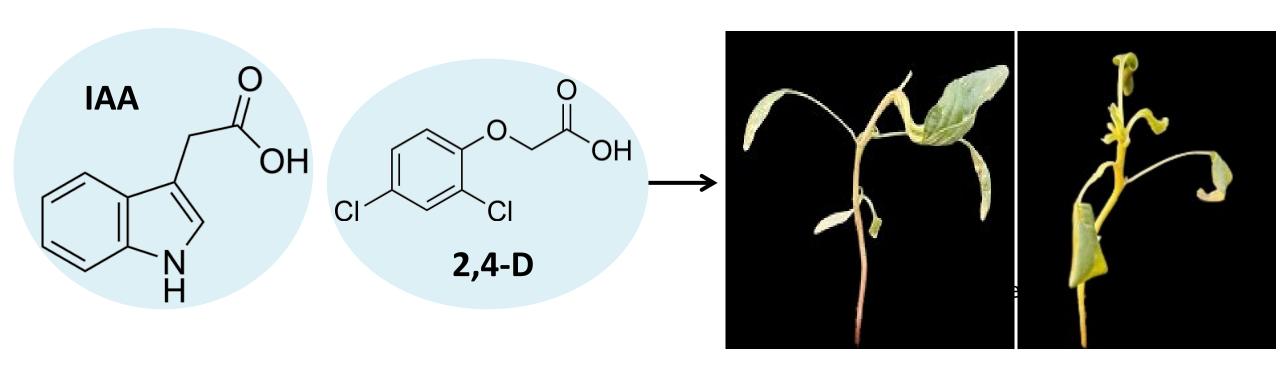
# Auxin-mimic herbicides and resistance in Weeds

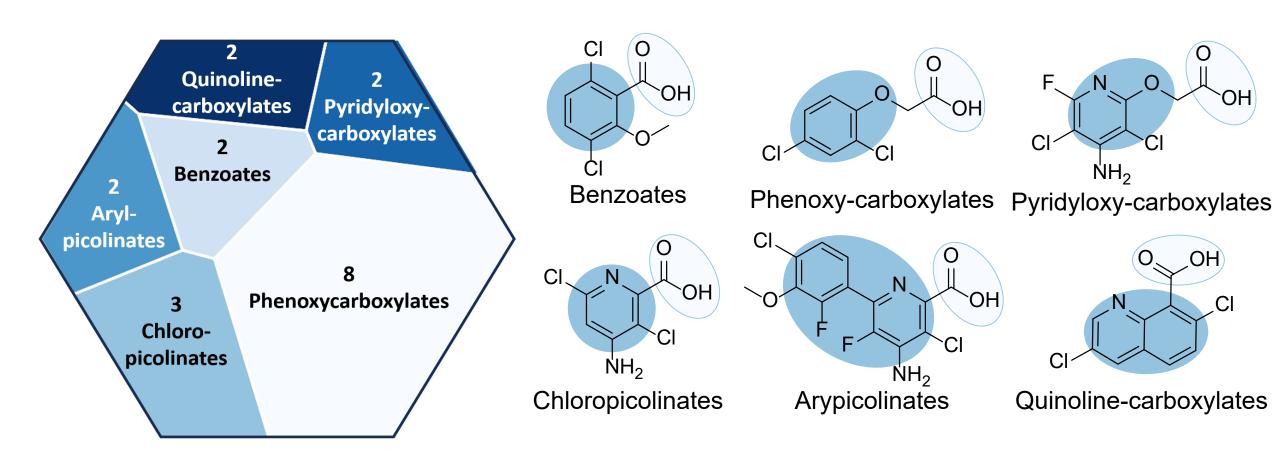
Dr. Franck Dayan, Professor Colorado State University

### What are auxin-mimic herbicides?

Auxin-mimic herbicides (AMHs) classified by HRAC as 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

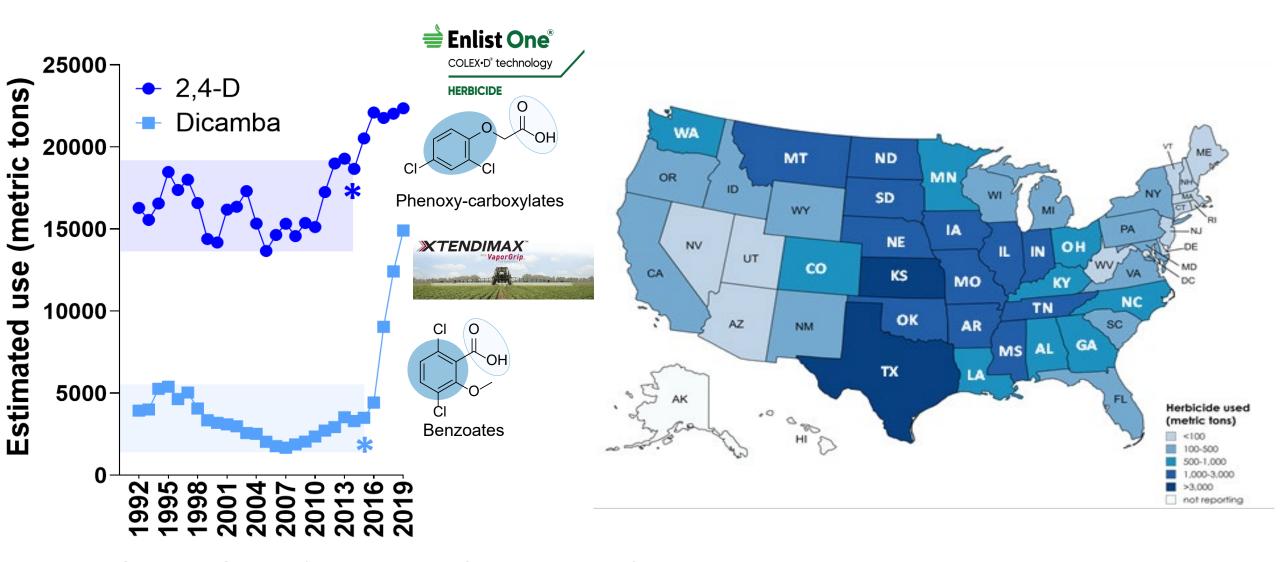


### Classification of AMH by chemical structure



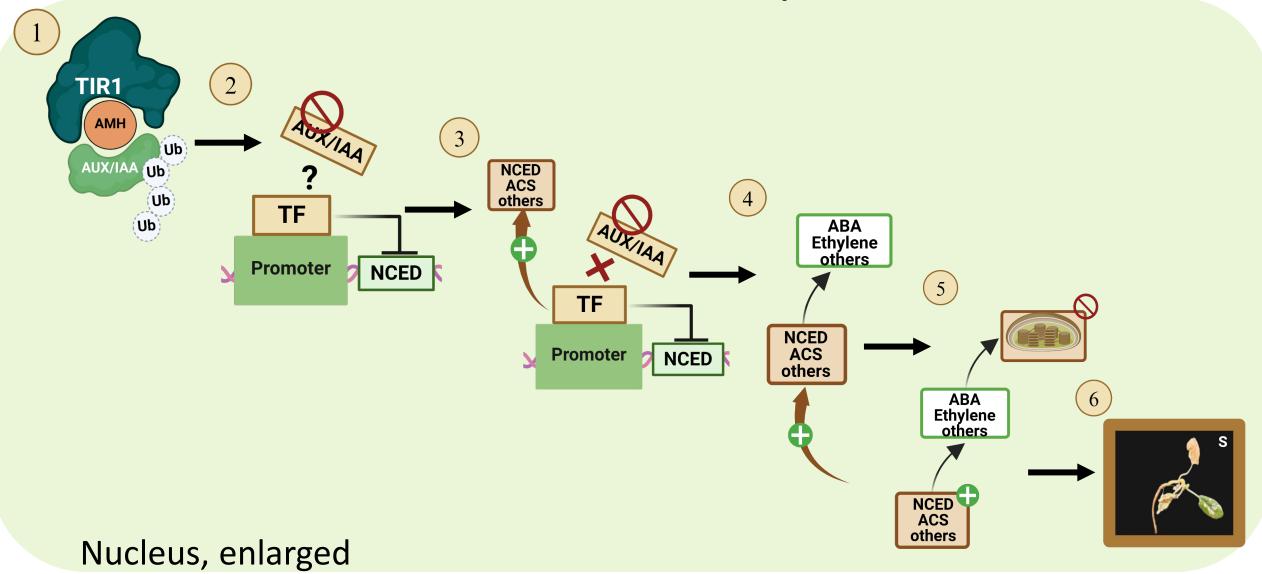
Moreno-Serrano, Gaines & Dayan 2024 Current status of auxin-mimic Herbicides. *Outlooks on Pest Management*, *35*(3), 105-112.

### Top two AMHs chemical classes use in crop production



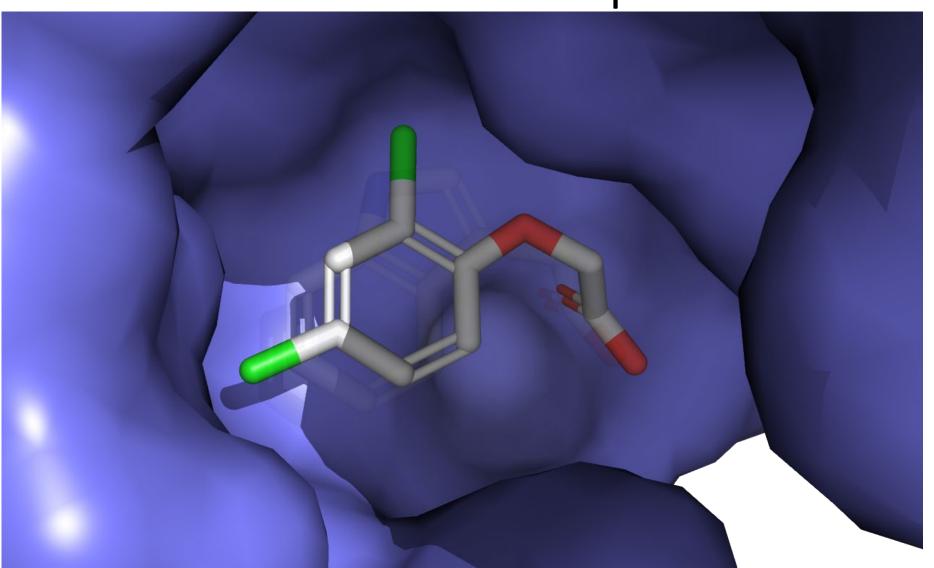
Moreno-Serrano, Gaines & Dayan 2024 Current status of auxin-mimic Herbicides. *Outlooks on Pest Management*, *35*(3), 105-112.

### How do AMHs act in plants?

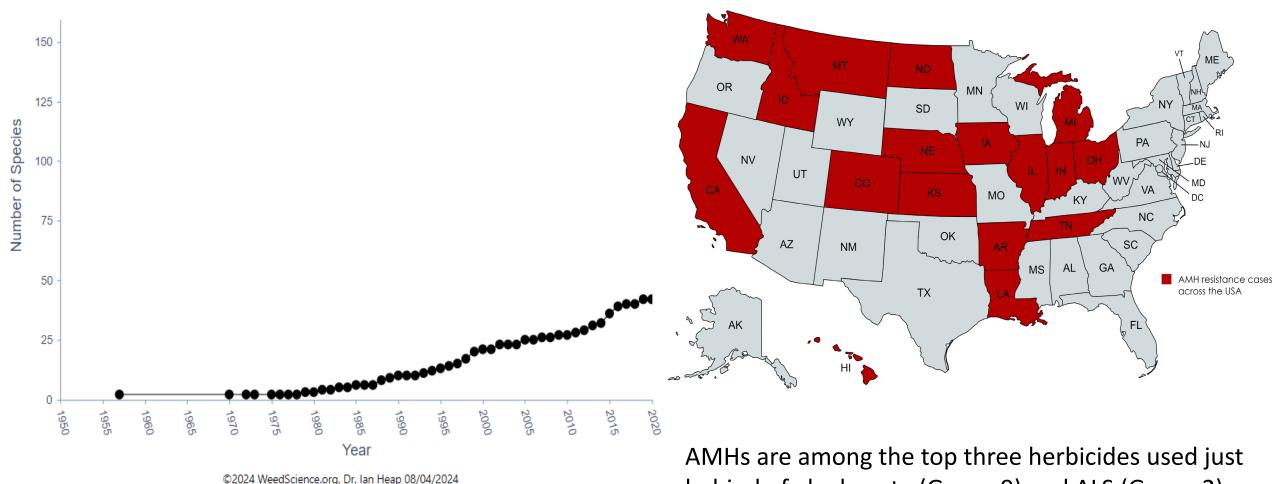


Adapted from: Gaines, 2006

### How do AMHs act in plants?



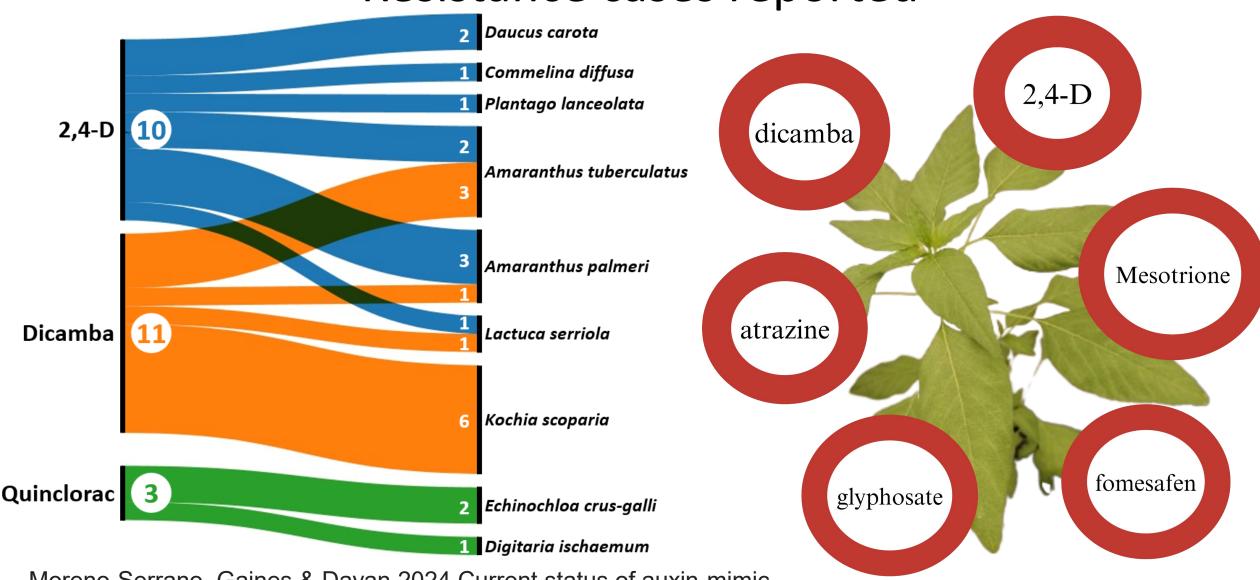
## AMHs resistance cases reported in the globally and in the USA



Moreno-Serrano, Gaines & Dayan 2024 Current status of auxin-mimic Herbicides. *Outlooks on Pest Management*, *35*(3), 105-112.

behind of glyphosate (Group 9) and ALS (Group 2)

Resistance cases reported



Moreno-Serrano, Gaines & Dayan 2024 Current status of auxin-mimic Herbicides. *Outlooks on Pest Management*, *35*(3), 105-112.

### Potential resistance mechanisms

Any trait which slows/prevents herbicide reaching the site of action, or reduces toxic effect at site of action

### **Non-Target Site**

Metabolism Altered translocation & sequestration

Reduced foliar uptake

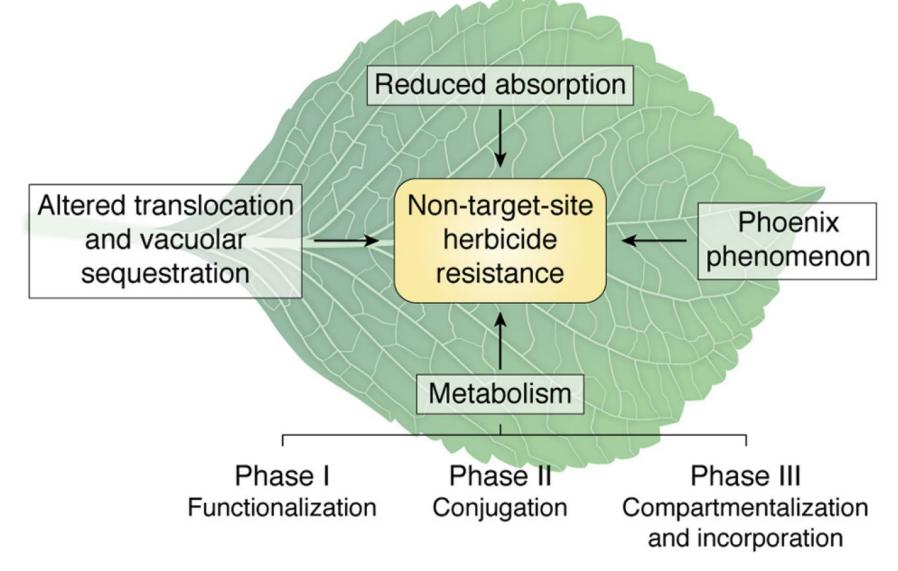
General oxidative stress response

### **Target Site**

Mutation/deletion

Increased expression/ Gene amplification

### Non-target site resistance mechanisms





### 2,4-D metabolic resistance in waterhemp

(Amaranthus tuberculatus)
Figueiredo et al 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, 70(49), 15380-15389.

Figueiredo et al 2018 Metabolism of 2, 4-dichlorophenoxyacetic acid contributes to resistance in a common waterhemp population. Pest Management Science, 74(10), 2356-2362.

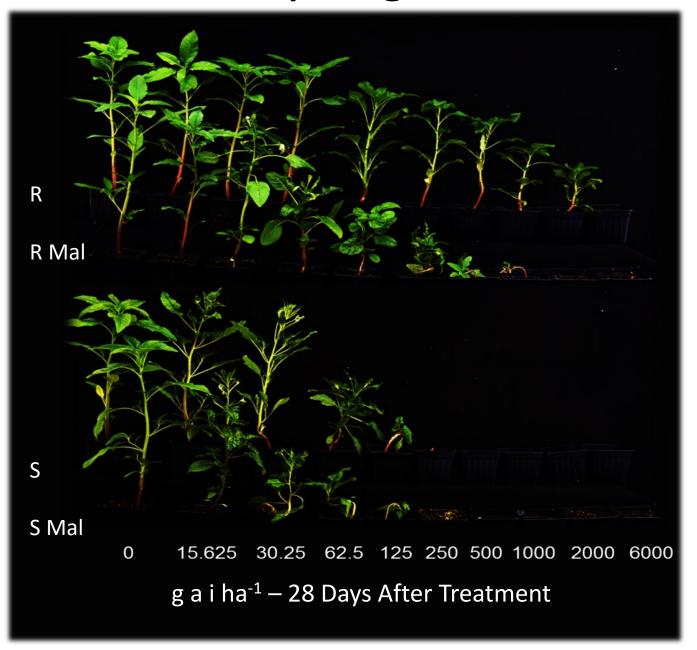


### Days After 2,4-D Treatment 500 g ae ha<sup>-1</sup>

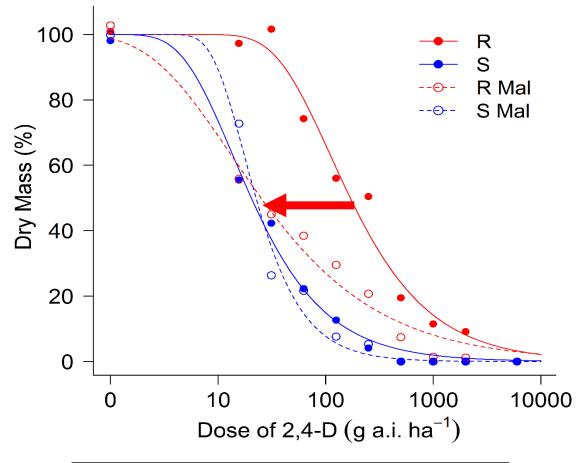




### Malathion synergizes 2,4-D



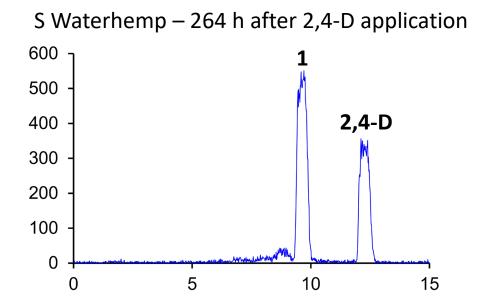
#### Malathion effect on 2,4-D resistance



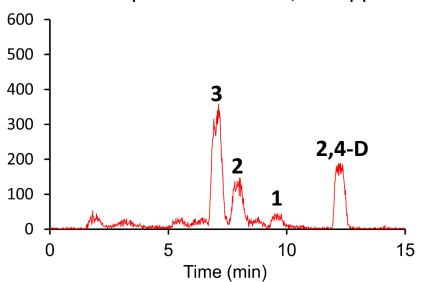
Estimate	ed effective dose: 50%	
	Estimate (g ai ha-1)	Std. Error
2	192	44
R Mal	24	8
S	21	5
S Mal	23	3

Figueiredo, et al. (2018) Pest management science

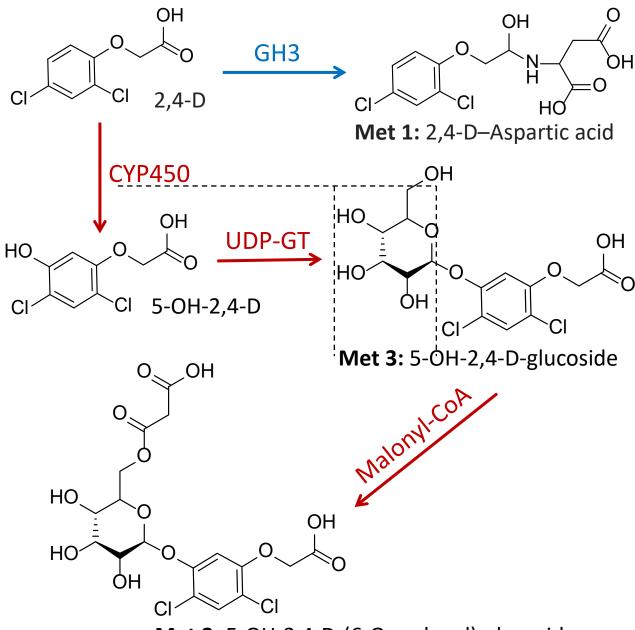
### 2,4-D Metabolism – Quantification



R Waterhemp – 264 h after 2,4-D application

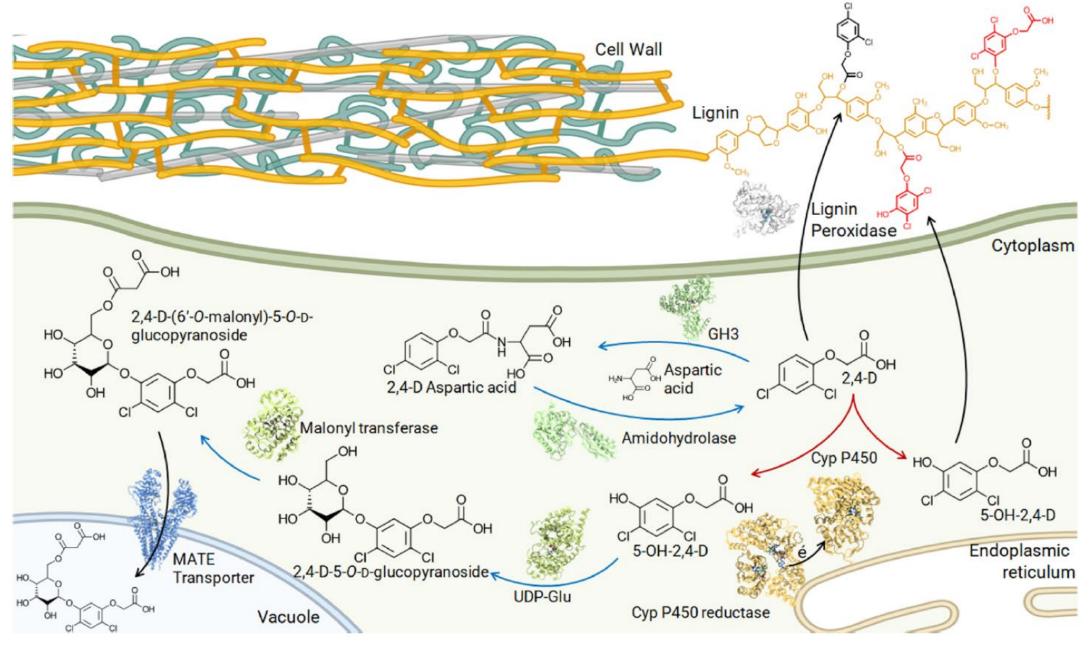


#### 2,4-D metabolism in Waterhemp



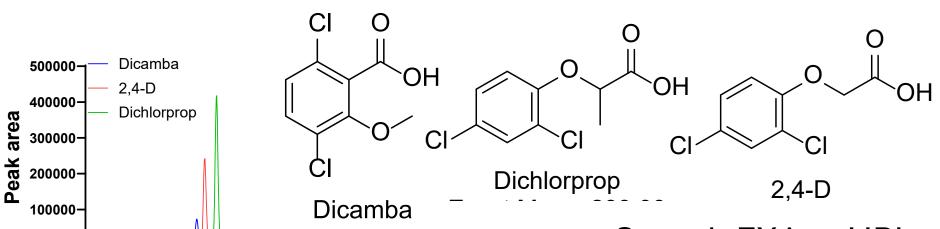
Met 2: 5-OH-2,4-D-(6-O-malonyl)-glucoside

Figueiredo, et al. (2018) Pest management science



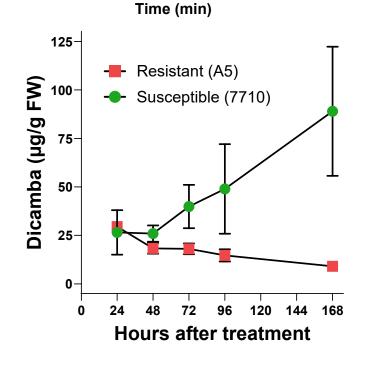
de Figueiredo et al 2022 Identification of a novel 2, 4-D metabolic detoxification pathway in 2, 4-D-resistant waterhemp JAFC 70:15380

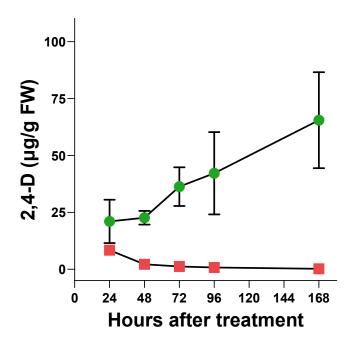
### Resistance to Scorch EX in kochia

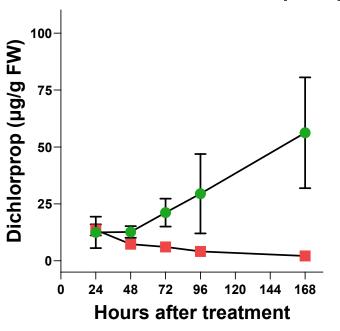




Scorch EX is a UPL product with dicamba, 2,4-D and dichlorprop







### Potential resistance mechanisms

Any trait which slows/prevents herbicide reaching the site of action, or reduces toxic effect at site of action

### **Non-Target Site**

Metabolism Altered translocation & sequestration

Reduced foliar uptake

General oxidative stress response

### **Target Site**

Mutation/deletion

Increased expression/ Gene amplification





# An in-frame deletion mutation in the degron tail of auxin coreceptor *IAA2* confers resistance to the herbicide 2,4-D in *Sisymbrium orientale*

Marcelo R. A. de Figueiredo (D a, Anita Küpper (D a,b), Jenna M. Malone (D c, Tijana Petrovic (D c, Ana Beatriz T. B. de Figueiredo a, Grace Campagnola (D d, Olve B. Peersen (D d, Kasavajhala V. S. K. Prasad (D e,f, Eric L. Patterson (D a,g, Anireddy S. N. Reddy e,f, Martin F. Kubeš (D h, Richard Napier (D h, Franck E. Dayan a,f, Christopher Preston (D c, and Todd A. Gaines (D a,f,1)

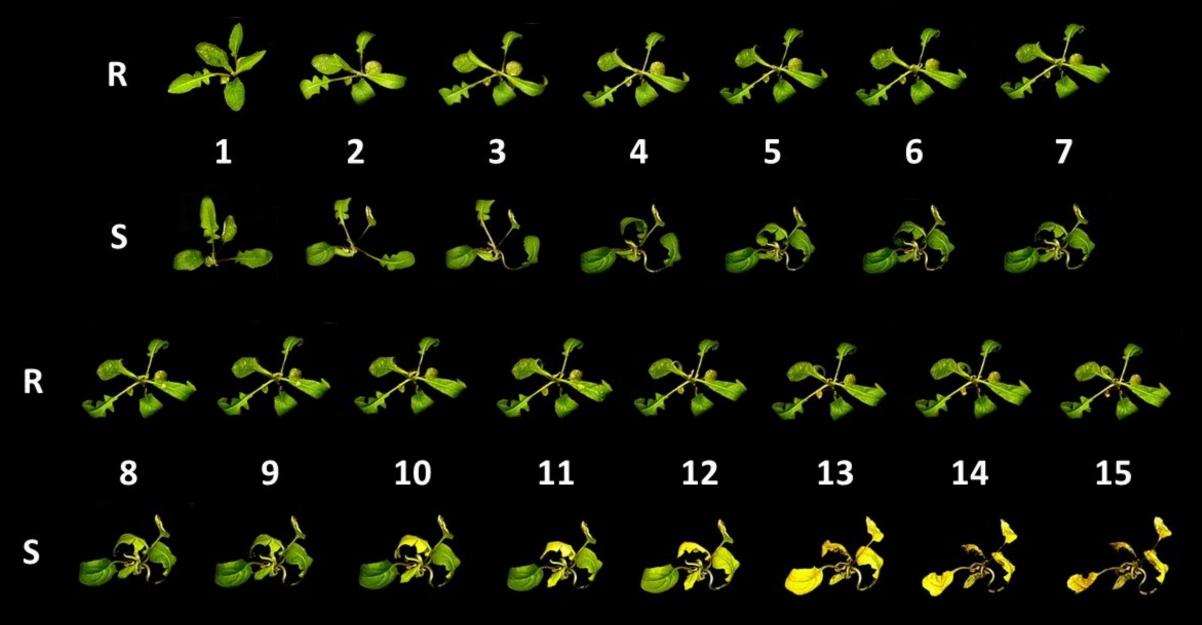
Edited by Sean Cutler, University of California, Riverside, CA; received March 25, 2021; accepted December 9, 2021

February 25, 2022

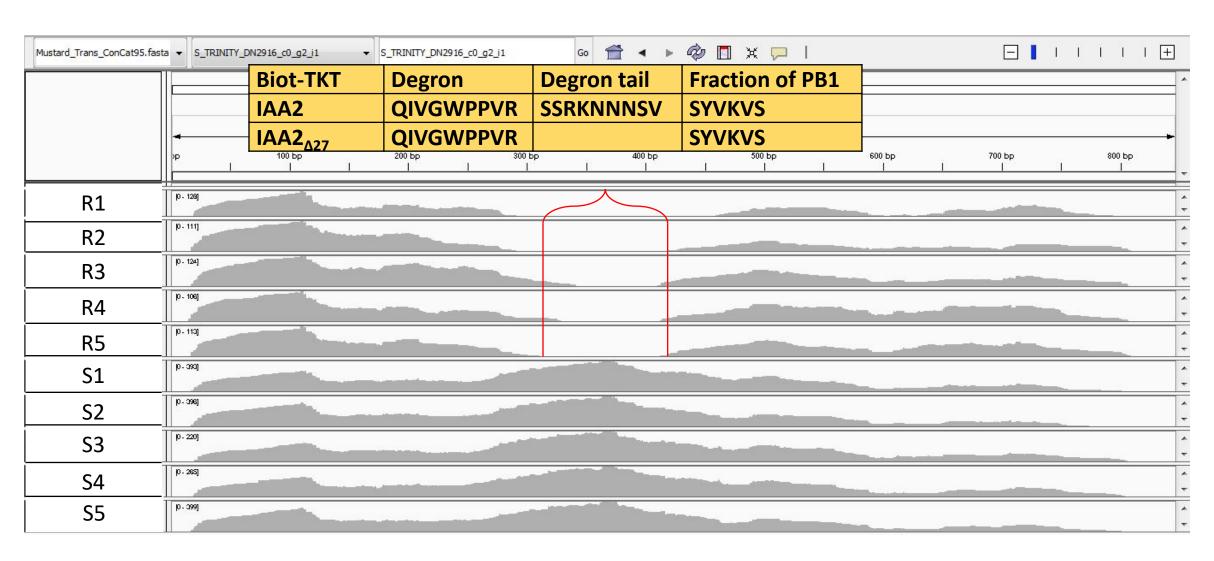
119 (9) e2105819119

https://doi.org/10.1073/pnas.2105819119

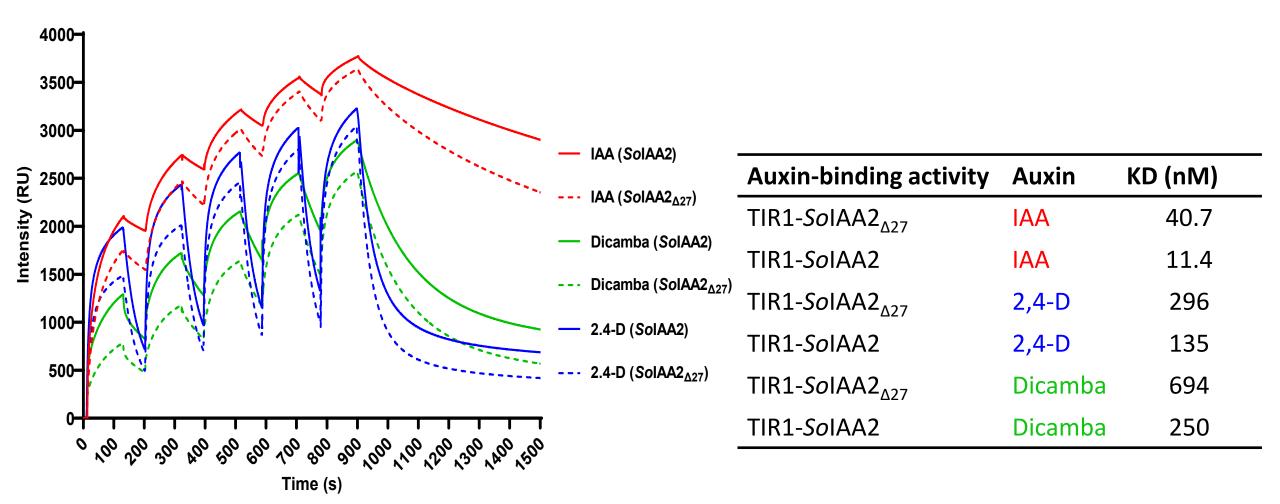
Days after treatment 2,4-D at 250 g a.i./ha



# RNA-Seq Alignment: lower expression in R RILs in IAA2, gap in coverage

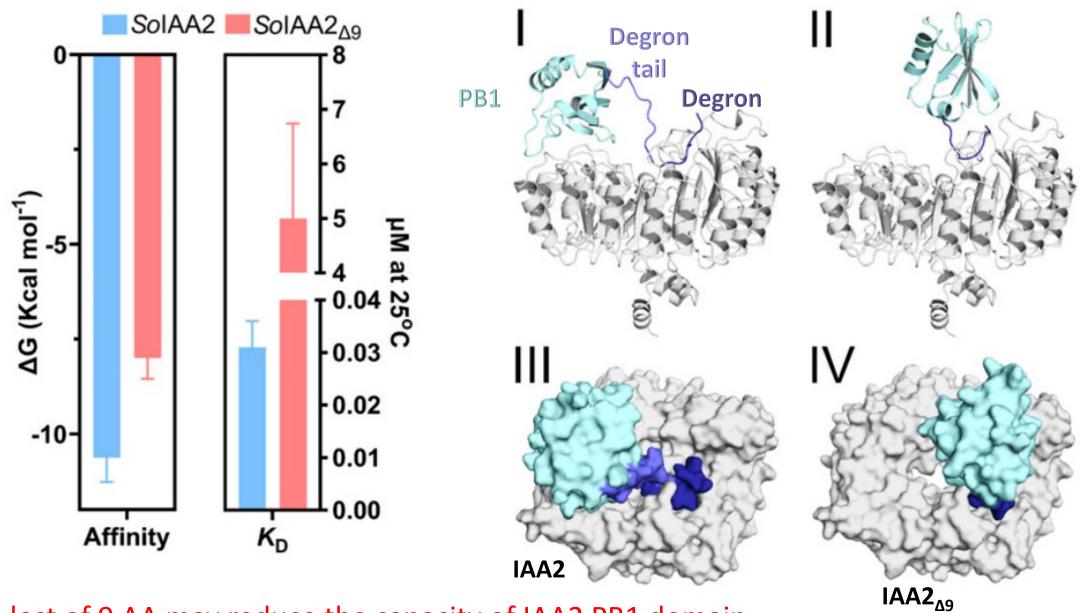


### SPR – Affinity binding assays (TIR1 protein)



Binding association (Ka) is lower for  $SolAA2_{\Delta 27}$  – Lower recognition and binding interactions Dissociation (Kd) is higher for  $SolAA2_{\Delta 27}$  – Higher instability of complex formation

### TIR1 and IAA2 binding prediction



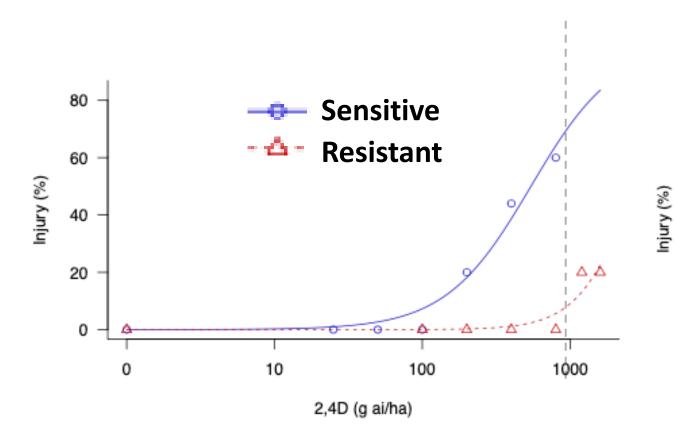
The lost of 9 AA may reduce the capacity of IAA2 PB1 domain

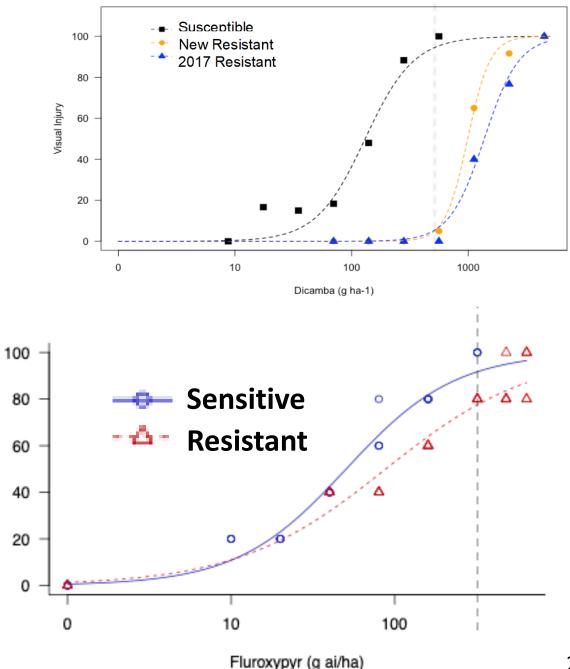
### A transposable element insertion in IAA 16 disrupts splicing and causes dicamba resistance in Bassia scoparia

Jacob S. Montgomery, Neeta Soni, Sofia Marques Hill, Sarah Morran, (D) Eric L. Patterson, Seth A. Edwards, Sandaruwan Ratnayake, Yu-Hung Hung, Pratheek H. Pandesha, R. Keith Slotkin, Richard Napier, (D) Franck Dayan, (D) Todd A. Gaines

Montgomery et al 2024 A transposable element insertion in IAA16 interrupts normal splicing and generates a novel dicamba resistance allele in Bassia scoparia. *Plant Journal, online* 

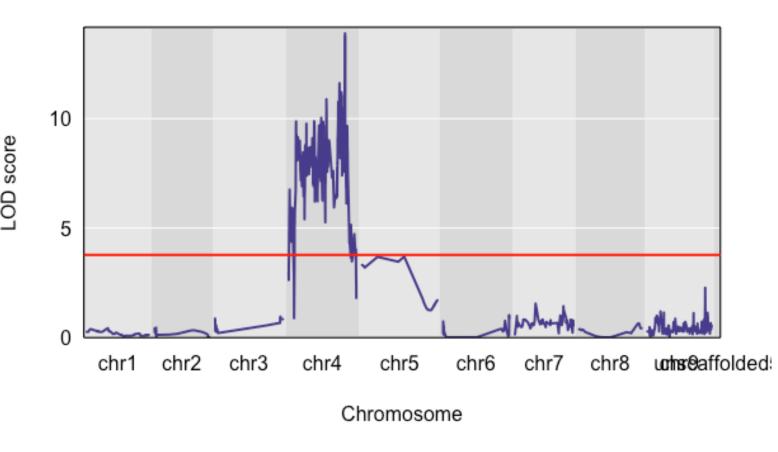
Montgomery et al 2024 A transposable element insertion in IAA16 interrupts normal splicing and generates a novel dicamba resistance allele in Bassia scoparia. *Plant Journal, online* 

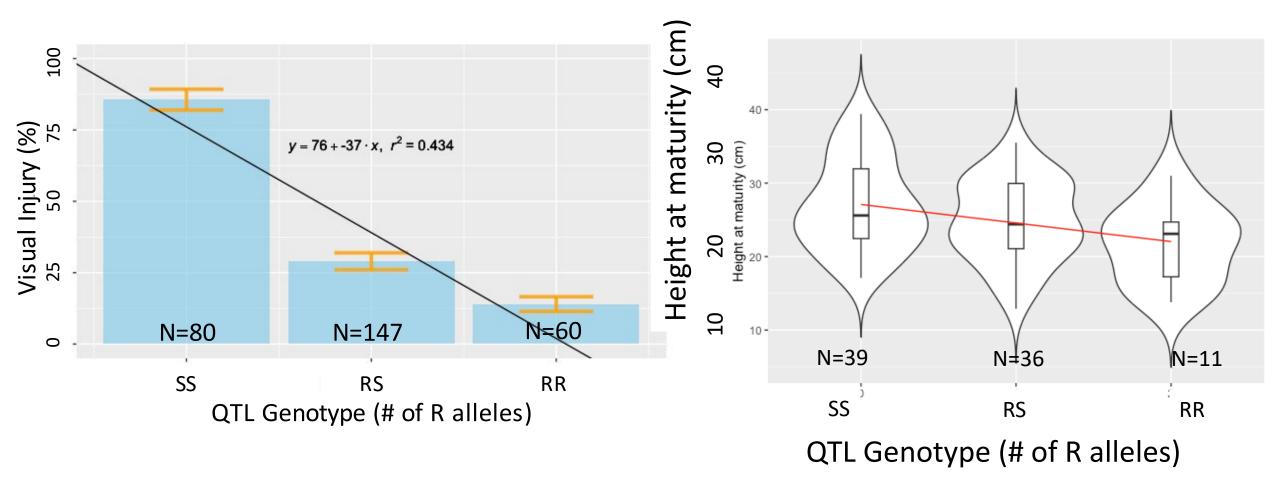




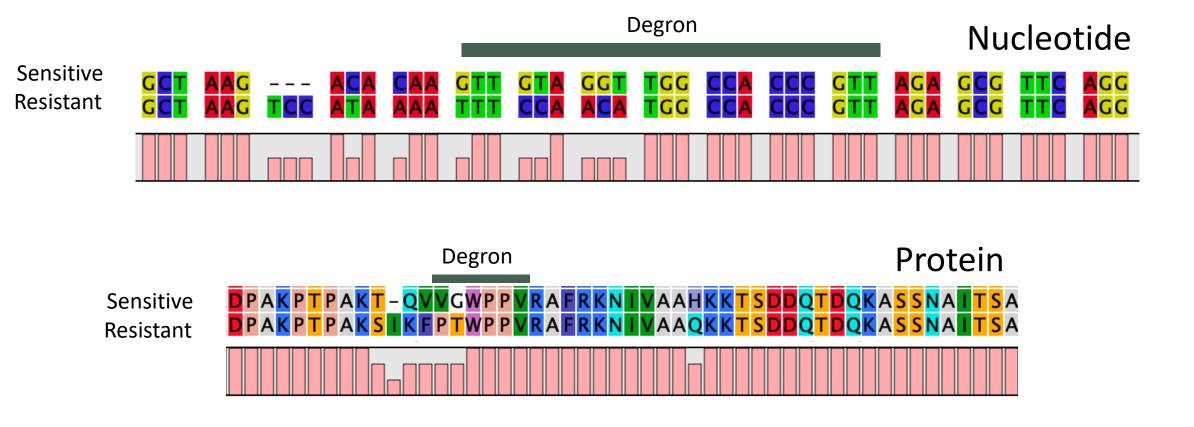
### One high-effect QTL exists on chr4

348,844 variants detected
2,473 variants passed filtering
Significance plotted in red
Peak centered at 88 Mbp
IAA16 located at 87.4 Mbp on
chr 4

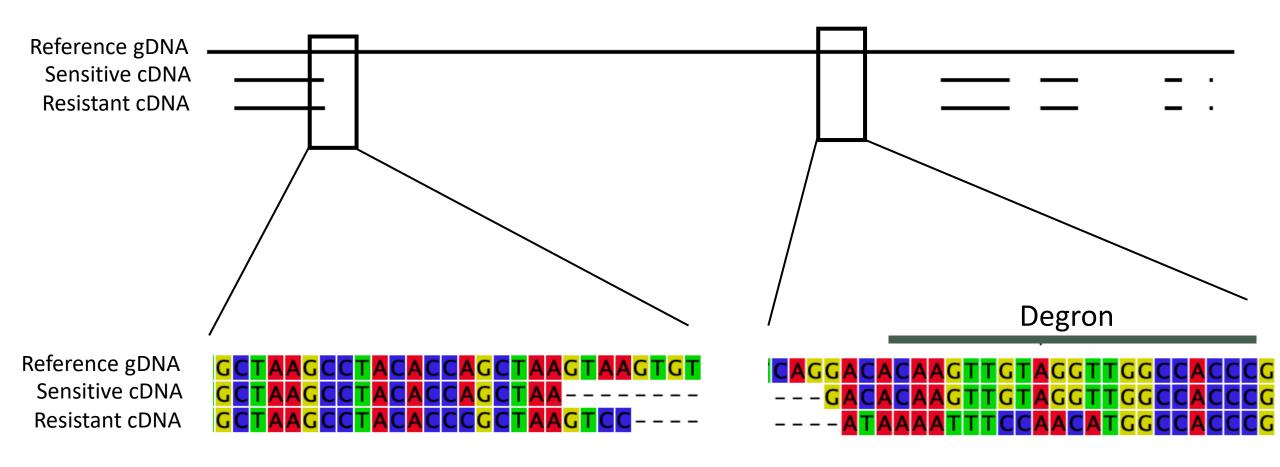




### $IAA16_{Mutant}$ is hyper variable around the degron



### Variable region of IAA16 flanks splice junction



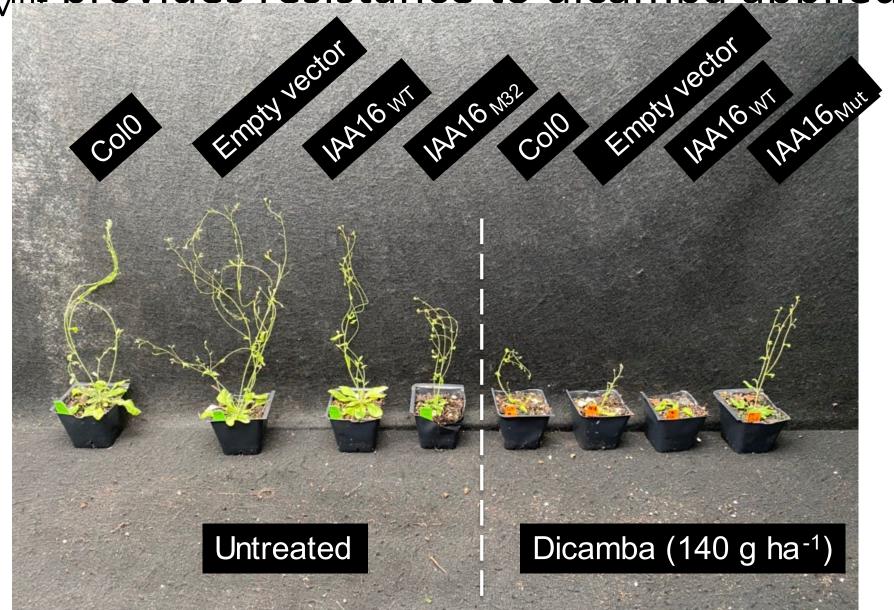
# Transposable element insertion at the beginning of exon 2 disrupts normal splicing

Tissue of homozygous resistant F 1 kb TGTAGGT**TGGCC**ACCGTTAGA plant used for HiFi resequencing IAA16 Exon 1 Reads assembled into contigs de **TSD TSD** TGGCC TGGCC novo 2608 bp 429 bp 429 bp M32 gDNA WT gDNA WT CDS M32 CDS M32 gDNA WT gDNA

WT CDS M32 CDS

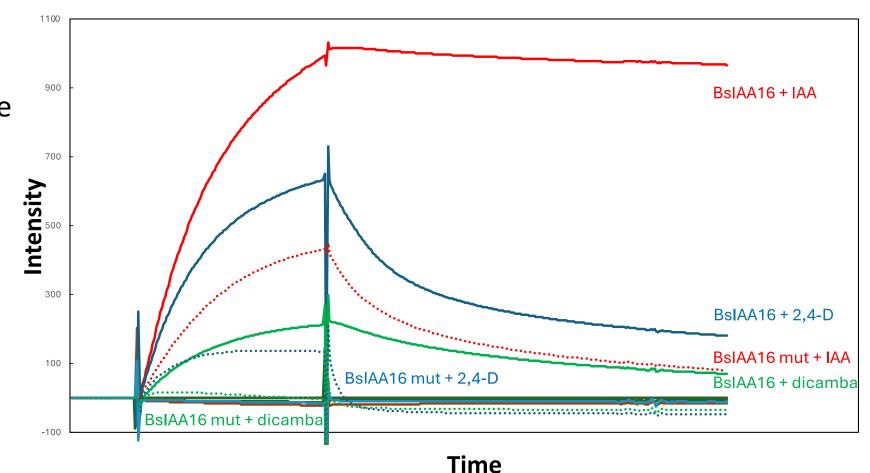
Degron

IAA16<sub>M...</sub> provides resistance to dicamba applied POST

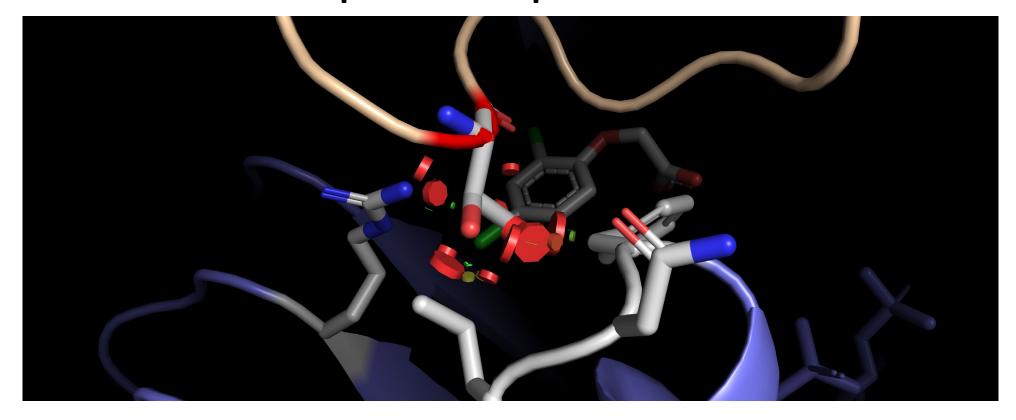


### IAA16mut has lower binding affinity to TIR1

SPR assay tested binding affinity and dissociation for IAA16 alleles with TIR1 in the presence of natural and synthetic auxins Dotted lines are the mutant IAA16 allele, solid are WT Shifts downward indicate lower binding affinity Quicker reduction after binding indicate quicker dissociation



### Mutation affects protein-protein interaction



Sensitive
Resistant

DPAKPTPAKT-QVVGWPPVRAFRKN

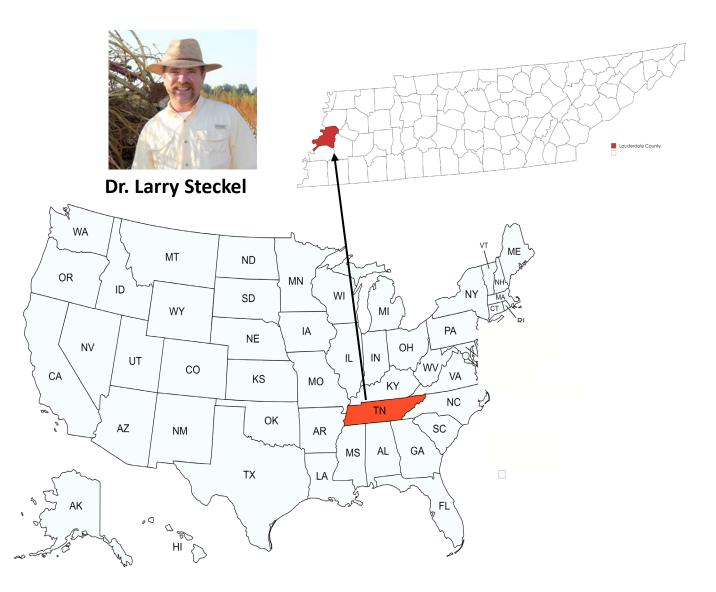
DPAKPTPAKSIKFPTWPPVRAFRKN

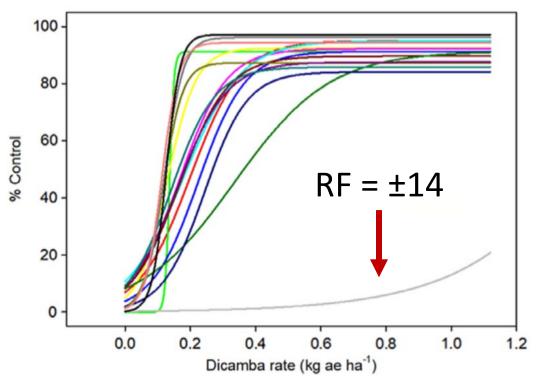
## Unraveling the resistance mechanism to dicamba in Palmer amaranth (*Amaranthus palmeri*)





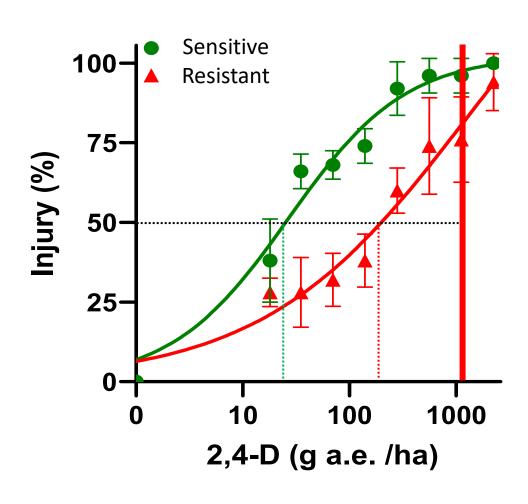
### A survey identified dicamba-resistance Palmer amaranth

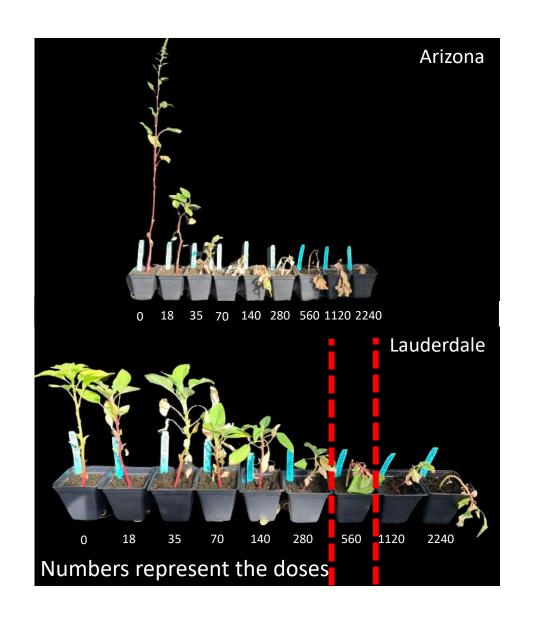




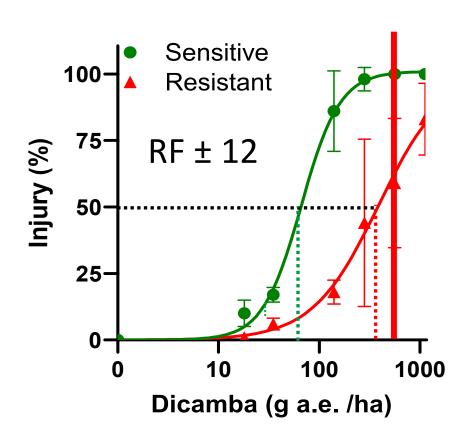
Foster, D., & Steckel, L. Weed Technol, (2022)

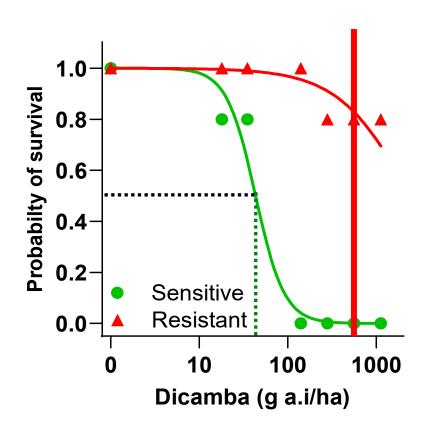
### DRC of the auxin mimic herbicides

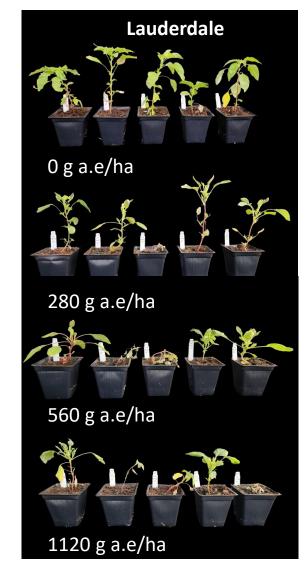




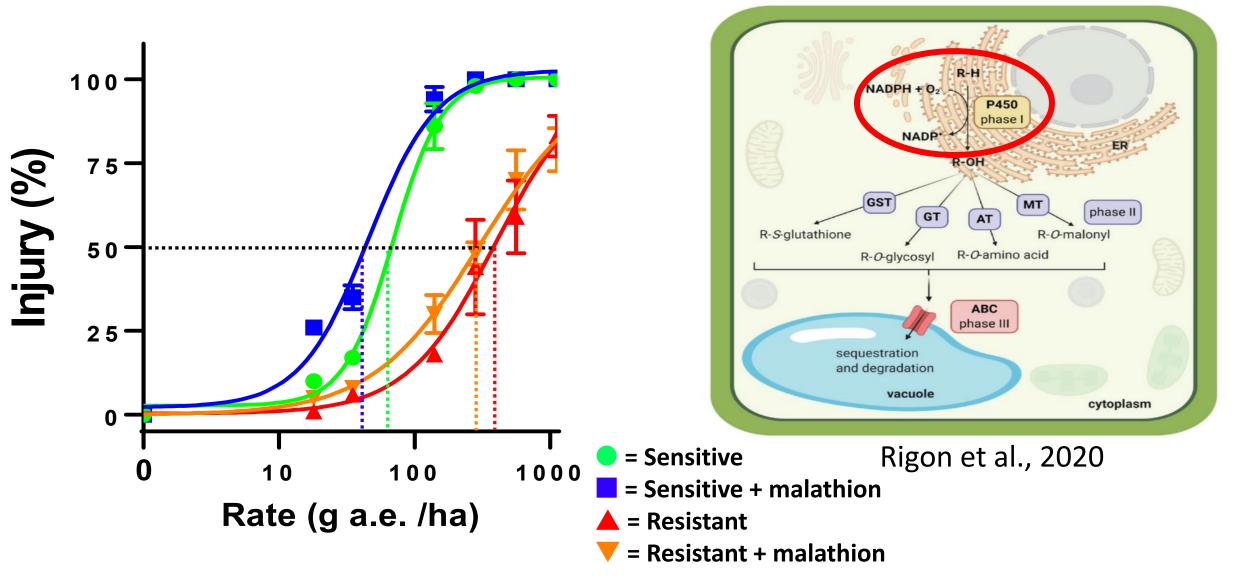
### Visual injury in response to dicamba



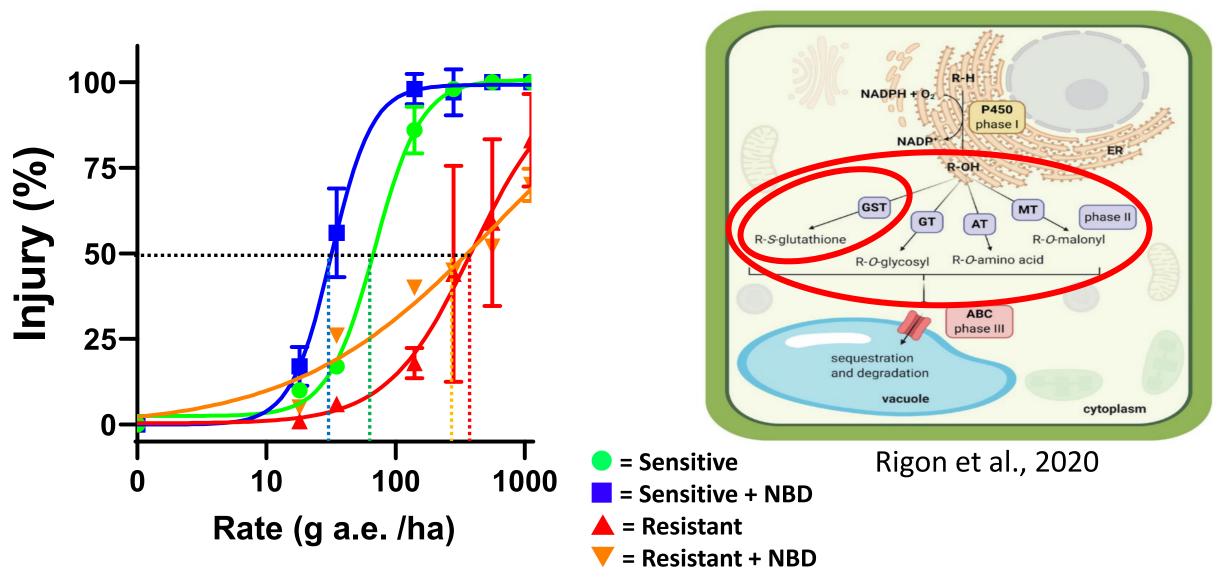




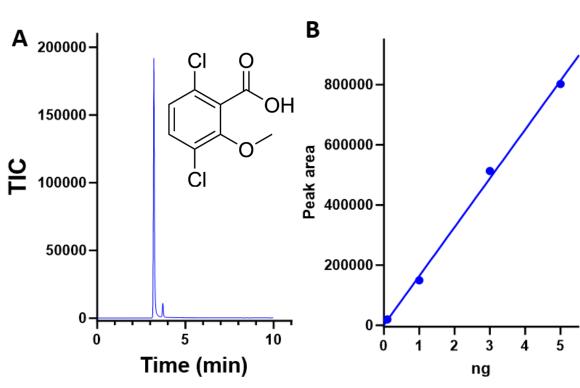
### Enhanced metabolism not mediated by P450



### Enhanced metabolism not mediated by GST

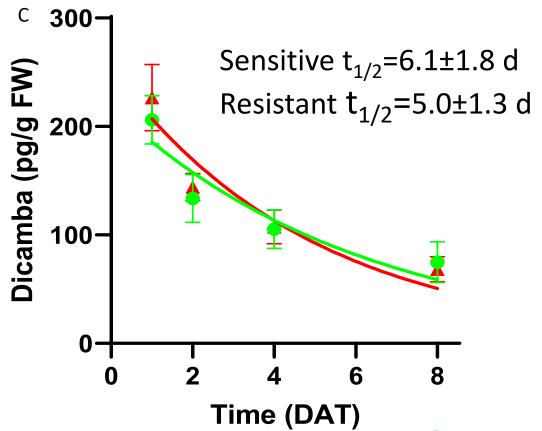


### NTSR mechanism is not mainly involved in the resistance



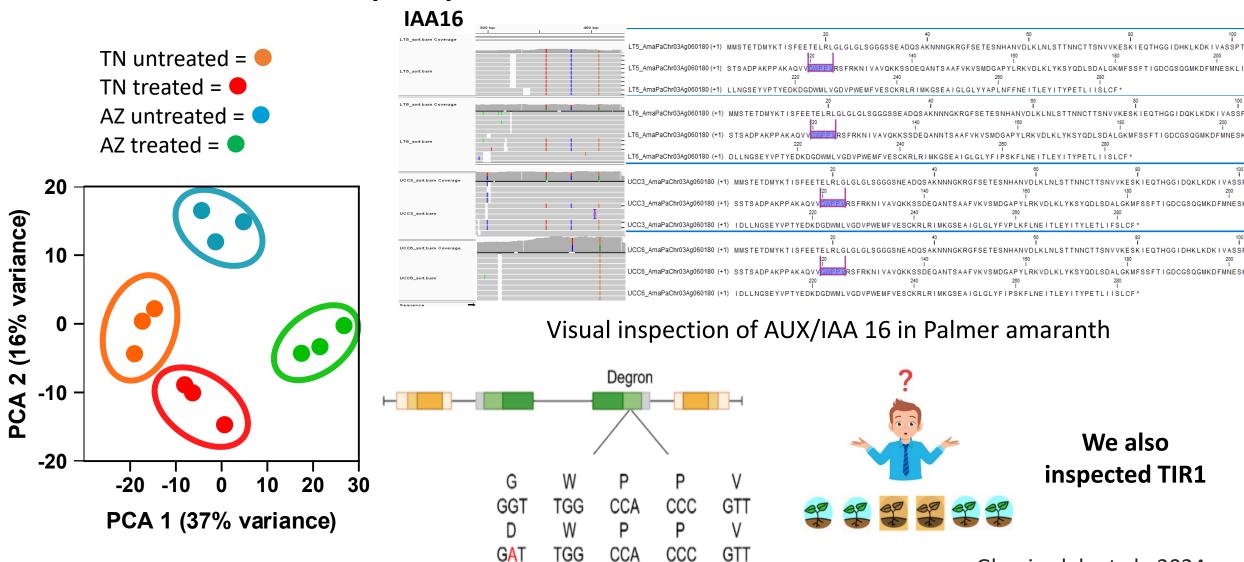
LC-MS/MS analysis of dicamba

- A) Chromatographic separation of the dicamba
- B) Calibration curve of dicamba



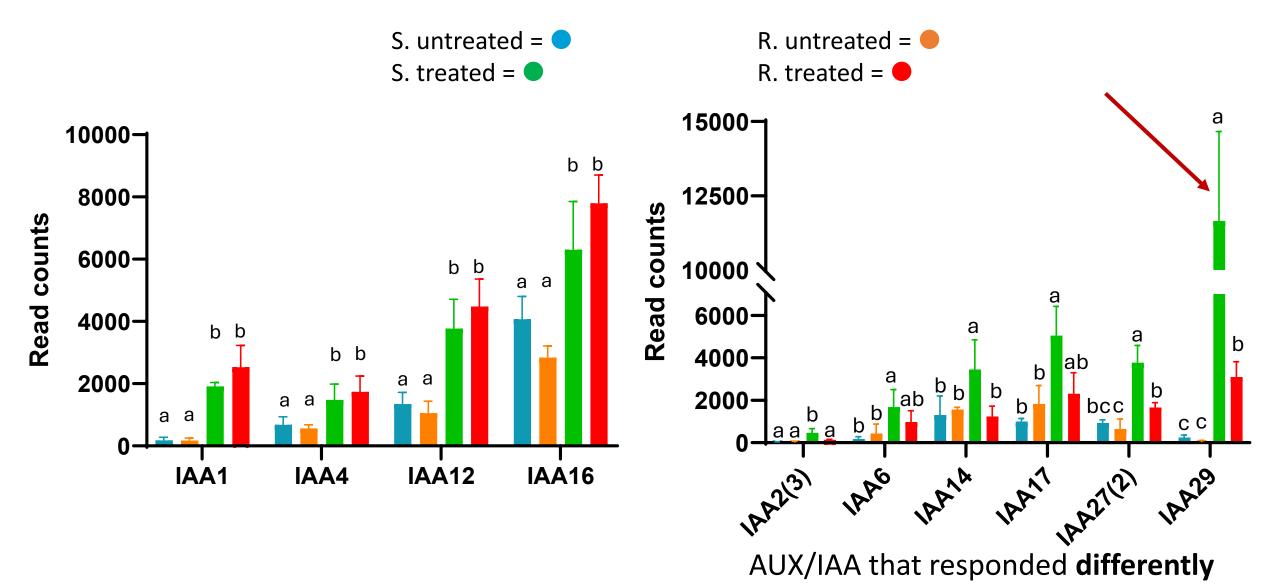
C) Metabolism of dicamba in sensitive (●=Sensitive) and resistant (▲=Resistant) Palmer amaranth biotypes. (P = 0.65)

### RNA-seq experiment and bioinformatic

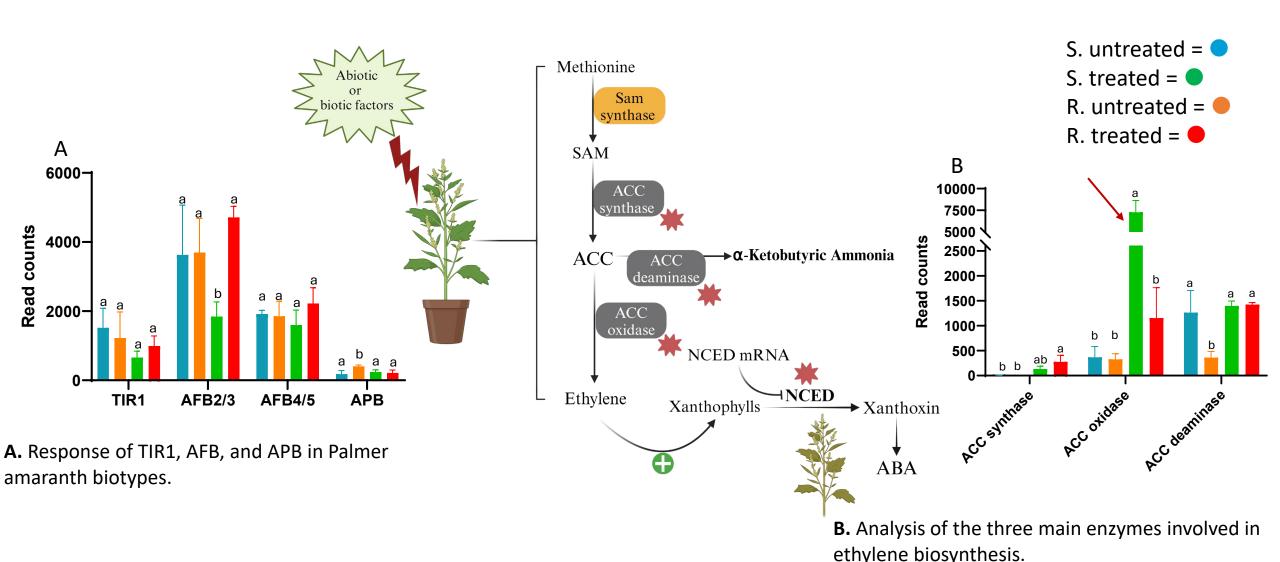


Ghanizadeh et al., 2024

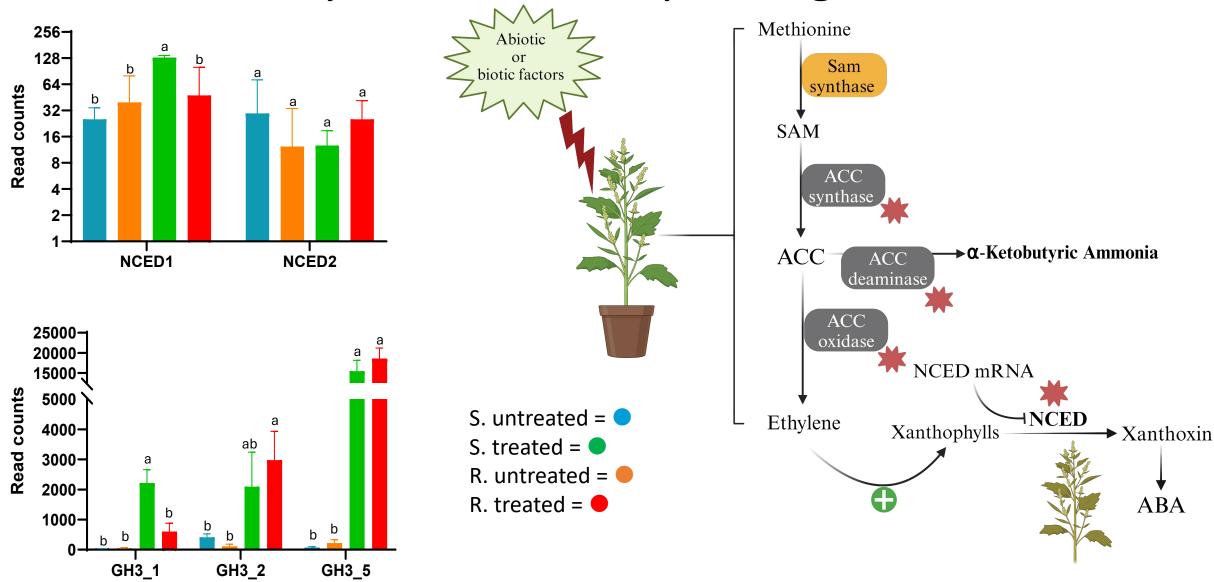
### Gene expression of AUX/IAA in Palmer

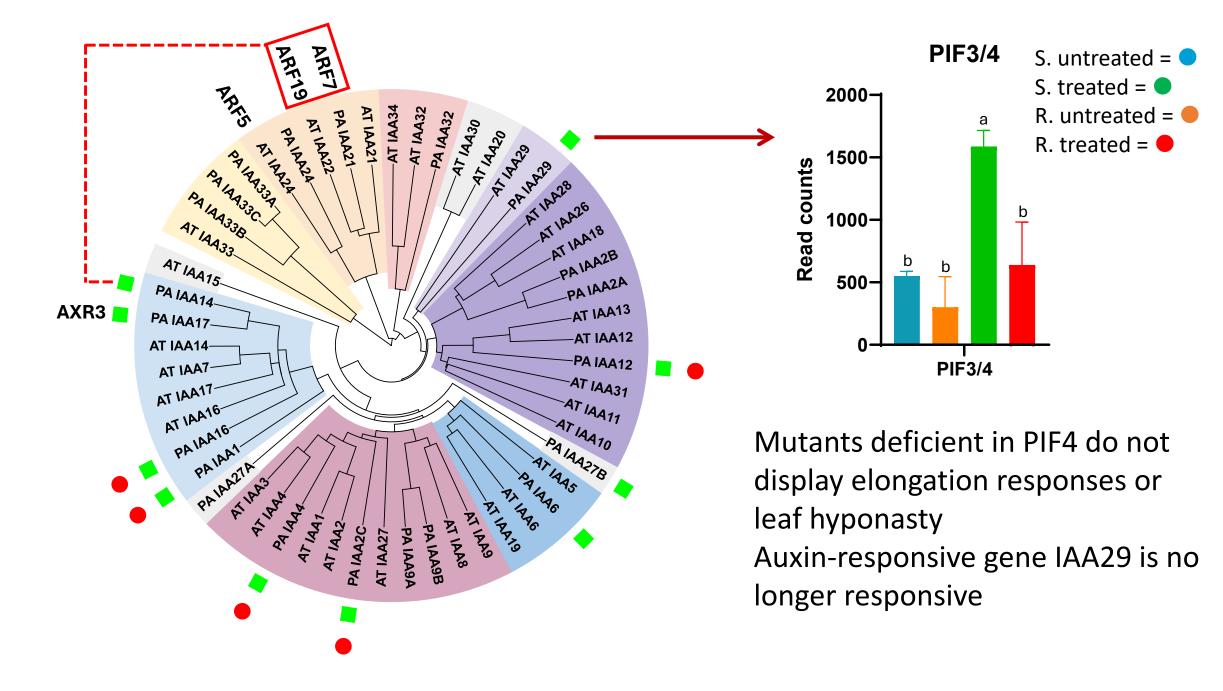


### AMHs interaction and auxin-response genes



### Analysis of auxin response-genes





# Questions





### Fall 2025

### **AB 509 Herbicide Selectivity and Action**

Online asynchronous course intended for graduate students and working professionals in industry, government, and consulting positions with interest in herbicide use.

Topics covered include the physicochemical properties of herbicides, their selectivity (placement and metabolic tolerance), their mechanism of action, uses in weed management, visual symptoms of herbicide treatment, how plants can evolve resistance to these compounds, why herbicide applications fail, and controversial topics related to the use of herbicides.

Online registration will open April 2025. Contact: Franck Dayan at <a href="mailto:franck.dayan@colostate.edu">franck.dayan@colostate.edu</a> or Todd Gaines at <a href="mailto:todd.gaines@colostate.edu">todd.gaines@colostate.edu</a>