Enhanced Metabolism of ALS Inhibitors in *Cyperus difformis*.

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Background

- Control of sedges in California rice has relied heavily on acetolactase synthase (ALS) inhibiting herbicides for decades.
- As a consequence, smallflower umbrella sedge (*Cyperus difformis* L.) (SF) populations resistant to ALS inhibitors are widespread in the region.
- Previous research has identified six major patterns of ALS cross-resistance in California populations of smallflower treated with bensulfuron-methyl (BEN), halosulfuron-methyl (HAL), bispyribac (BIS), and penoxsulam (PEN) (*Fig 1*).
- Dose-response and malathion inhibition studies strongly suggest increased rates of ALS inhibitor metabolism in resistant SF populations.

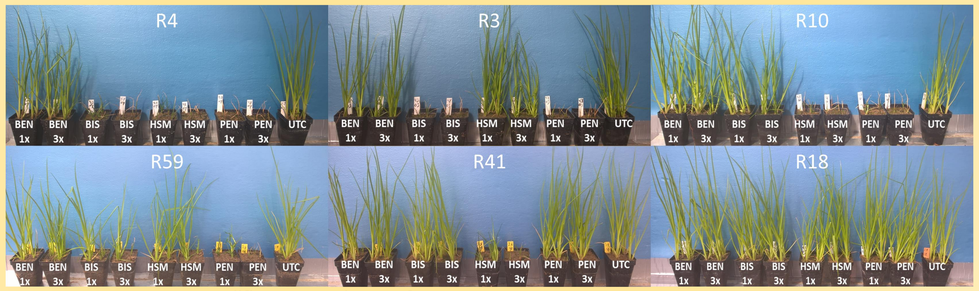

Fig. 1. Representative smallflower populations from six major ALS cross-resistance patterns.

Objective

Screen representative smallflower populations that are cross-resistant to ALS inhibitors for evidence of enhanced herbicide metabolism, using liquid chromatography and mass spectrometry (LC-MS) techniques.

Experimental Design

- Study was conducted at UC Davis, Davis CA, and at the Multi-User Analytical Lab (MUAL) at Clemson University, Clemson SC.
- Seed from six ALS cross-resistant SF populations, plus a known susceptible (SUS), were sown onto ricefield soil in 36-plug trays, in a CRD.
  - Glasshouse temperatures were 33/ 23°C day/ night, with a 16h photoperiod.
- Herbicides were applied at 3.5ls at label-recommended rates, plus 0.25% v/v NIS.
  - Bensulfuron-methyl (Londax®) @ 70 g a.i. ha^{-1}
  - Halosulfuron-methyl (Halomax 75®) @ 70 g a.i. ha^{-1}
  - Bispyribac-sodium (Regiment CA®) @ 37 g a.i. ha^{-1}
  - Penoxsulam (Granite SC®) @ 42 g a.i. ha^{-1}
- Seedlings were harvested at 12, 24, and 48 HAT.
  - Seedlings were rinsed with 50% methanol to wash away unabsorbed herbicides.
  - Rinsed seedlings were flash-frozen in liquid nitrogen, and stored at -80°C until shipping to MUAL for LC-MS analysis.
- LC-MS analyzed absolute concentrations of herbicide parent molecules, and relative concentrations of known and suspected metabolites, for each herbicide.
- Data analyzed using JMP® software, and LSDs calculated from LS means at $\alpha = 0.05$.

Results & Discussion

- **Two isomeric O-demethylated bensulfuron metabolites were identified (Table 1).**
  - Population (Pop.) R59 had a 2.4-fold increase in total isomers over susceptible (SUS) by 48HAT.
    - O-demethylation is commonly catalyzed by cytochrome P450s, which are known to literature as causes of some enhanced herbicide metabolism in plants.
- **Two major (HAL-III, HA-RE) and four minor halosulfuron metabolites were identified.**
  - O-demethylated HAL-III concentrations in Pops. R4 and R10 were 1.6 and 1.9-fold greater, respectively, than in SUS at 48HAT.
  - CD-582, a likely glycosylated halosulfuron molecule, was present in R4 and R59 at levels 1.9 and 1.7-fold greater than SUS.
    - Glycosylation is commonly catalyzed by glycosyl transferases (GTs), another common herbicide detoxifying enzyme.
- **Two major (desmethyl-BIS, Me2BA) and two minor bispyribac metabolite were identified.**
  - Population R10 concentrations of desmethyl-BIS were 2.25-fold greater than SUS at 48HAT.
  - No populations had detected increases of Me2BA over SUS at any timepoint.
- **Four penoxsulam metabolites were detected.**
  - 2-HP-PEN was present at levels approximately 2.0-fold over SUS in Pops. R3, R41, and R59.
    - 2-HP-PEN may be a product of glutathione-S-transferase (GST) mediated dehalogenation.
    - Enhanced GST activity is known to literature as a cause of increased herbicide metabolism in plants.
  - CD-645, a probable glycosylated penoxsulam, was detected in Pop. R59 at 1.75-fold concentration over SUS.

Table 1. LC-MS analysis results of ALS inhibitor metabolism in six ALS-R and one susceptible (SUS) smallflower populations, from samples harvested 12, 24, and 48 hours after treatment (HAT). Units are in ng molecules per g sample fresh weight. Concentrations of parent molecules (bensulfuron, halosulfuron, bispyribac, penoxsulam) are absolute, while each metabolite concentration is relative across samples, using analytical-grade samples of parent molecules as reference standards. LSDs ($\alpha = 0.05$) can be used to compare metabolite concentrations across samples.

Population	R3			R4			R10			R18			R41			R59			SUS			LSD
HAT	12	24	48	12	24	48	12	24	48	12	24	48	12	24	48	12	24	48	12	24	48	
Bensulfuron	1480	1879	1365	1628	1439	841	1305	1588	1091	1357	1724	1072	1665	2212	1160	1910	2326	1090	1922	1896	1138	**350**
Desmethyl-BEN	3.6	4.6	10.5	6.1	5.1	11.2	3.8	5.2	12.9	3.1	4.9	7.9	2.8	6.7	10.3	7.7	5.6	25.0	4.8	5.5	10.5	**4.0**
Halosulfuron	231	182	135	128	210	170	266	227	147	164	161	129	175	174	149	235	205	168	231	173	147	**38**
HAL-III[a]	2.7	3.6	6.0	4.1	9.3	15.0	5.1	12.7	17.7	1.5	2.1	4.2	1.1	1.4	2.4	3.1	6.5	9.3	2.5	3.6	9.5	**1.3**
HAL-V	0.1	0.2	0.3	0.4	0.8	1.1	0.3	0.7	1.0	0.0	0.1	0.2	0.0	0.0	0.1	0.4	0.6	1.1	0.2	0.4	0.8	**0.1**
HAL-VIII	0.1	0.3	0.3	0.4	0.4	0.7	0.2	0.3	0.5	0.1	0.1	0.4	0.1	0.2	0.3	0.4	0.5	0.5	0.3	0.3	0.7	**0.1**
HAL-RE	2.9	5.3	7.1	2.7	7.0	10.8	5.5	13.7	19.8	3.7	13.0	19.4	1.5	5.7	15.2	4.1	8.4	7.1	4.1	5.7	9.6	**4.6**
CD-341	0.2	0.4	0.5	0.4	0.5	0.6	0.3	0.4	0.6	0.2	0.4	0.9	0.3	0.4	1.0	0.5	0.7	0.7	0.4	0.6	0.9	**0.3**
CD-582	0.1	0.1	0.3	0.4	1.1	2.3	0.2	0.9	1.6	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.6	2.0	0.1	0.4	1.2	**0.1**
Bispyribac	43	46	26	51	28	17	56	45	31	39	37	29	35	30	26	36	36	22	48	31	19	**12**
5-OH-BIS	0.2	0.2	0.1	0.2	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	**0.0**
Desmethyl-BIS	1.1	1.2	1.0	0.9	0.4	0.6	1.4	1.3	1.8	0.8	0.6	0.8	0.6	0.6	1.0	0.9	1.0	0.9	1.2	0.6	0.8	**0.5**
Me2BA	2.7	2.7	2.9	2.5	2.7	2.4	2.1	2.5	2.2	1.3	2.2	2.1	0.9	2.3	3.1	1.4	3.5	2.2	3.6	3.3	3.5	**0.9**
CD-592	0.4	0.5	0.6	0.4	0.3	0.3	1.0	1.1	0.9	0.3	0.5	0.4	0.2	0.3	0.4	0.3	0.7	0.4	0.3	0.4	0.3	**0.2**
Penoxsulam	108	153	145	118	176	158	209	218	190	166	156	144	102	107	102	173	146	133	153	144	175	**30**
2-HP-PEN	0.0	0.4	1.0	0.0	0.2	0.4	0.1	0.1	0.4	0.2	0.1	0.4	0.3	0.4	1.0	0.2	0.3	1.0	0.0	0.2	0.5	**0.2**
BSTCA	0.0	0.0	0.3	0.0	0.1	0.5	0.0	0.2	0.4	0.0	0.2	0.6	0.0	0.1	0.4	0.0	0.0	0.5	0.0	0.0	0.5	**0.1**
2-amino-TP	0.4	0.5	0.6	0.2	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.3	0.5	0.5	0.4	0.8	0.3	0.4	0.6	**0.1**
CD-645	0.0	0.1	0.4	0.0	0.1	0.5	0.0	0.1	0.3	0.0	0.1	0.4	0.0	0.0	0.3	0.1	0.1	0.7	0.0	0.0	0.4	**0.2**

[a]HAL-III, desmethyl halosulfuron; HAL-V, glycosylated halosulfuron; HAL-VIII, halosulfuron guanidine; HAL-RE, halosulfuron rearrangement ester; CD-341, unknown halosulfuron metabolite; CD-582, likely glycosylated HAL-III; CD-592, likely glycosylated bispyribac; 2-HP-PEN, 2-hydrozyphenyl-penoxsulam; CD-645, likely glycosylated penoxsulam.

Conclusions & Future Work

- Aggregated results suggest that enhanced P450 and GT activity are present in populations R4, R10, R41, and R59, leading to differential ALS inhibitor cross-resistance. Enhanced GST activity may also be a factor in enhanced herbicide metabolism in R3, R41, and R59.
- Knowledge of differential resistance can aid California growers and advisers in tailoring field-specific herbicide programs to minimize yield loss and slow the spread of resistance.
- ALS gene sequencing may also reveal target-site alterations as a cause of resistance.

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