NEW INCIDENCES OF WEED RESISTANCE TO ALS INHIBITORS

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Over the past 20 years, an important discovery in herbicide chemistry has been herbicides that inhibit acetolactate synthase (ALS). Although there are 15 classes of chemistry which have been described as inhibitors of ALS, only four classes have been commercialized: sulfonylurea, imidazolinone, triazolopyrimidine, and pyrimidinylthiobenzoates (Saari et al. 1994). These four classes of herbicides have been widely used due to their relatively low use rates, limited environmental impact, low mammalian toxicity, wide crop selectivity, and high efficacy. The rapid adoption and persistent use of these herbicides has selected for weeds with resistance to ALS-inhibiting herbicides. Currently there are 43 monocots and 20 dicots reported worldwide to be resistant to ALS-inhibiting herbicides (Heap 1999). Within the Midwestern states, there are 14 species with reported resistance to ALS-inhibiting herbicides (Heap 1999).

Giant foxtail with putative resistance to ALS inhibitors was identified recently in Wisconsin (WI), Minnesota (MN), and Illinois (IL). These populations were identified in fields with a history of ALS inhibitor use in both corn and soybean. In addition, eastern black nightshade and green foxtail with putative resistance to ALS inhibitors were also identified recently in Wisconsin. Therefore, our objectives were to confirm and quantify resistance of giant foxtail from WI, MN, and IL, eastern black nightshade from WI, and green foxtail from WI to imidazolinone and sulfonylurea herbicides.

Materials and Methods

Seed Sources

Giant foxtail. Putatively resistant giant foxtail seeds were collected from a single location in each state. At each location, putative susceptible seeds were collected in an adjacent field which had no known use history of imidazilinone or sulfonlyurea herbicides.

Eastern black nightshade. Putatively resistant eastern black nightshade seeds were collected from a soybean field in south central Wisconsin. Putatively susceptible eastern black nightshade seeds were collected from a soybean field on the University of Wisconsin Arlington Agricultural Experiment Station.

Green foxtail. Putative resistant green foxtail seeds were collected from a soybean field in west central Wisconsin. Putatively susceptible green foxtail seeds were collected in an alfalfa field in southeastern Wisconsin which had no known use history of imidazilinone or sulfonylurea herbicides.

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Whole-Plant Dose Response Experiments

Giant foxtail. Herbicide dose-response experiments were conducted in a greenhouse at the University of Wisconsin using three- to four-leaf stage giant foxtail plants. A stationary pot sprayer with one 8002E nozzle calibrated to deliver a carrier volume of 187 L ha^{-1} at 275 kPa was used for all herbicide applications. Imazethapyr was applied at 0, 0.62, 1.25, 2.5, 5, and 10 g ae ha^{-1} to susceptible plants or 0, 17.5, 35, 70, 140, and 1120 g ae ha^{-1} to resistant plants. Nicosulfuron was applied at 0, 0.15, 0.31, 0.62, 1.25, and 2.5 g ai ha^{-1} to susceptible plants or 0, 8.8, 17.5, 35, 70, 1120 g ai ha^{-1} to resistant plants. All herbicide treatments contained 0.25% (by vol) non-ionic surfactant (NIS) and 1.25% (by vol) 28% nitrogen (N).

Eastern black nightshade. Herbicide dose-response experiments were conducted in a greenhouse at the University of Wisconsin using three- to four-leaf stage eastern black nightshade plants. Imazethapyr was applied at 0, 0.35, 0.70, 1.4, 2.8, 5.6 and 11.2 g ha^{-1} to susceptible plants or 0, 35, 70, 140, 280, 560, and 1120 g ha^{-1} to resistant plants. Imazamox was applied at 0, 0.44, 0.88, 1.76, 3.52, and 7.04 g ae ha^{-1} or 0, 44, 88, 176, 352, and 704, g ha^{-1} to resistant plants. Primisulfuron was applied at 0, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 g ai ha^{-1} to susceptible or resistant plants. Herbicide treatments were applied with a stationary pot sprayer as described above. All herbicide treatments contained 0.25% NIS and 1.25% N.

Green foxtail. Three- to four-leaf susceptible or resistant green foxtail plants were treated with 0, and 704 g ha^{-1} imazamox. Herbicide treatments were applied with a stationary pot sprayer as described above. All herbicide treatments contained 0.25% NIS and 1.25% N.

Data Collection and Experimental Design

Shoot biomass was harvested 14 days after treatment, dried at 70 C for 72 hours, and dry mass quantified. The experimental design was completely randomized for each experiment, with three or more replicates per treatment. Experiments were conducted one to two times.

***In-vivo* ALS Assay**

Giant foxtail. Three- to four-leaf resistant or susceptible giant foxtail plants were treated with 0, 0.007, 0.07, 0.7, 7, 70, and 700 g ha^{-1} imazethapyr or equal rates of nicosulfuron. Herbicide treatments were applied with a stationary pot sprayer as described above. All herbicide treatments contained 0.25% NIS and 1.25% N.

Eastern black nightshade. Resistant or susceptible three- to four-leaf stage eastern black nightshade plants were treated with of 0, 0.007, 0.07, 0.7, 7, 70, and 700 g ha^{-1} imazethapyr or equal rates of primisulfuron. Herbicides were applied with a stationary pot sprayer as previously described. All herbicide treatments contained 0.25% NIS and 1.25% N.

Plants were treated with 1, 2-cyclopropanedicarboxylic acid (CPCA) at 766 g ha^{-1} containing 0.25% NIS 21 h following herbicide application. ALS activity was determined 24 h after treatment using the *in vivo* ALS assay developed by Simpson et al. (1995) in which ALS activity is measured by accumulation of acetolactate. The acetolactate is decarboxylated to acetoin, which can be quantified spectrophotometrically. The experiment was conducted as a completely randomized design with four replications per treatment. The experiment was conducted one to two times.

RESULTS

Giant foxtail. The whole plant response of the putatively resistant Wisconsin accession was 15-and 20-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 1). The effective dose of imazethapyr that reduced shoot biomass 50% relative to non-treated plants was 14.8 g ha^{-1} for the resistant accession, whereas the ED_{50} value for the susceptible accession was 1.0 g ha^{-1}. The ED_{50} value for nicosulfuron was 8.2 g ha^{-1} for the resistant accession and 0.4 g ha^{-1} for the susceptible accession.

Table 1. Whole plant response (ED_{50}) and acetolactate synthase (ALS) enzyme response (I_{50}) of resistant (R) and susceptible (S) Wisconsin giant foxtail to imazethapyr and nicosulfuron.

	ED_{50}[a]			I_{50}[b]		
	Accession			Accession		
Herbicide	R	S	R/S	R	S	R/S
	g ha^{-1}			g ha^{-1}		
Imazethapyr	14.8 ± 3.6[c]	1.0 ± 0.2	15	> 700	1.6 ± 2.3	> 438
Nicosulfuron	8.2 ± 1.9	0.4 ± 0.8	20	200 ± 28	0.1 ± 0.2	2000

[a]ED_{50} value indicates effective herbicide dose that reduced shoot biomass by 50% relative to non-treated plants.

[b]I_{50} value indicated herbicide dose that inhibited ALS activity by 50% relative to non-treated plants.

[c]Mean ± 95% confidence interval.

The ALS enzyme response (I_{50}) of the resistant Wisconsin accession was greater than 438- and 2000-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 1). At the highest imazethapyr dose, 700 g ha^{-1}, the enzyme from the resistant accession was not inhibited by 50%. In comparison, only 1.6 g ha^{-1} of imazethapyr inhibited the enzyme from the susceptible accession by 50%. The I_{50} for the resistant accession to nicosulfuron was 200 g ha^{-1} and the I_{50} for the susceptible accession was 0.1 g ha^{-1}.

The whole plant response of the putatively resistant Minnesota accession was 14-and 19-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 2). The effective dose of imazethapyr that reduced shoot biomass 50% relative to non-treated plants was 26.2 g ha^{-1} for the resistant accession, whereas the ED_{50} value for the susceptible accession was 1.9 g ha^{-1}. The ED_{50} value for nicosulfuron was 7.6 g ha^{-1} for the resistant accession and 0.4 g ha^{-1} for the susceptible accession.

Table 2. Whole plant response (ED_{50}) and acetolactate synthase (ALS) enzyme response (I_{50}) of resistant (R) and susceptible (S) Minnesota giant foxtail to imazethapyr and nicosulfuron.

	ED_{50}[a]			I_{50}[b]		
	Accession			Accession		
Herbicide	R	S	R/S	R	S	R/S
	g ha^{-1}			g ha^{-1}		
Imazethapyr	26.2 ± 1.9[c]	1.9 ± 0.4	14	> 700	3.8 ± 2.3	> 184
Nicosulfuron	7.6 ± 2.2	0.4 ± 0.3	19	> 700	0.5 ± 0.5	> 1400

[a]ED_{50} value indicates effective herbicide dose that reduced shoot biomass by 50% relative to non-treated plants.

[b]I_{50} value indicated herbicide dose that inhibited ALS activity by 50% relative to non-treated plants.

[c]Mean ± 95% confidence interval.

The ALS enzyme response (I_{50}) of the resistant Minnesota accession was greater than 184- and greater than1400-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 2). At the highest imazethapyr dose, 700 g ha^{-1}, the enzyme from the resistant accession was not inhibited by 50%. In comparison, only 3.8 g ha^{-1} of imazethapyr inhibited the enzyme from the susceptible accession by 50%. The I_{50} for the resistant accession to nicosulfuron was greater than 700 g ha^{-1} and the I_{50} for the susceptible accession was 0.5 g ha^{-1}.

The whole plant response of the putatively resistant Illonois accession was 13- and 7-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 3). The effective dose of imazethapyr that reduced shoot biomass 50% relative to non-treated plants was 15.5 g ha^{-1} for the resistant accession, whereas the ED_{50} value for the susceptible accession was 1.2 g ha^{-1}. The ED_{50} value for nicosulfuron was 2.1 g ha^{-1} for the resistant accession and 0.3 g ha^{-1} for the susceptible accession.

Table 3. Whole plant response (ED_{50}) and acetolactate synthase (ALS) enzyme response (I_{50}) of resistant (R) and susceptible (S) Illinois giant foxtail to imazethapyr and nicosulfuron.

	ED_{50}[a]			I_{50}[b]		
	Accession			Accession		
Herbicide	R	S	R/S	R	S	R/S
	g ha^{-1}			g ha^{-1}		
Imazethapyr	15.5 ± 5.3[c]	1.2 ± 0.4	13	> 700	1.6 ± 2.1	> 437
Nicosulfuron	2.1 ± 1.5	0.3 ± 0.1	7	46 ± 13	0.7 ± 1.1	66

[a]ED_{50} value indicates effective herbicide dose that reduced shoot biomass by 50% relative to non-treated plants.

[b]I_{50} value indicated herbicide dose that inhibited ALS activity by 50% relative to non-treated plants.

[c]Mean ± 95% confidence interval.

The ALS enzyme response (I_{50}) of the resistant Illinois accession was greater than 437- and 66-fold resistant to imazethapyr and nicosulfuron, respectively, compared to the susceptible accession (Table 3). At the highest dose, 700 g ha^{-1} of imazethapyr, the enzyme from the resistant accession was not inhibited by 50%. In comparison, only 1.6 g ha^{-1} of imazethapyr inhibited the enzyme from the susceptible accession by 50%. The I_{50} for the resistant accession to nicosulfuron was 46 g ha^{-1} and the I_{50} for the susceptible accession was 0.7 g ha^{-1}.

The whole plant response data and the *in-vivo* data are not quantitatively equivalent, but the data are qualitatively similar. Other studies have also shown quantitative differences of whole plant and enzyme responses in ALS resistant accessions of common cocklebur (Sprague et al. 1997a), palmer amaranth (Sprague et al. 1997c), common waterhemp (Foes et al. 1998; Sprague et al. 1997b), and kochia (Foes et al. 1999). The data, however from the whole plant and the ALS *in-vivo* assays are consistent for resistant and susceptible accessions. The whole plant and ALS *in-vivo* assay data both quantify that the WI, MN, and IL accessions are ALS resistant.

Eastern black nightshade. Based on whole plant response, the putatively resistant eastern black nightshade was 161, 133- and 6-fold resistant to imazethapyr, imazamox, and primisulfuron, respectively, compared to the susceptible accession (Table 4). The effective dose of imazethapyr that reduced shoot biomass 50% relative to non-treated plants was 112 g ha^{-1} for the resistant accession, whereas the ED_{50} value for the susceptible accession was 0.7 g ha^{-1}. The ED_{50} value for imazamox was 37.3 g ha^{-1} for the resistant accession and 0.3 g ha^{-1} for the susceptible accession. The resistant and susceptible accession were both sensitive to primisulfuron. The ED_{50} value for the resistant accession was 1.3 g ha^{-1} and the ED_{50} for the susceptible accession was 0.2 g ha^{-1}.

Table 4. Whole plant response (ED_{50}) and acetolactate synthase (ALS) enzyme response (I_{50}) of resistant (R) and susceptible (S) eastern black nightshade to imazethapyr, imazamox, and primisulfuron..

	ED_{50}[a]			I_{50}[b]		
	Accession			Accession		
Herbicide	R	S	R/S	R	S	R/S
	g ha^{-1}			g ha^{-1}		
Imazethapyr	112 ± 13.5[c]	0.7 ± 1.1	161	22.4 ± 13.1	0.1 ± 0.2	172
Imazamox	37.3 ± 10.6	0.3 ± 0.1	133	nd[d]	nd	nd
Primisulfuron	1.3 ± 0.3	0.2 ± 0.1	6	0.1 ± 0.1	< 0.007	< 14

[a]ED_{50} value indicates effective herbicide dose that reduced shoot biomass by 50% relative to non-treated plants.

[b]I_{50} value indicated herbicide dose that inhibited ALS activity by 50% relative to non-treated plants.

[c]Mean ± 95% confidence interval.

[d]nd indicates not determined.

The ALS enzyme response (I_{50}) of the resistant accession was 172 - and 14-fold resistant to imazethapyr and primisulfuron, respectively, compared to the susceptible accession (Table 4). The I_{50} value was 22.4 and 0.2 g ha^{-1} of imazethapyr for the resistant and susceptible accessions. The I_{50} value for the resistant accession to primisulfuron was 0.1 g ha^{-1} and for the susceptible accession the I_{50} value was less than 0.007 g ha^{-1}.

Green foxtail. Resistant plants all survived treatment with 704 g ha^{-1} of imazamox, whereas all susceptible plants died at the same rate.

Summary

The Wisconsin, Minnesota, and Illinois giant foxtail accessions were cross-resistant to imazethapyr and nicosulfuron. Resistance in these giant foxtail accessions is associated with a less sensitive ALS enzyme. Resistance to ALS inhibitors has developed in three geographically isolated populations. The eastern black nightshade accession is resistant to imazethapyr and imazamox. There is lack of cross-resistance as the black nightshade is sensitive to primisulfuron. Resistance is associated with a less sensitive ALS enzyme. The green foxtail accession is resistant to imazamox.

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