FIELD-EVOLVED IMAZAPYR RESISTANCE IN *IXOPHORUS UNISETUS* AND *ELEUSINE INDICA* IN COSTA RICA

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ABSTRACT

Field-evolved resistance to acetolactate synthase inhibitors has been previously documented for sulfonylurea herbicides but not for imidazolinones. The first confirmation of imazapyr resistance, found in populations of *Ixophorus unisetus* growing along ditchbanks in rice producing areas in the Northern Pacific region of Costa Rica, is described. These populations were subjected to imazapyr applications for over five years before becoming resistant. A second case was detected in the Central Valley of Costa Rica in a poultry operation, where goosegrass (*Eleusine indica*) became the most prevalent weed after continuous overuse of imazapyr for more than 4 years. Screenhouse studies indicate that selected biotypes of *I. unisetus* are 5 to 80 times more resistant to imazapyr than the most susceptible biotype. The resistant biotype of goosegrass required 14 times more imazapyr for a 50% growth reduction. There was some degree of cross resistance to other imidazolinones and some sulfonylureas.

INTRODUCTION

Weed populations that have evolved resistance to sulfonylurea herbicides, which inhibit acetolactate synthase (ALS), have been previously characterized. Sulfonylurea-resistant biotypes of four broadleaf weeds species (*Lactuca serriola*, *Kochia scoparia*, *Salsola iberica*, and *Stellaria media*) were selected in North America by this class of herbicides (Thill *et al.*, 1991). In Australia and in England, two grasses (*Lolium rigidum* and *Alopecurus myosuroides*, respectively) evolved cross-resistance to sulfonylureas after being selected with other non-related herbicide families (Thill *et al.*, 1991).

Imidazolinone herbicides also inhibit ALS but no field- evolved resistance has been documented until now. Some sulfonylurea-resistant biotypes of *L. serriola* and *K. scoparia* exhibit cross-resistance to imidazolinones (Mallory-Smith *et al.*, 1990; Primiani *et al.*, 1990). Imidazolinone-resistant maize is being commercially developed (Newhouse *et al.*, 1991).

Herbicide resistance in grasses is less common than resistance in broadleaves, accounting for about 30% of all reported cases (LeBaron, 1991). *Eleusine indica* (goosegrass), an important weed with worldwide distribution, evolved resistance to dinitroanilines after repeated use of trifluralin in cotton over a period of 10 years in South Carolina, USA (Mudge *et al.*, 1984). *Ixophorus unisetus* (Panicoideae) is not considered a major weed, except along ditchbanks in rice and sugar cane in some areas of Central America. In a few instances, it invades rice fields outcompeting the crop and reducing yields. However, crop losses due to this weed have not been quantified. In Costa Rica, *I. unisetus* is reported at elevations from sea level to 1,200 m. It is also present in Mexico and the rest of Central America, as well as in Colombia, Venezuela, and Cuba (Pohl, 1980).

The objective of the bioassay studies reported here was to determine if field populations of both *I. unisetus* and *E. indica* from Costa Rica have evolved resistance to imazapyr and other ALS inhibitors.

MATERIALS AND METHODS

Plant material

Seed of the allegedly resistant (R) populations of *I. unisetus* were collected from plants growing along ditchbanks on a rice-producing farm in Puntarenas province. These populations (identified as Tijo Blanco and Escuadra) had been subjected to imazapyr applications at commercial rates (0.5-0.7 kg a.e./ha) for over five years. Other populations where imazapyr failed to control this weed after repeated applications were those named Taboga and CATSA-2, collected from sugar cane farms in Guanacaste province. Susceptible (S) populations, probably treated at least once with imazapyr, also came from Guanacaste (El Viejo, CATSA-1, and Palmira); the Carrillos biotype, never treated with imidazolinones, was used as the unexposed control for all biotype comparisons.

R-goosegrass seed was collected from a poultry farm located in the Central Valley, where this weed became prevalent after continuous overuse of imazapyr for more than four years. The S-biotype of goosegrass was obtained from a roadside about 3 km away from the poultry farm.

None of the populations studied of either species had been treated with other imidazolinones or any sulfonylurea herbicide.

Herbicides

I. unisetus was treated postemergence with nine dose rates of imazapyr, sulfometuron-methyl, triasulfuron, and preemergence with chlorsulfuron, imazethapyr, and imazaquin. Goosegrass was sprayed early postemergence with imazapyr, imazethapyr, imazaquin, sulfometuron-methyl, chlorimuron-ethyl, and metsulfuron methyl. Rates used are indicated in Table 1. Commercial formulations of the herbicides were applied in a spray booth (R & D Sprayers, Opelousas, Louisiana, USA) or by a portable CO_2-operated sprayer, both equipped with a flat fan nozzle delivering 200 l/ha.

Experimental procedures

For postemergence treatments, *I. unisetus* seed was germinated in soil at room temperature and seedlings transplanted 10-12 days after emergence to pots containing approximately 0.5 kg of soil. Goosegrass seed was mechanically dehulled and caryopses were germinated in Petri dishes containing Whatman No. 2 filter paper moistened with 0.2% KNO_3. Dishes were placed in a growth chamber at 30°C/20°C day/night temperatures (12 h photoperiod). Seedlings (2-3 cm long) were individually transplanted to pots. Pots were maintained in a screenhouse or outside before and after herbicide treatment. Plants (10 per pot) were sprayed with the corresponding herbicide at the 5-6 leaf stage for *I. unisetus* or at the 2-3 leaf stage for goosegrass. Above-ground tissue of *I. unisetus* plants treated with imazapyr was harvested 7 days after application (DAA); and those treated with triasulfuron or sulfometuron-methyl, at 15 DAA to determine both fresh and dry weights. Goosegrass plants were harvested 15 DAA. Two weeks after initial harvesting, clipped plants that resprouted were counted and weighed.

TABLE 1. Rates of herbicides for bioassays with *I. unisetus* and *E. indica*[1].

Herbicide	Dose range (g/ha)[2]	Species	Biotype
Imazapyr	0 - 2400	Ei, Iu	S
	0 - 19200	Ei, Iu	R
Imazaquin	0 - 640	Ei, Iu	S
	0 - 2560	Ei, Iu	R
Imazethapyr	0 - 1200	Ei, Iu	S
	0 - 9600	Ei, Iu	R
Chlorsulfuron	0 - 60	Iu	S
	0 - 240	Iu	R
Chlorimuron-ethyl	0 - 40	Ei	S
	0 - 160	Ei	R
Metsulfuron-methyl	0 - 40	Ei	S
	0 - 160	Ei	R
Sulfometuron-methyl	0 - 300	Ei	S
	0 - 1200	Ei	R
	0 - 75	Iu [3]	S
	0 - 150	Iu	R
Triasulfuron	0 - 200	Iu	S
	0 - 800	Iu	R

1 Abbreviations: Ei: *Eleusine indica*, Iu: *Ixophorus unisetus*, S: susceptible, R: resistant.

2 Rates for imidazolinone herbicides are given in acid equivalent; for sulfonylureas, in active ingredient.

3 El Viejo, S-biotype of Iu, was treated with the same rates used for S-Ei.

For preemergence herbicides, *I. unisetus* seed was germinated in a 50:50 mixture of soil:sand and transplanted (10 each) into pots with soil, when the radicles were about 1-3 mm long. The following day, pots were sprayed as before and surviving plants were counted and weighed 30 DAA. The response of biotypes CATSA-1 and Taboga to imazethapyr and chlorsulfuron could not be determined because seed failed to germinate.

A completely randomized design with four replications was used for the bioassays, unless initial plant growth differences were noticeable when a randomized complete block design was more suitable. Data were analyzed by regression using appropriate transformations when needed and GR_{50} (herbicide rate required to reduce fresh weight by 50%) or LD_{50} values were calculated from regression equations. Most responses were described by exponential or polynomial functions. Data presented here correspond to fresh weight evaluations and regrowth counts.

RESULTS AND DISCUSSION

A wide range of responses to ALS inhibitors was observed among *I. unisetus* biotypes. GR_{50} values and resistance indexes (RI: ratio of GR_{50} of each biotype over Carrillos biotype) are presented in Tables 2 and 3. Biotypes originally considered as susceptible were affected by imazapyr similarly to the control (Carrillos) biotype as indicated by GR_{50} values. Taboga, a biotype collected from a sugar cane farm with imazapyr-use history was susceptible. The most resistant biotypes (Tijo Blanco and Escuadra) came from the farm (Hacienda San Agustín) where imidazolinone resistance was originally suspected. Escuadra is almost 80 times more resistant than Carrillos and plant regrowth could be inhibited only after spraying a rate 32 times higher than that required for the same effect in the susceptible biotype. Only Tijo Blanco and Escuadra exhibited cross-resistance to other imidazolinones (Table 3). El Viejo and Palmira had negative cross-resistance (greater sensitivity) to imazethapyr. All biotypes were equally or more susceptible to sulfometuron-methyl and chlorsulfuron than the control biotype. It was not possible to calculate a GR_{50} value for triasulfuron because plants were only slightly affected by this herbicide. However, Tijo Blanco was relatively more susceptible to triasulfuron than the other biotypes (data not shown). Lack of efficacy of sulfonylureas against *I. unisetus* was expected since these herbicides are commercially used to control broadleaves and show selectivity to grass crops and weeds.

Primiani et al. (1990) found a resistance index of 30 by visually comparing a chlorsulfuron-resistant biotype of *K. scoparia* with a susceptible one, and observed different degrees of cross-resistance to other sulfonylureas and imazapyr.

TABLE 2. Relative response of *I. unisetus* biotypes to postemergence application of imazapyr and sulfometuron-methyl.

Biotype	Imazapyr			Sulfometuron-methyl		
	GR_{50} (g/ha)	RI[1]	RIAR	GR_{50} (g/ha)	RI	RIAR
Carrillos	22.0	1.00	300	10.5	1.00	>75
El Viejo	22.8	1.04	75	1.9	0.18	150
CATSA-1	33.9	1.54	150	0.6	0.06	>75
Palmira	47.4	2.15	38	17.7	1.69	>75
CATSA-2	<150.0	<6.82	150	1.0	0.09	150
Tijo Blanco	117.6	5.35	4800	0.5	0.05	>150
Taboga	45.6	2.11	300	2.6	0.25	>150
Escuadra	1725.0	78.80	9600	12.0	1.15	>150

[1] Abbreviations: RI: Resistance index, RIAR: Lowest regrowth-inhibiting applied rate in g/ha.

TABLE 3. Relative response of *I. unisetus* biotypes to preemergence application of chlorsulfuron, imazaquin and imazethapyr.

Biotype	Chlorsulfuron		Imazaquin		Imazethapyr	
	GR_{50} (g/ha)	RI[1]	GR_{50} (g/ha)	RI	GR_{50} (g/ha)	RI
Carrillos	14.04	1.00	10.5	1.00	53.2	1.00
El Viejo	<1.47	<0.03	7.6	0.72	6.5	0.12
CATSA-1	-	-	14.4	1.37	-	-
Palmira	3.01	0.21	9.1	0.86	11.6	0.22
CATSA-2	1.08	0.08	15.6	1.48	66.9	1.26
Tijo Blanco	6.13	0.44	155.7	14.83	475.2	8.93
Taboga	-	-	14.0	1.33	-	-
Escuadra	21.28	1.52	151.9	14.47	628.0	11.80

[1] RI: Resistance index.

Imazapyr-resistant goosegrass showed cross-resistance to imazaquin, sulfometuron-methyl, chlorimuron-ethyl and, especially, to imazethapyr (Table 4). Metsulfuron-methyl had little effect on goosegrass growth; this herbicide is commonly used for selective broadleaf weed control in rice and other crops. The level of resistance to imazapyr in goosegrass (RI = 14-21) is moderate in comparison to that observed with the most resistant biotype of *I. unisetus* (RI = 79).

TABLE 4. Response of two *E. indica* biotypes from Costa Rica to imidazolinone and sulfonylurea herbicides.

Herbicide[1]	--------- GR_{50} (g/ha) --------- S-Biotype		R-Biotype		RI		Regrowth LD_{50} (g/ha)		
	PFW	RFW	PFW	RFW	PFW	RFW	R	S	RI
IPR	34	49	460	1030	14	21	1460	46	32
ITR	150	210	>9600	>9600	>64	>46	>9600	390	>25
IQN	525	592	>2560	>2560	>5	>4	LI	LI	-
SFM	10	9	62	156	6	18	921	21	43
CME	28	30	>160	>160	>6	>5	LI	LI	-
MSM	ND	LI	ND	LI	-	-	LI	LI	-

[1] Abbreviations: IPR: imazapyr, ITR: imazethapyr, IQN: imazaquin, SFM: sulfometuron-methyl, CME: chlorimuron-ethyl, MSM: metsulfuron-methyl, PFW: plant fresh weight, RFW: regrowth fresh weight, RI: Resistance index (R/S), S: susceptible, R: resistant, ND: not determined (data did not allow calculation), LI: Low growth inhibition even at highest rates precluded calculation.

The mechanism of resistance to imidazolinones in *I. unisetus* and goosegrass has not been studied. Resistance to sulfonylureas in resistant broadleaf weeds is due to decreased sensitivity of the ALS enzyme to these herbicides (Thill *et al.*, 1991). The discovery of imazapyr resistance in these two weeds offers the opportunity to further study physiological aspects of ALS-inhibiting herbicides.

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