

Rapid Communication

Lack of Cross-Resistance of Imazaquin-Resistant *Xanthium strumarium* Acetolactate Synthase to Flumetsulam and Chlorimuron

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Acetolactate synthase (ALS) was isolated from a field population of cocklebur (*Xanthium strumarium*) that developed resistance to the herbicide Scepter following three consecutive years of application. The active ingredient of Scepter, imazaquin, gave an inhibitor concentration required to produce 50% inhibition of the enzyme activity that was more than 300 times greater for the resistant enzyme than for the wild-type cocklebur ALS. Tests with flumetsulam and chlorimuron show that the resistant ALS was not cross-resistant to these two other classes of ALS inhibitors.

In recent years, target-site resistance has surfaced as a potential barrier to the long-term successful use of three highly active herbicidal chemical families, namely the imidazolinones (Los, 1987), sulfonylureas (Beyer et al., 1988), and triazolopyrimidine sulfonanilides (Gerwick and Kleschick, 1991). The structure of one member of each of these three families of herbicides is shown in Figure 1. These compounds have been shown to act by inhibiting the enzyme ALS (EC 4.1.3.18) (Shaner et al., 1984; Beyer et al., 1987; Subramanian et al., 1989), which catalyzes the first common step in the biosynthesis of Leu, Ile, and Val in plants and microorganisms.

Resistance to all three chemical classes has been selected for in a wide variety of species including cotton (Subramanian et al., 1990), wheat (Newhouse et al., 1992), *Datura innoxia* (Saxena and King, 1988), maize (Anderson and Georgeson, 1989), and tobacco (Chaleff and Ray, 1984). Also, selection for resistant populations of *Lactuca serriola*, *Kochia scoparia*, *Salsola iberica*, and *Stellaria media* has occurred in the field after continuous use of sulfonylureas (Mallory-Smith et al., 1990). Almost all cases of resistance to ALS inhibitors have been due to an altered ALS enzyme. Schloss et al. (1988) demonstrated that all three chemical classes of herbicides compete for binding to ALS. However, ALS isolated from many mutant plants has shown varying levels of cross-resistance among these classes of inhibitors (Saxena and King, 1988, 1990; Hall and Devine, 1990), suggesting that these compounds share an overlapping but not identical binding site on ALS. In addition, instances have been recorded where mutants selected for resistance to one chemical class were

found to be hypersensitive to other classes of ALS inhibitors (Saxena and King, 1988).

Scepter-resistant *Xanthium strumarium* was first observed in 1991 in a Mississippi field that had received Scepter only between crop rows (banded treatments) since 1989. Each year, subsequent treatments of Scepter were applied to the field as cocklebur appeared. The resistance of these plants was confirmed in 1991 by Dr. William Barrentine (Delta Research and Extension Center, Stoneville, MS). In the present article, we provide, for the first time, ALS inhibition data and cross-resistance comparisons for a naturally occurring weed population resistant to the imidazolinone imazaquin.

MATERIALS AND METHODS

Chemicals

Flumetsulam and imazaquin used in the present study were synthesized by chemists at DowElanco (Indianapolis, IN). Chlorimuron was isolated from the formulated herbicide Classic. Pyruvate, DTT, FAD, TPP, Val, Leu, Cys, 2-naphthol, and creatine were purchased from Sigma. Stock solutions of inhibitors were prepared in DMSO, which was purchased from Fisher Scientific Co. (Fair Lawn, NJ).

Plant Material

The Scepter-resistant *Xanthium strumarium* seeds were a gift from Dr. William Barrentine. They were harvested in December 1991 from a field in Bolivar County, MS. Susceptible seeds were purchased from Azlin Seed Co. (Leland, MS). The germination of the cocklebur seeds was increased by soaking them in 72 μM GA₃ for 24 h, draining, and refrigerating for at least 5 d before planting. The resistant and wild-type plants were grown in 25-cm-diameter pots containing a sandy loam soil in a greenhouse with 25 to 30°C days and 15 to 20°C nights.

Enzyme Extraction and Assay

Preparation and assay of crude extracts were based on a modification of the method described by Singh et al. (1988).

Abbreviations: ALS, acetolactate synthase; FAD, flavin adenine dinucleotide; I_{50} , inhibitor concentration required to produce 50% inhibition of the enzyme activity; TPP, thiamine pyrophosphate; PVPP, polyvinylpyrrolidone.

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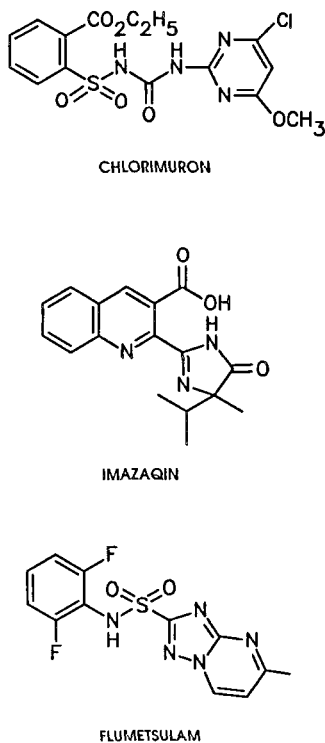


Figure 1. The chemical structures of chlorimuron, a sulfonylurea; imazaquin, an imidazolinone; and flumetsulam, a triazolopyrimidine sulfonanilide.

Young leaf tissue (5 g) was ground to a fine powder in liquid nitrogen. The powder was added to 150 mL of cold extraction buffer containing 20 mM Hepes, pH 7.2, 5 mM $MgCl_2$, 10 mM sodium pyruvate, 5 mM EDTA, 1 mM Val, 1 mM Leu, 10 mM Cys, 100 μM FAD, 10% glycerol, and 1% (w/v) PVPP and stirred at 4°C for 5 min. The homogenate was filtered through two layers of Miracloth and centrifuged at 25,000g for 20 min at 4°C. The supernatant was brought to 50% saturation with $(NH_4)_2SO_4$ and stirred for 20 min at 4°C. The mixture was then centrifuged at 25,000g for 30 min at 4°C, and the supernatant was discarded. The remaining pellet was resuspended in 20 mM Hepes (pH 7.2) containing 1 mM TPP, 10 μM FAD, and 1 mM DTT and used immediately.

Enzyme activity was assayed colorimetrically by measuring the amount of acetoin formed from acetolactate using the method of Westerfeld (1945). Standard reaction mixtures contained 20 mM Hepes (pH 7.2), 5 mM $MgCl_2$, 1 mM TPP, 10 μM FAD, 20 mM sodium pyruvate, and various concentrations of the inhibitors in a final DMSO concentration of 4.2%. The reaction was initiated with the enzyme and the mixture was incubated at 37°C for 90 min. The reaction was stopped with the addition of H_2SO_4 to produce a final concentration of 0.5% H_2SO_4 and the mixture was heated at 60°C for 20 min to decarboxylate the acetolactate. The acetoin concentration produced was determined by adding creatine and 1-naphthol to the reaction mixture in final concentrations of 2.0 mg/mL and 20 mg/mL, respectively. The mixture was

made alkaline with the addition of NaOH to a final concentration of 0.5 N and was incubated at 37°C for 40 min. The A_{530} was measured to determine the amount of acetoin produced. The reaction was determined to be linear for at least 90 min.

Inhibition of the enzyme activity was determined by testing 10 concentrations of the inhibitors spanning a range of 28,000 μM . Two sets, containing two repetitions per set, were tested for each inhibitor.

RESULTS AND DISCUSSION

Figure 2 shows the inhibition curves of the susceptible and Scepter-resistant enzymes for the three ALS-inhibiting compounds. The I_{50} values for the three classes of herbicides for inhibition of the wild-type cocklebur ALS varied greatly, but all three showed significant levels of inhibitory activity at appropriate concentrations (Table I). Flumetsulam and chlorimuron inhibited the activity of the Scepter-resistant cocklebur ALS at levels comparable to the inhibition of the wild-type enzyme, with resistance ratios of 1.4 and 1.1, respectively (Table I). The resistant cocklebur ALS showed a resistance ratio of greater than 324 to the active ingredient of Scepter, imazaquin (Table I). No cross-resistance was seen for at least one other member of the triazolopyrimidine family (data not shown). The differential susceptibility of ALS isolated from the cocklebur biotype resistant to imazaquin inhibition is consistent with the observed resistance in the field. The sensitivity of the ALS from the resistant cocklebur to both flumetsulam and chlorimuron is also consistent with the sensitivity seen in the field (W. Barrentine, unpublished data).

Although imidazolinone-resistant plants have been isolated through mutagenesis (Saxena and King, 1990; Sathasivan et al., 1991; Newhouse et al., 1992), this is the first report of an enzyme-based resistance to an imidazolinone occurring

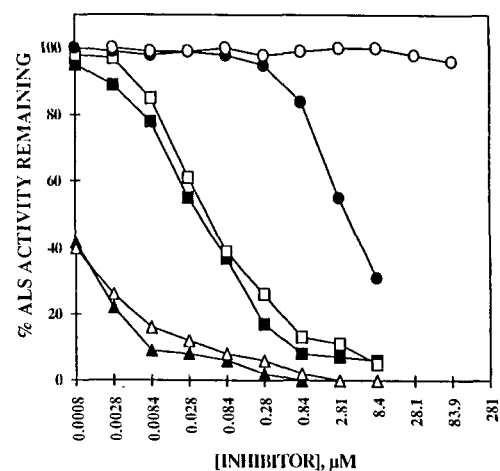


Figure 2. Inhibition curves for the the three ALS-inhibiting compounds. The susceptible (closed symbols) and resistant (open symbols) enzymes were assayed at varying concentrations of chlorimuron (Δ , \blacktriangle), flumetsulam (\square , \blacksquare), and imazaquin (\circ , \bullet). The inhibition data were determined as described in "Materials and Methods." Results are the average of two sets containing two replications per set.

Table I. I_{50} values for three classes of ALS inhibitors determined on the crude extracts of both wild-type and Scepter-resistant *X. strumarium* ALS

Inhibitors	I_{50}		Resistance Ratio ^a
	Sensitive	Resistant	
	<i>nM</i>		
Flumetsulam	350 ± 70	500 ± 23	1.4
Chlorimuron	1.8 ± 0.33	2.0 ± 0.16	1.1
Imazaquin	7400 ± 2300	>2.4 × 10 ⁶	>324

^a The resistance ratio was calculated by dividing the I_{50} of the resistant ALS by the I_{50} of the sensitive ALS.

through field use of the formulated herbicide. Because this population was identified after only three successive years of Scepter use, the likelihood of additional biotypes emerging with resistance to herbicides that inhibit ALS seems high. A major difference between the imidazolinone resistance identified in this report and naturally occurring sulfonyleurea resistance is the lack of cross-resistance to the two other classes of ALS-inhibiting chemicals. Hall and Devine (1990) showed that a naturally occurring chlorsulfuron-resistant biotype of *S. media* was totally cross-resistant to D489, a member of the triazolopyrimidine sulfonanilide family, and was slightly cross-resistant to imazamethabenz, an imidazolinone herbicide. Given the structural dissimilarities between chemical families of ALS inhibitors, it is possible that the various families of inhibitor molecules employ different structural elements of the same inhibitor-binding domain. Our results, along with those of Hall and Devine (1990), strongly suggest that the inhibitor-binding domain of ALS does contain structural elements that are unique to imazaquin in addition to the shared elements of the sulfonyleurea- and triazolopyrimidine-binding site. It follows that deactivation of the unique imazaquin-binding element(s) within the inhibitor-binding domain has not affected the binding of the sulfonyleurea and triazolopyrimidine classes of inhibitors in Scepter-resistant *X. strumarium*.

The continued appearance of weeds resistant to any of the ALS inhibitors is reason for concern, but the above data show that resistance of a weed biotype to a single class of ALS inhibitors does not necessarily mean that there will be cross-resistance to other classes of ALS-inhibiting herbicides.

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