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**THE INTERACTION OF ACETOLACTATE SYNTHASE INHIBITING
HERBICIDES WITH GRAMINICIDES AND INSECTICIDES**

By

Antonio Castro-Escobar

A THESIS

**Submitted to
Michigan State university
in partial fulfillment of the requirements
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ABSTRACT

THE INTERACTION OF ACETOLACTATE SYNTHASE INHIBITING HERBICIDES WITH GRAMINICIDES AND INSECTICIDES

BY

ANTONIO CASTRO-ESCOBAR

The acetolactate synthase inhibitors may interact antagonistically with acetyl-CoA carboxylase inhibitors to reduce grass control. ALS inhibitors may also interact synergistically with soil applied insecticides such as terbufos and may injure corn. Greenhouse studies were conducted to evaluate the antagonistic interaction between imazethapyr, technical and formulated, with graminicides in giant foxtail control. The effect of ammonium sulfate in the interaction was also evaluated. 2-Ketobutyrate may accumulate as a result of the blocking of the ALS enzyme by ALS inhibitors. Thus, the role of excess 2-ketobutyrate on the interaction of imazethapyr and fluzifop-butyl was also studied in giant foxtail. In addition, field studies were conducted to evaluate the interaction of the ALS inhibitors nicosulfuron and primisulfuron with the insecticide terbufos. A rapid pesticide detection kit was evaluated for terbufos detection in corn shoot extract.

An antagonistic interaction resulting from the formulated imazethapyr and the imazethapyr technical product with the graminicides, fluzifop-butyl, sethoxydim, quizalofop, and UBI-C4874 was observed on giant foxtail control. No antagonistic

interaction was observed with the formulation blank and the graminicides suggesting that a chemical antagonism was unlikely. The addition of ammonium sulfate to the tank mixture overcame the antagonistic interaction.

The inclusion of 2-ketobutyrate caused a reduction in giant foxtail shoot fresh weight. A significant antagonistic interaction was observed between fluazifop-butyl and 2-ketobutyrate applied at 10^{-2} M. The metabolic intermediates 2-ketobutyrate, 2-aminobutyrate, or pyruvate combined with imazethapyr increased injury to giant foxtail. Addition of 2-ketobutyrate appeared to overcome the antagonistic interaction between imazethapyr and fluazifop-butyl.

Postemergence applications of nicosulfuron and primisulfuron to corn grown in a field previously treated with terbufos for corn rootworm control resulted in a synergistic interaction injurious to corn. A strong correlation between corn injury and the amount of terbufos level detected with the pesticide detector kit in the shoot extract was obtained. The pesticide detector kit was an efficient method to detect the presence of terbufos in the corn plants. **Nomenclature:** Imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; fluazifop, ()-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]-oxy]phenoxy]propanoic acid; sethoxydim, 2-[1-(ethoxymino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; quizalofop, ()-2[4-[CG-chloro-2-quinoxalinyloxy]phenoxy]propanoic acid; UBI-C4874, ()-tetrahydrofuryl(R)-2-[4-(-6chloroquinoxalin-2-yloxy)phenoxy]propanoic acid; nicosulfuron, 2[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide; primisulfuron, 2-[3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl]-benzoic acid methylester; terbufos, S-[[[(1,1-

dimethylethylthio)methyl]O,O-diethylphosphorodithionate; corn, [*Zea mays* L.]; giant
foxtail, [*Setaria faberi* Herrm.].

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INTRODUCTION

Herbicides have been the most frequently used chemicals in modern crop management systems. Today, the use of more than one chemical in the same crop is a common production practice.

Herbicides might be applied as a mixture with adjuvants, fertilizers, fungicides, insecticides, nematicides, or other herbicides. The increase in no-till and minimum tillage acreage, improved biological performance, environmental and economic incentives, and weed resistance management are some of the factors that have sparked the use of chemical mixtures.

Undesired interactions among pesticides can occur. The interaction between ALS inhibitors with graminicides and insecticides has become the focus of considerable research. The interaction of ALS inhibitors with graminicides may result in loss of weed control; the interaction of ALS inhibitors with insecticides may result in crop injury.

The objectives of these research were: 1) to determine the extent and basis of the imazethapyr and graminicide interaction, 2) to determine whether the imazethapyr formulation contributes to the interaction, 3) to determine whether tank-mixing ammonium sulfate with imazethapyr and a graminicide will overcome the observed interaction, 4) to determine whether 2-ketobutyrate was involved in the observed interaction, and 5) to evaluate a quick and easy to use kit to detect the presence of terbufos in corn seedlings at levels that result in corn injury from postemergence applications of nicosulfuron and primisulfuron.

CHAPTER ONE
OVERCOMING THE ANTAGONISTIC INTERACTION
OF IMAZETHAPYR AND GRAMINICIDES

Abstract. The effect of imazethapyr formulations on the activity of several graminicides was studied under greenhouse conditions. The effect of imazethapyr formulation and ammonium sulfate on the interaction of imazethapyr and graminicides was also evaluated. Formulated imazethapyr, imazethapyr formulation blank, and imazethapyr technical product were applied in a tank-mixture with various graminicides at one-fourth and one-half of the normal application rate to giant foxtail. An antagonistic interaction of the formulated imazethapyr and the imazethapyr technical product with the graminicides, fluazifop-butyl, sethoxydim, quizalofop-ethyl, and UBI-C4874 on giant foxtail control was observed. Since this antagonistic interaction was not observed with the formulation blank and the graminicides, a chemical interaction is unlikely. The addition of 1.12 kg/ha of ammonium sulfate to the spray mixtures overcame the observed antagonistic interaction. The explanation for the observed effect of ammonium sulfate appeared to be the enhancement of imazethapyr activity on giant foxtail control. **Nomenclature:** Fluazifop, (\pm)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]-oxy]phenoxy] propanoic acid; imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; quizalofop, (\pm)-2-[4-[CG-chloro-2-quinoxalinyloxy] phenoxy]propanoic acid; sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; UBI-C4874, (\pm)-tetrahydrofurfuryl(*R*)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propanoic acid; giant foxtail, *Setaria faberi* Herrm. # SETFA.

Additional index words: Ammonium sulfate, imazethapyr, quizalofop, sethoxydim, UBI-C4874, SETFA.

INTRODUCTION

The use of two or more agrochemicals on the same crop has become a common production practice (3). Sequential applications of agrochemical mixtures or combinations such as mixtures of herbicides with adjuvants, fertilizers, fungicides, insecticides, nematicides, or other herbicides may be used as part of modern pest management practices.

The growing number of herbicides that are off patent, the increased demand for greater weed control, and the increase in no-till and minimum tillage acreage are some of the contributing factors for the use of more herbicide mixtures. However, a broader spectrum of weed control and decreased costs of application are the primary reasons for using herbicide mixtures (3). Other economic and environmental incentives have also increased the importance of herbicide mixtures. The use of mixtures decreases the number of trips across the field, saves fuel, decreases labor, and reduces the mechanical damage to the crop and soil. Herbicide combinations may also prevent the development of resistant weed species which result from the long-term use of a single effective herbicide.

Despite the biological and economic advantages, problems are also associated with herbicide mixtures. An example of these problems is the interaction of imazethapyr with various graminicides.

Soybean growers have found imazethapyr an effective postemergence herbicide; however, grass control may not be as good as desired. Tank-mixing graminicides effective for grass control in soybeans with imazethapyr has been reported to cause antagonism in the control of grasses (4). The extent and basis or the role of the imazethapyr formulation in this interaction have not been determined. Sequential applications of the graminicide and imazethapyr prevent the interaction but is more costly and demanding of time than the application of tank-mixture. The addition of ammonium sulfate to bentazon effectively

overcame the antagonistic interaction between bentazon and sethoxydim (8). The potential for ammonium sulfate to facilitate tank-mixing graminicides with imazethapyr merits investigation. It would be beneficial if a system could be found to mix imazethapyr with grass herbicides without reducing the performance of such herbicides.

The objectives of this research were: a) to determine the extent and basis of the imazethapyr and graminicide interaction, b) to determine whether the imazethapyr formulation contributes to the interaction, and c) to determine whether tank-mixing ammonium sulfate with imazethapyr and a graminicide will overcome the observed interaction.

MATERIALS AND METHODS

Imazethapyr and graminicide interaction studies. Giant foxtail was grown in 945-ml plastic pots containing Baccto potting media. The pots were placed in the greenhouse at $25 \pm 2^\circ\text{C}$ with 16-h days. Natural plus supplemental lighting with high pressure sodium lighting provided an average of $1200 \mu\text{E} \cdot \text{m}^2 \cdot \text{s}^{-1}$ in the greenhouse. After emergence, plants were thinned to three plants per pot. Herbicide treatments were applied postemergence to the grass plants at the three- to four-leaf stage. The herbicide treatments included 12.9 and 25.0 g ai/ha of imazethapyr formulated product, formulation blank, and 4.25 and 8.5 g/ha of technical product, respectively. Graminicides used were fluazifop-butyl (53.4 and 106.8 g ai/ha), sethoxydim (53.9 and 107.8 g ai/ha), UBI-C 4874 (24.9 and 49.9 g ai/ha) and quizalofop-ethyl (24.9 and 49.9 g ai/ha), respectively. Each of the graminicides and imazethapyr formulation experiments were conducted separately. For that reason, a separate LDS value is presented for each study. All herbicide treatments were applied with 1% v/v of crop oil concentrate except quizalofop-ethyl which was applied with 1/4% of the non-ionic

surfactant X-77¹. Ammonium sulfate at rate of 1.12 kg/ha was also applied as tank mixture with the herbicides.

Herbicide treatments were applied with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume. A completely randomized design was used and each treatment was replicated four times. Data presented are the means of two experiments.

Visual injury 14 days after herbicide treatment and shoot height 16 days after treatment were used as a measure of grass control. Visual injury rating scale of 0 to 100% was used with complete death of grass plants receiving a rating of 100%. Following the factorial analysis of variance, means were separated with Fisher's protected LSD at the 5% level of significance.

RESULTS AND DISCUSSION

An antagonistic interaction of the imidazolinone, imazethapyr, and the graminicides, fluazifop-butyl, sethoxydim, quizalofop-ethyl, and UBI-C4874 on annual grass control was observed in the greenhouse study (Table 1). Myers and Coble (4) have previously reported observing the antagonistic interaction with tank-mixtures of imazethapyr with fluazifop-butyl, sethoxydim, and quizalofop-ethyl. They were able to overcome the interaction with sequential applications of graminicides and imazethapyr. The antagonistic interaction of N-bentazon with sethoxydim was similarly overcome with sequential applications (2, 5). Wanamarta et al. (7) identified this interaction as a chemical interaction in which the Na^+ from the formulated bentazon associated with the sethoxydim to form Na-sethoxydim. Imazethapyr is commercially formulated as the NH_4^+ salt. Since fluazifop-butyl and quizalofop-ethyl are esters, they are unlikely to compete with imazethapyr for the NH_4^+

¹X-77 nonionic surfactant is a mixture of alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol marketed by Valent U.S.A. Corp., 1333 N. California Blvd., Walnut Creek, CA 94596.

leaving imazethapyr vulnerable to the formation of Ca^{++} and Mg^{++} salts. These salts are less readily absorbed by plants resulting in a reduced weed control.

Gerwick, et al. (1) could not explain the imidazolinone herbicide interaction with the graminicide on the basis of decreased absorption of the graminicide. They suggested that the antagonism resulted from a physiological link between the effect of the imidazolinone on acetolactate synthase and the graminicide effect on acetyl-CoA carboxylase. The results presented in Table 2 indicate that the antagonistic interaction occurred with the technical as well as the formulated imazethapyr. The formulation blank did not affect graminicide activity. Thus, a chemical interaction between the imazethapyr formulation and the graminicide is unlikely.

The addition of ammonium sulfate to the spray solution helped overcome the antagonistic interaction on annual grass control (Table 3). The explanation for the observed effect of ammonium sulfate appears to be the enhancement of imazethapyr activity on grass control. Fluazifop-butyl activity was not enhanced by the ammonium sulfate. This was expected since the fluazifop-butyl is an ester and not a salt. The presence of Ca^{++} and Mg^{++} in hard water may overwhelm the NH_4^+ from the NH_4^+ imazethapyr and result in the formation of Ca^{++} and Mg^{++} salts of imazethapyr. The Ca^{++} and Mg^{++} salts of herbicides are usually not absorbed as well as the NH_4 salts (6, 7).

The potential for such a link are shown in Figures 1 and 2.

Table 1. The interaction of imazethapyr on the effect of various graminicides on the visual injury and shoot growth of giant foxtail 14 days after postemergence herbicide application.

Herbicide treatment	Rate	Fluazifop-butyl				Sethoxydim				UBI-C4874				Quizalofop-ethyl			
		Visual injury		Shoot height		Visual injury		Shoot height		Visual injury		Shoot height		Visual injury		Shoot height	
		14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT	14 DAT	16 DAT
		%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant
Untreated control	0	0 g	59 a	0 f	62 a	0 f	62 a	0 f	62 a	0 g	62 a	0 g	62 a	0 g	62 a	0 g	62 a
Imazethapyr ^a	1/4x	46 f	44 b	50 e	41 b	48 e	45 b	48 e	45 b	50 f	44 b	50 f	44 b	50 f	44 b	50 f	44 b
	1/2x	62 d	26 cd	64 cd	22 cd	62 d	19 c	62 d	19 c	63 e	19 c	63 e	19 c	63 e	19 c	63 e	19 c
Graminicide ^b	1/4x	83 b	10 e	94 a	9 f	98 a	9 e	98 a	9 e	100 a	8 d	100 a	8 d	100 a	8 d	100 a	8 d
	1/2x	91 a	9 e	96 a	8 e	100 a	9 e	100 a	9 e	100 a	9 d	100 a	9 d	100 a	9 d	100 a	9 d
Imaz + Gram ^c	1/4x + 1/4x	58 e	28 cd	58 d	28 c	62 d	9 e	62 d	9 e	71 c	10 d	71 c	10 d	71 c	10 d	71 c	10 d
Imaz + Gram	1/2x + 1/2x	74 c	14 e	72 b	16 de	74 b	10 de	74 b	10 de	75 b	10 d	75 b	10 d	75 b	10 d	75 b	10 d
Imaz + Gram	1/4x + 1/2x	56 e	29 c	75 b	14 ef	70 c	9 e	70 c	9 e	71 cd	9 d	71 cd	9 d	71 cd	9 d	71 cd	9 d
Imaz + Gram	1/2x + 1/4x	61 d	23 d	69 bc	17 de	67 c	14 d	67 c	14 d	68 d	11 d	68 d	11 d	68 d	11 d	68 d	11 d
LDS 0.05		3.5	5.2	7.0	7.0	4.5	3.2	4.5	3.2	3.3	3.0	3.3	3.0	3.3	3.0	3.3	3.0

Means are from two experiments with four replications each. The data for each herbicide was analyzed separately. Any means connected by the same letter are not significantly different from each other at the 5% level by LSD.

^aImazethapyr: 1/4x = 12.9 g ai/ha; 1/2x = 25.7 g ai/ha

^bGraminicide: Fluazifop: 1/4x = 53.4 g ai/ha, 1/2x = 106.7 g ai/ha; Sethoxydim: 1/4x = 53.9 g ai/ha, 1/2x = 107.8 g ai/ha; UBI-C4874: 1/4x = 24.9 g ai/ha, 1/2x = 49.9 g ai/ha; Quizalofop: 1/4x = 24.9 g ai/ha, 1/2x = 49.9 g ai/ha.

^cImazethapyr + Graminicide

Table 2. The effect of imazethapyr formulations on the interaction of imazethapyr and several graminicides.

Herbicide treatment	Rate	Fluazifop-butyl				Sethoxydim				UBI-C4874	
		Visual injury 14 DAT	Shoot height 16 DAT	Visual injury 14 DAT	Shoot height 20 DAT	Visual injury 14 DAT	Shoot height 20 DAT	Visual injury 14 DAT	Shoot height 16 DAT	Visual injury 14 DAT	Shoot height 16 DAT
		%	cm/plant								
Untreated control		0 l	55 ab	0 i	59 abc	0 m	55 ab				
Imazethapyr ^a	1/4x	35 i	40 e	48 h	43 bcd	35 j	40 e				
Formulated	1/2x	43 g	31 g	52 g	34 de	43 i	31 f				
Graminicide ^b	1/4x	80 bcd	9 j	74 d	7 f	91 c	9 i				
	1/2x	84 ab	9 j	85 b	7 f	95 a	9 i				
Imaz F + Gram ^c	1/4x + 1/4x	35 i	38 ef	48 h	42 bcd	68 f	15 h				
Imaz F + Gram	1/2x + 1/2x	56 e	20 i	56 f	62 ab	63 g	11 hi				
Imaz F + Gram	1/4x + 1/2x	36 hi	29 gh	51 g	33 de	70 e	9 i				
Imaz F + Gram	1/2x + 1/4x	53 i	23 hi	55 f	42 bcd	25 l	22 g				
Imazethapyr ^d	1/4x	23 k	47 cd	46 h	37 cdf	25 l	47 cd				
Technical	1/2x	28 g	42 de	61 i	27 def	30 k	42 de				
Imaz T + Gram ^e	1/4x + 1/4x	40 gh	33 fg	55 f	20 ef	63 fg	13 hi				
Imaz T + Gram	1/2x + 1/2x	47 f	32 fg	71 d	21 def	69 ef	9 i				
Imaz T + Gram	1/4x + 1/2x	36 hi	29 g	56 f	32 de	74 d	9 i				
Imaz T + Gram	1/2x + 1/4x	36 hi	33 fg	71 d	20 ef	64 h	9 i				
Imazethapyr ^a	1/4x	0 l	50 bc	0 i	64 ab	0 m	50 bc				
Blank	1/2x	0 l	52 a	0 i	65 a	0 m	51 a				
Imaz B + Gram ^f	1/4x + 1/4x	78 d	9 j	79 c	8 f	93 b	9 i				
Imaz B + Gram	1/2x + 1/2x	83 abc	8 j	89 a	7 f	94 ab	8 i				

Herbicide treatment	Rate	Fluazifop-butyl			Sethoxydim			UBI-C4874		
		Visual injury	Shoot height	Visual injury	Shoot height	Visual injury	Shoot height	Visual injury	Shoot height	
		14 DAT	16 DAT	14 DAT	20 DAT	14 DAT	16 DAT	14 DAT	16 DAT	
		%	cm/plant	%	cm/plant	%	cm/plant	%	cm/plant	
Imaz B + Gram	1/4x + 1/2x	85 a	9 j	85 b	8 f	95 a	9 i			
Imaz B + Gram	1/2x + 1/4x	79 cd	9 j	80 c	8 f	93 b	9 i			
LDS 0.05		4.3	6.1	2.9	22.2	1.6	5.4			

Means are from two experiments with four replications each. Any means connected by the same letter are not significantly different from each other at the 5% level by LSD.

^aImazethapyr formulated/blank: 1/4x = 12.9 g ai/ha; 1/2x = 25.7 g ai/ha

^bGraminicide: Fluazifop-butyl: 1/4x = 53.4 g ai/ha, 1/2x = 106.7 g ai/ha; Sethoxydim: 1/4x = 53.9 g ai/ha, 1/2x = 107.8 g ai/ha; UBI-C4874: 1/4x = 8.25 g ai/ha, 1/2x = 16.5 g ai/ha; Imazethapyr technical: 1/4x = 4.25 g ai/ha, 1/2x = 8.5 g ai/ha.

^cImazethapyr + Graminicide

^dImazethapyr technical: 1/4x = 4.25 g ai/ha; 1/2x = 8.5 g ai/ha

^eImazethapyr Technical + Graminicide

^fImazethapyr Blank + Graminicide

Table 3. The effect of ammonium sulfate on the interaction of imazethapyr and several graminicides.

Herbicide treatment	Rate	Fluazifop-butyl				Sethoxydim				UBI-C4874			
		-		+		-		+		-		+	
		Visual injury 14 DAT	Shoot height 16 DAT										
Untreated control		0 m	55 a	0 h	52 a	0 h	59 a	0 k	57 a	0 k	55 a	52 a	
Imazethapyr ^b	¼x	35 l	40 b	48 g	14 e	83 c	42 b	35 j	9 e	63 h	40 b	14 ef	
	½x	43 j	31 c	53 ef	9 e	92 b	34 c	43 i	7 e	84 d	31 c	9 g	
Graminicide ^c	¼x	79 c	9 f	74 d	11 ef	85 c	7 e	91 bc	8 e	93 ab	9 g	8 g	
	½x	84 b	10 ef	84 c	9 f	86 c	7 e	96 ab	7 e	96 ab	11 fg	9 g	
Imaz + Gram ^d	¼x + ¼x	35 l	38 b	48 g	11 ef	86 c	42 b	68 fg	8 e	71 fg	15 ef	18 de	
	½x + ½x	56 h	20 d	56 e	9 f	95 a	27 d	66 gh	7 e	92 abc	11 fg	8 g	
Imaz + Gram	¼x + ½x	38 k	29 c	51 fg	12 ef	90 b	36 c	73 ef	7 e	76 e	9 g	9 g	
	½x + ¼x	53 i	23 d	55 e	13 ef	93 ab	26 d	64 g	7 e	88 cd	22 d	8 g	
LSD 0.05		2.1	5.3	3.3	4.7	4.5	5.0						

Means are from two experiments with four replications each. Any means connected by the same letter are not significantly different from each other at the 5% level by LSD.

^a Ammonium sulfate^e = 1135 gr/ha

^b Imazethapyr: ¼x = 12.9 g a.i./ha; ½x = 25.7 g a.i./ha

^c Graminicide: Fluazifop-butyl: ¼x = 53.4 g a.i./ha, ½x = 106.7 g a.i./ha; Sethoxydim: ¼x = 53.9 g a.i./ha, ½x = 107.8 g a.i./ha.

^d Imazethapyr + Graminicide

Figure 1. Role of ALS in synthesis of branched chain amino acids and pantothenate.

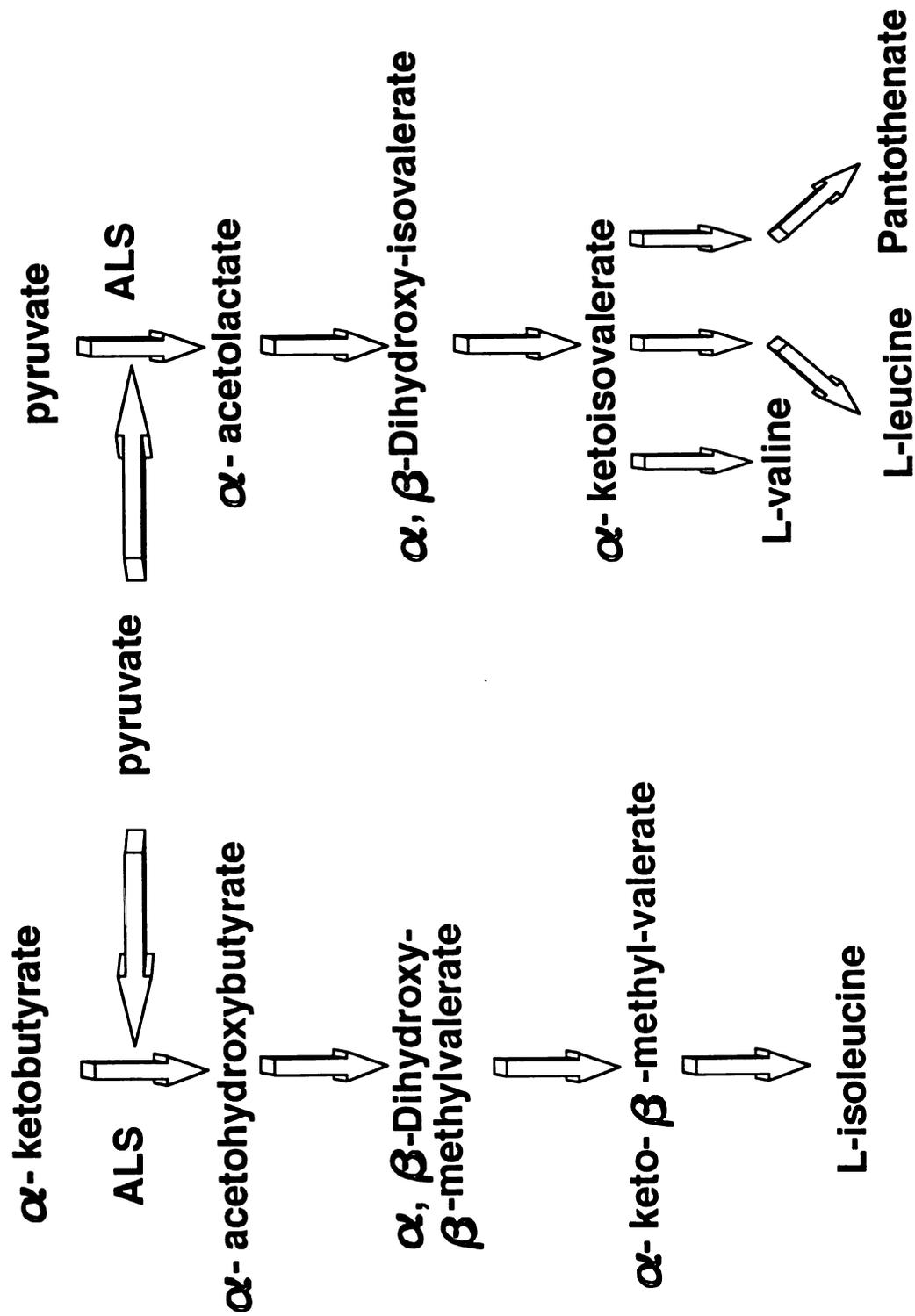


Figure 1.

Figure 2. Role of ACCase in the metabolism pathway of pantothenate to malonyl CoA.

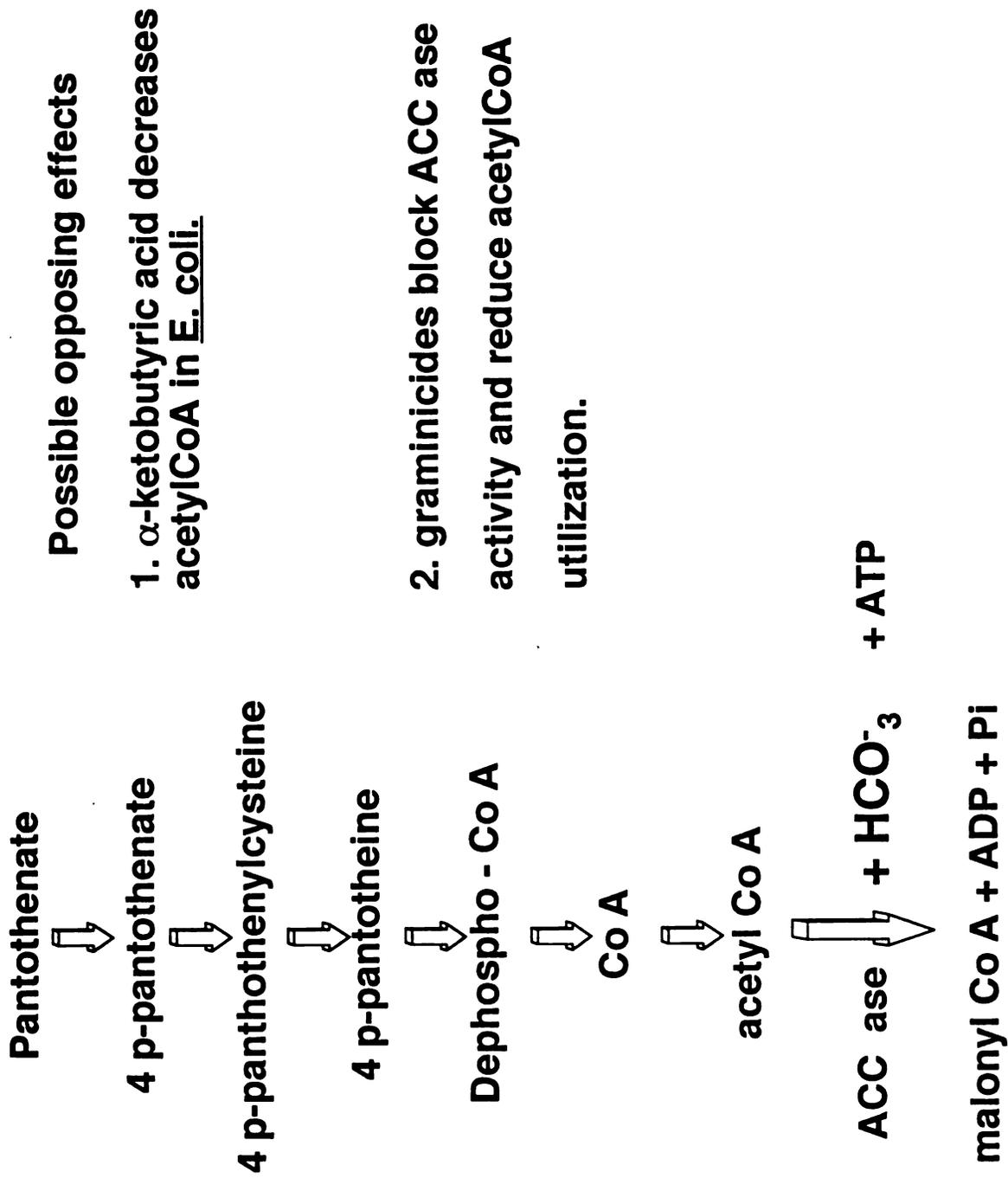


Figure 2.

LITERATURE CITED

1. Gerwick, B.C., P.C. Thompson, and R. Noveroske. 1988. Potential mechanism in antagonism with arloxyphenoxypropionate herbicides. *Abstr. Weed Sci. Soc. Amer.* 28:284.
2. Hartzler, R.G., and C.L. Foy. 1983. Compatability of BAS 9052OH with acifluorfen and bentazon. *Weed Sci.* 31:597-599.
3. Hatzios, K. K. and D. Penner. 1985. Interactions of herbicides with other agrochemicals in higher plants. *Rev. Weed Sci.* 1:1-63.
4. Myers, F. and D. Coble. 1992. Antagonism of graminicide activity on annual grass species by imazethapyr. *Weed Technol.* 6:333-338.
5. Rhodes, G.N., Jr., and H.D. Coble. 1984. Influence of bentazon on absorption and translocation of sethoxydim in goosegrass (*Eleusine indica* L.). *Weed Sci.* 32:555-597.
6. Thelen, K.D., E.P. Jackson, and D. Penner. 1992. Use of proton magnetic resonance spectrometry for determining chemical-based herbicide antagonism. *Proc. North Central Weed Sci. Soc.* 47:108.
7. Wanamarta, G., D. Penner, and J. J. Kells. 1989. The basis of bentazon antagonism on sethoxydim absorption and activity. *Weed Sci.* 37:400-404.
8. Wanamarta, G., D. Penner, and J. J. Kells. 1993. Overcoming antagonistic effects of Na-bentazon on sethoxydim absorption. *Weed Technol.* 7:322-325.

CHAPTER TWO
MODULATORS OF IMAZETHAPYR ACTIVITY AND THE INTERACTION OF
IMAZETHAPYR AND FLUAZIFOP-BUTYL

Abstract. The herbicides that inhibit acetolactate synthase and herbicides that inhibit acetyl-CoA carboxylase interact antagonistically to reduce grass control. The role of excess 2-ketobutyrate on the interaction of imazethapyr and fluazifop-butyl was studied in giant foxtail. In sand cultivar studies in the greenhouse, the inclusion of 10^{-4} M 2-ketobutyrate in the nutrient solution caused a 44% reduction in giant foxtail shoot fresh weight. A significant antagonistic interaction was observed following foliar application of 53.4 g/ha fluazifop-butyl to plants provided with 10^{-4} M 2-ketobutyrate. Foliar application of 10^{-3} M 2-ketobutyrate, 2-aminobutyrate, or pyruvate had no effect on giant foxtail growth. However, these metabolic intermediates combined with imazethapyr foliarly applied at 12 g/ha increased injury to giant foxtail. The addition of 2-ketobutyrate to the spray solution appeared to overcome the antagonistic-interaction between imazethapyr and fluazifop-butyl. **Nomenclature:** Fluazifop-butyl, (\pm)-2-[4-[[5-(trifluoromethyl)-2-pyridimyl]oxy]phenoxy]propanoic acid butyl ester; imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; giant foxtail, *Setaria faberi* Herrm. # SETFA. **Additional index words:** ACCase, acetolactate synthase, ALS, giant foxtail, SETFA.

INTRODUCTION

Imazethapyr is an imidazolinone herbicide identified as a potent inhibitor of ALS (22). Imazethapyr has utility for weed control in soybean (*Glycine max* (L.) Merr.) and imidazolinone-resistant corn (*Zea mays* L.) (1, 5, 7, 10, 11). Tank-mixtures of herbicides which inhibit ALS with the aryloxyphenoxypropanoate and the cyclohexenedione herbicides may result in loss or at least partial loss of grass control (17, 18). The basis for this antagonism has not been determined but is not considered to involve uptake, translocation, or molecular fate of the grass herbicide (16).

Two hypotheses have been proposed to explain the death of plants as a consequence of ALS inhibition. The first is that inhibition of the ALS enzyme depletes the supply of the amino acids leucine, isoleucine, and valine resulting in a starvation for these amino acids (23). The second hypothesis is based on studies with microorganism and proposes that inhibiting the ALS enzyme results in an accumulation of 2-ketobutyrate to toxic levels (15). From a series of studies designed to disprove the second hypothesis, Shaner and Singh (21) concluded that imazaquin injured corn as a consequence of starvation for valine and leucine and that the accumulation of 2-aminobutyrate or 2-ketobutyrate was not accountable for the herbicidal activity of imazaquin. The 2-ketobutyrate has a high vapor pressure and is very volatile.

Hofgren et al. (12) failed to observe an accumulation of 2-ketobutyrate in potato (*Solanum tuberosum* L. cv Desiree), a dicot, following treatment with the ALS inhibitor.

The aryloxyphenoxypropanoate and cyclohexenedione herbicides inhibit ACCase (4, 20). Substrates for this enzyme are pyruvate and acetate. The antagonistic interaction on the control of broadleaf signalgrass (*Bracharia platyphylla* (Griseb.) Nash) by the tank mixture of quizalofop and chlorimuron was significantly reduced by supplementing the nutrition of the broadleaf signalgrass with L-leucine, L-isoleucine, and L-valine (9). Excess pyruvate that might have accumulated from ALS inhibition by chlorimuron apparently is not involved in the antagonistic interaction between quizalofop and chlorimuron (3).

Suggested bases for the antagonism observed between herbicides that inhibit ALS and those that inhibit ACCase include a) the accumulation of intermediates as a consequence of ALS inhibition that overcome the block imposed by the ACCase inhibitors, b) inhibition by the ALS inhibitors of growth necessary for the phytotoxic action of the ACCase inhibitors, and c) the requirement for the synthesis of leucine, isoleucine, and valine for the phytotoxic action of the ACCase inhibition (3, 8). Since Bjelk and Monaco (3) showed that excess pyruvate did not appear to be involved in the interaction of the ALS inhibitors and ACCase inhibitors, it was the objective of this study to determine whether 2-ketobutyrate was involved in this antagonistic interaction.

MATERIALS AND METHODS

Evaluation of 2-ketobutyrate activity on giant foxtail in nutrient culture. Giant foxtail (*Setaria faberi* Herrm.) was grown in a 224-ml styrofoam cups containing silica sand. Filter paper was placed at the bottom of the cups to prevent the loss of sand and grass seed at watering. Grass plants were maintained under greenhouse conditions at 25 ± 2 C with 16-h day. Natural plus supplemental lighting with high pressure sodium lighting provided an average of $1200 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ in the greenhouse. Grass plants were watered every other day with Hoagland's nutrient solution with a pH of 6.6. After emergence, grass seedlings were transplanted into 20-ml glass vials, three plants per vial. Vials were previously covered with aluminum foil to prevent light penetration and algae growth. The vials contained 20 ml of 0, 10^{-2} , 10^{-3} , and 10^{-4} M solutions of 2-ketobutyrate applied in Hoagland's nutrient solution, and the pH of the solution was adjusted to 6.6. When the grass plants reached the three to four-leaf stage, fluazifop-butyl was applied POST to the grass plants at rates of 26.7 and 53.4 g ai/ha, respectively, with 1% v/v crop oil concentrate¹. Herbicide treatments were applied

¹Herbimax, a product of Loveland Industries, Inc., P. O. Box 906, Loveland, CO 80539.

with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume. The experimental design was a completely randomized design with four replications, and the experiment was repeated. Fourteen days after herbicide treatment, the three grass plants per vial per replication were harvested and fresh weight taken. Following the analysis of variance, means were separated using the Fisher's protected LSD at the 5% level of significance.

The effect of potential modulators of imazethapyr activity on giant foxtail. Giant foxtail was grown in 945-ml plastic pots containing Baccto potting media. The pots were placed in the greenhouse at 25 ± 2 C with 16-h day. Natural plus supplemental lighting with high pressure sodium lighting provided an average of $1200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ in the greenhouse. After emergence, plants were thinned to three plants per pot. At time of treatment, grass plants were at the three-leaf stage. 2-Ketobutyrate, 2-aminobutyrate, and pyruvate at 10^{-3} M were dissolved in Hoagland's nutrient solution which served as a buffer and the pH of each solution was adjusted to 6.6. The solutions were applied with 0.25% v/v Sylgard 309 about 10 to 20 minutes prior to imazethapyr application. Imazethapyr was applied POST to giant foxtail at 12.9 g ai/ha with 1% v/v of crop oil concentrate. Treatments were applied with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume. A completely randomized design was used and each treatment was replicated four times. Data presented are the means of two experiments. Visual injury and shoot height 14 days after treatment were used as measure of grass control. Visual injury rating scale of 0 to 100% was used with complete death of grass plants receiving a rating of 100%. Following the factorial analysis of variance, means were separated with Fisher's protected LSD at the 5% level of significance.

The effect of 2-ketobutyrate on the interaction of fluazifop-butyl and imazethapyr in giant foxtail. Giant foxtail was grown in 945-ml plastic pots containing Baccto potting media. The pots were placed in the greenhouse at 25 ± 2 C with 16-h day. Natural plus supplemental lighting with high pressure sodium lighting provided an average of $1200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ in the greenhouse. After emergence, plants were thinned to three plants per

pot. At time of treatment, grass plants were at the two- to three-leaf stage. 2-Ketobutyrate at 10^{-3} M was dissolved in Hoagland's nutrient solution and the pH was adjusted to 6.6. 2-Ketobutyrate was applied POST to grass plants about 5 to 10 minutes prior to herbicide treatment with 0.25% v/v Sylgard 309. The herbicide treatments included imazethapyr at 12.9 g ai/ha and fluazifop-butyl at 53.4 g ai/ha, respectively. Both herbicides were applied POST to grass plants alone or in combination with 1% v/v of crop oil concentrate. The treatments were applied with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume. A completely randomized design was used and each treatment was replicated four times. Visual injury and shoot height 14 days after treatment were taken as an indicator of grass control. Data presented are the means of two experiments. Following the factorial analysis of variance, means were separated with Fisher's protected LSD at the 5% level of significance.

The effect of 2-ketobutyrate on imazethapyr activity on soybean. Elgin 87 soybean was grown in 945-ml plastic pots containing Baccto potting media. The pots were placed in the greenhouse at 25 ± 2 C with 16-h day. Natural plus supplemental lighting with high pressure sodium lighting provided an average of $1200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ in the greenhouse. After emergence, plants were thinned to three plants per pot. At time of treatment, soybean plants were at the two trifoliolate leaf stage and about 20 cm tall. 2-Ketobutyrate at 10^{-3} M concentration was dissolved in Hoagland's nutrient solution which served as a buffer and the pH of each solution was adjusted to 6.6. 2-Ketobutyrate was applied with 0.25% v/v Sylgard 309 about 5 to 10 minutes prior to imazethapyr application. Imazethapyr was applied POST to soybean plants at 51.5 g ai/ha with 1% v/v of crop oil concentrate. The herbicide and 2-ketobutyrate treatments were applied with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume. A completely randomized design was used and each treatment was replicated six times. Data presented are plant height means from two experiments 14 days after treatment. Following the factorial analysis of variance, means were separated with Fisher's protected LSD at the 5% level of significance.

RESULTS AND DISCUSSION

Nutrient culture study.

Fluazifop-butyl inhibited fresh weight accumulation of giant foxtail in the expected rate dependent manner in the absence of 2-ketobutyrate (Table 1). 2-Ketobutyrate also inhibited giant foxtail growth in a rate dependent manner in the absence of POST application of fluazifop-butyl (Table 1). At the low rate of 2-ketobutyrate (10^{-4} M) and the high rate of fluazifop-butyl (53.4 g/ha) an antagonistic interaction was very evident. Assuming they act at the same target site and applying an additive interaction model, all combination values for the 10^{-3} M and 10^{-4} M 2-ketobutyrate plus either rate of fluazifop-butyl are greater than expected indicating antagonism. Antagonism of ALS inhibiting herbicides where action may result in accumulation of 2-ketobutyrate with ACCase inhibitors has been documented (6). Butyrate has been documented as being phytotoxic (14).

Foliar studies.

Foliar application of potential modulators of ALS inhibiting herbicides, 2-ketobutyrate, 2-aminobutyrate, or pyruvate, had no effect on giant foxtail (Table 2). This is consistent with the results of Shaner and Singh (21). However, if giant foxtail received both imazethapyr and one of the potential modulators, injury to giant foxtail increased (Table 2). These results could be interpreted to support the hypothesis of LaRossa et al. (15) that ALS inhibiting herbicide exert their action through the accumulation of phytotoxic intermediates.

In a separate study the antagonistic interaction of the tank-mixture of imazethapyr and fluazifop-butyl on giant foxtail injury was observed (Table 3). 2-Ketobutyrate applied foliarly did not injure giant foxtail (Table 3) or soybean (Table 4), however, if both 2-ketobutyrate and imazethapyr were applied, injury to giant foxtail was enhanced (Table 3) but no injury to soybean was evident (Table 4). The application of fluazifop-butyl, imazethapyr, and 2-ketobutyrate resulted in giant foxtail injury similar to that obtained with imazethapyr plus 2-ketobutyrate but slightly less than with fluazifop-butyl with or without 2-ketobutyrate (Table 3). These results raise the question whether the interaction between the ALS

inhibiting herbicides and the ACCase inhibiting herbicides involve potential modulators of ALS activity. Banas et al. (2) have reported that ACCase inhibitors rapidly increase the ratio of linolenic:oleic+linoleic fatty acids in wheat (*Triticum aestivum* L.) roots. In contrast, the ALS inhibitor, chlorsulfuron [2-chloro-N-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide] promoted oleic acid synthesis via the pyruvate dehydrogenase complex in the chloroplasts of spinach (*Spinacia oleracea* L.) (13). This shift was related to the presence of high levels of pyruvate. Although these opposing effects may in part explain the antagonistic interaction of the ALS-inhibiting and ACCase inhibiting herbicides it does not explain the modulating role of 2-ketyobutyrate or 2-aminobutyrate nor does it consider the effect of ACCase inhibiting herbicides on membrane depolarization documented by Shimabukuro and Hoffer (24).

Table 1. The effect of 2-ketobutyrate and its role on fluazifop-butyl activity on giant foxtail in nutrient culture.

Herbicide treatment	Rate	2-ketobutyrate			
		0	10^{-4} M	10^{-3} M	10^{-2} M
	g/ha	----- Fresh weight mg/3 plants -----			
Untreated	0	800	450	370	180
Fluazifop-butyl	26.7	240	210	190	50
Fluazifop-butyl	53.4	80	210	190	100
LSD_{0.05}				126	

Table 2. The effect of potential modulators of imazethapyr action on giant foxtail.

Treatment	Rate	Visual injury	Shoot height
		%	cm
Untreated	0	0	63
Pyruvate	10^{-3} M	0	63
2-Ketobutyrate	10^{-3} M	0	61
2-Aminobutyrate	10^{-3} M	0	59
Imazethapyr ^a	1/4 X	48	38
Imazethapyr + pyruvate	1/4 X + 10^{-3} M	63	22
Imazethapyr + 2-ketobutyrate	1/4 X + 10^{-3} M	73	11
Imazethapyr + 2-aminobutyrate	1/4 X + 10^{-3} M	78	11
LSD _{0.05}		4	5

^aImazethapyr 1/4X = 12.9 g ai/ha

Table 3. The effect of 2-ketobutyrate on the interaction of fluazifop-butyl and imazethapyr in giant foxtail.

Treatment	Rate	Visual injury 14 DAT ^a
	g/ha	%
Untreated	0.0	0
2-Ketobutyrate ^b		0
Imazethapyr	12.9	32
Fluazifop-butyl	53.4	78
Imazethapyr + fluazifop-butyl	12.9 + 53.4	53
Imazethapyr + 2-ketobutyrate	12.9 + 10 ⁻³ M	73
Fluazifop-butyl + 2-ketobutyrate	53.4 + 10 ⁻³ M	79
Imazethapyr + fluazifop-butyl + 2-ketobutyrate	12.9 + 53.4 + 10 ⁻³ M	70
LSD _{0.05}		8

^aDAT - days after treatment.

^b2-Ketobutyrate was applied at 10⁻³ M concentration.

Table 4. The effect of 2-ketobutyrate on imazethapyr activity on Elgin 87 soybean.

Treatment	Rate	Plant height 14 DAT ^a
		cm
Untreated	0	46
Imazethapyr ^b	1X	48
2-Ketobutyrate	10 ⁻³ M	50
Imazethapyr + 2-ketobutyrate	1X + 10 ⁻³ M	49
LSD _{0.05}		3

^aDAT = days after treatment

^bImazethapyr 1X = 51.5 g ai/ha.

LITERATURE CITED

1. Arnold, F. J., W. A. Smith, and F. R. Taylor. 1994. Imazethapyr plus dicamba for weed control in imi-corn. *Proc. North Cent. Weed Sci. Soc.* 49:54.
2. Banas, A., I. Johansson, G. Stenlid, and S. Stymne. 1993. The effect of haloxyfop and alloxymidim on growth and fatty acid composition of wheat roots. *Swedish J. Agric. Res.* 23:55-65.
3. Bjelk, L.A. and T.J. Monaco. 1992. Effect of chlorimuron and quizalofop on fatty acid biosynthesis. *Weed Sci.* 40:1-6.
4. Burton, J.D., J.W. Gronwald, D.A. Somers, J.A Connelly, B.G. Gengenback, and D.L. Wyse. 1988. Inhibition of plant acetyl- Coenzyme A carboxylase by the herbicide sethoxydim and haloxyfop. *Biochem. Biophys. Res. Commun.* 148:1039-1044.
5. Cartwell, J. R., R. A. Liebl, and F. W. Slife. 1989. Imazethapyr for weed control in soybeans (*Glycine max.*). *Weed Technol.* 3:596-681.
6. Ferreira, K. L., J. D. Burton, and H. D. Coble. 1995. Physiological basis for antagonism of fluazifop-p by DPX-PE350. *Weed Sci.* 43:184-191.
7. Frasier, A. L. and D. Penner. 1994. New opportunities for weed control using imidazolinone resistant corn. *Proc. North Cent. Weed Sci. Soc.* 49:108.
8. Gerwick, B.C., P. Thompson, and R. Noveroske. 1988. Potential mechanisms in antagonism with aryloxyphenoxypropionate herbicides. *Abstr. Weed Sci. Soc. Amer.* 28:100.
9. Hahn, K.L. and H.D. Coble. 1989. The effect of exogenously supplied amino acids on the antagonistic interaction between quizalofop and chlorimuron. *Abstr. Weed Sci. Soc. Amer.* 29:86.
10. Hart, S. E., L. M. Wax, J. B. Carey, and D. L. Zinck. 1994. Imazethapyr based weed control systems in imidazolinone resistant corn. *Proc. North Cent. Weed Sci. Soc.* 49:63.

11. Hayden, T. A. and S. Wendy. 1994. Weed control in imi-corn with imazethapyr and imazethapyr combinations. *Proc. North Cent. Weed Sci. Soc.* 49:56.
12. Hofgren, R., B. Laber, I. Schuttke, A-K. Klonus, W. Streber, and H-D. Pohlenz. 1995. Repression of acetolactate synthase activity through antisense inhibition. *Plant Physiol.* 107:469-477.
13. Homeyer, U., D. Schulze-Siebert, and G. Schultz. 1985. On the specificity of the herbicide chlorsulfuron in intact spinach chloroplasts. *Z. Naturforsch.* 40c:917-918.
14. Lanyzagarta, A., J. M. de la Torre, and P. Alle. 1988. The effect of butyrate on cell cycle progression in *Allium cepa* root meristems. *Plant Physiol.* 72:775-781.
15. LaRossa, R.A., T.K. Van Dyke, and D.R. Smulsky. 1987. Toxic accumulation of 2-ketobutyrate caused by inhibition of branched-chain amino acid biosynthetic enzyme acetolactate synthase in *Salmonella typhimurium*. *J. Bacteriol.* 169:1372-1378.
16. Liebl, R. and A.D. Worsham. 1987. Effect of chlorsulfuron on the movement and fate of diclofop in Italian ryegrass (*Lolium multiflorum*) and wheat (*Triticum aestivum*). *Weed Sci.* 35:623-628.
17. Minton, B. W., D. R. Shaw, and M. E. Kurtz. 1989. Postemergence grass and broadleaf herbicide interactions for red rice (*Oryza sativa*) control in soybeans (*Glycine max.*). *Weed Technol.* 3:329-334.
18. Myers, F. and D. Coble. 1992. Antagonism of graminicide activity on annual grass species by imazethapyr. *Weed Technol.* 6:333-338.
19. Ray, T. B. 1984. Site of action of chlorsulfuron: inhibition of valine and leucine biosynthesis in plants. *Plant Physiol.* 75:827-831.
20. Secor, J. and C. Cseke. 1988. Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. *Plant Physiol.* 86:10-12
21. Shaner, D.L. and B.K. Singh. 1993. Phytotoxicity of acetohydroxyacid synthase inhibitors is not due to accumulation of 2-ketobutyrate and/or 2-aminobutyrate. *Plant Physiol.* 103:1221-1226.

22. Shaner, D. L., P. C. Anderson, and M. A. Stidham. 1984. Imidazolinones potent inhibitors of acetohydroxyacid synthase. *Plant Physiol.* 76:545-546.
23. Sheel, D. and J.E. Casida. 1985. Acetohydroxyacid synthase inhibitors as herbicides. In K. Neumann, W. Bary, E. Reinhart, eds. *Primary and Secondary Metabolism of Plant Cell Cultures*. Springer Verlag, Berlin. pp. 344-355.
24. Shimabukuro, R. H. and B. L. Hoffer. 1995. Enantiomers of diclofop-methyl and their role in herbicide mechanisms of action. *Pestic. Biochem. and Physiol.* 51:68-82.

CHAPTER THREE
USE OF ORGANOPHOSPHATE INSECTICIDE LEVELS IN CORN SEEDLINGS
AS AN INDICATOR OF INJURY POTENTIAL FROM
POSTEMERGENCE APPLICATIONS OF
SULFONYLUREA HERBICIDES

Abstract. Postemergence applications of nicosulfuron and primisulfuron may injure corn plants depending on the level of terbufos present in the young corn plants from prior application of terbufos for corn rootworm control. Field studies were conducted in 1992 and 1993 to evaluate the interaction of nicosulfuron and primisulfuron with terbufos. Terbufos was applied in-furrow at 0, 186, 375, and 750 g ai/100 m of row. Nicosulfuron was applied at 35 and 70 g ai/ha and primisulfuron at 40 and 80 g ai/ha when the corn was at the four-leaf stage. Prior to herbicide application, plant samples both fresh and frozen from each treatment were subjected to terbufos analysis. Terbufos levels in the plant samples were determined with a rapid detection kit¹. The correlation coefficient for terbufos detected in the shoot extract with observed herbicide injury to corn was 0.89 in 1992 and 0.94 in 1993. Injury ratings showed a greater correlation with terbufos levels than did corn shoot height. Thus, the rapid detection kit provided an efficient method to determine whether an injurious terbufos-herbicide interaction might occur. **Nomenclature:** Nicosulfuron, 2-[[[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide; primisulfuron, methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-

¹The Ticket, Agri Screen Product Line, Neogen Corp., Lansing, MI 48912.

pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate; terbufos, S-[(tert-butylthio)methyl]-O,O-diethylphosphoridithioate; corn, *Zea mays* L.

Additional index words: Detection kit, interaction, nicosulfuron, primisulfuron, terbufos.

INTRODUCTION

The use of more than one pesticide on the same crop during the growing season has become a frequent occurrence in modern crop production.

Despite the numerous advantages derived from the use of more than one chemical, adverse effects to the crop may also occur. Several researchers have reported detrimental interactions between the sulfonylurea herbicides, nicosulfuron and primisulfuron, with terbufos (2, 3, 5, 6, 9, 11, 13, 15, 16, 17, 18, 19, 20, 21, 22). These herbicides were introduced in 1991 for the control of grass and some broadleaf species in corn.

Northern and western corn rootworm can be very damaging in corn fields in Michigan, especially in fields where corn follows corn. Thus, the use of a soil insecticide in corn production is often necessary for the control of corn rootworm. The insecticide terbufos is often the insecticide of choice for this purpose. Corn may be injured when nicosulfuron and primisulfuron are applied to corn fields that received a prior soil application of terbufos. Corn injury symptoms range from slight growth inhibition and leaf curl to death of the corn plants.

Terbufos absorbed by the corn plant and translocated to the shoot appears to interfere with the metabolism of these two herbicides. Both the insecticide and the two herbicides involved in this interaction are metabolized by mixed function oxidase.

The observed interaction may be affected by soil organic matter content, methods of application of the insecticide, insecticide formulation, and environmental conditions (5, 16, 25), thus, making it difficult to predict the severity of the interaction. One way of assuring safety to corn from the use of sulfonylurea herbicides after using the insecticide terbufos would be assurance that the level of terbufos in the corn plants was below the critical level

that causes a detrimental interaction. The determination of that critical level in the corn tissue could be provided by a low cost kit designed for that purpose that gave a color reaction at the critical level plus a safety factor. Other procedures have also been used for detection of organophosphate residue levels on leaf and soil surfaces to determine reentry into treated fields (4, 7, 12, 23). These procedures include soil surface residue analysis (23) and leaf disc analysis (4, 7, 12). However, these methods require laboratory manipulation and more time to obtain the results.

The kit evaluated in this research was originally developed by the Midwest Research Institute for the United States Army. The purpose was to provide the army with an easy and reliable test to test the safety of drinking water in the field. This procedure required no scientific experience, no instrumentation. In addition, secure storage in extreme weather conditions and high sensitivity made the kit attractive for use in other areas such as agriculture, food processing, and food production. This kit is based on a colorimetric reaction carried out by the enzyme acetylcholinesterase (1)

The objective of this research was to evaluate a quick and easy-to-use kit that could detect the presence of terbufos in corn seedlings at levels that result in corn injury from POST applications of nicosulfuron and primisulfuron.

MATERIALS AND METHODS

'Pioneer 3573' corn was planted at East Lansing, Michigan, in 1992 and 1993 in a Spinks loamy sand soil containing 2.6% OM using a completely randomized block design. The insecticide, terbufos, was applied in-furrow at planting at 186, 375, and 750 g/100 m of row. Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] at 2.2 kg/ha and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] at 1.1 kg/ha were applied PRE. When the corn plants approached the four-leaf stage, five plants from each of the four replications of each treatment were collected and frozen for terbufos residue analysis in 1992 and 1993. In 1993 fresh samples were also

collected and analyzed. Samples for terbufos residue analysis were collected prior to POST herbicide application.

POST herbicide application treatments included 35 (1x rate) and 70 (2x rate) g/ha nicosulfuron and 40 (1x rate) and 80 (2x rate) g/ha primisulfuron. These herbicides were applied with 0.25% nonionic adjuvant² when corn was at the four-leaf stage.

Corn injury was determined 14 DAT. Plant height was measured at 21 DAT. Correlation coefficient was determined for both corn injury and plant height with the concentration of terbufos detected in the plants. Analysis of variance for corn injury and plant height was also conducted. After analysis of variance, means were separated by the Fisher's protected LSD at the 5% level of significance. Data presented are the means of three replications.

Terbufos detection procedure. Frozen corn plants were thawed. Physical pressure was used to obtain an extract for analysis from both thawed and fresh samples. The plant extract (1 ml) was partitioned with 1 ml hexane. Five drops of the hexane were placed on the test ticket disc. A heat gun was used to evaporate the hexane. The activator ampule was placed in the beaker with 20 ml of distilled water, broken with the glass rod, and the glass rod used to place 3 drops of this solution on the test ticket disk. After 2 min., the two disks were pressed together for 3 min. to allow color development. The development of blue color on the test ticket disk was indicative of the absence of an organophosphate insecticide. The terbufos concentration was determined visually by color comparison with a terbufos standard curve (Figure 1).

²X-77, nonionic surfactant is a mixture of alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol marketed by Loveland Industries, Inc., P. O. Box 906, Loveland, CO 80539.

RESULTS AND DISCUSSION

An interaction between the sulfonyleurea herbicides, nicosulfuron and primisulfuron, and the insecticide terbufos was observed in corn field studies conducted in 1992 and 1993. Corn injury was greater when nicosulfuron was applied at 1/2 and 1x rates to rows that received 1/2 or 1x rate of terbufos (Table 1). Other researchers have reported injury to corn caused by the nicosulfuron and terbufos interaction (5, 16, 25). The same trend of injury was observed with primisulfuron applied at 1/2 and 1x rates with the greater injury resulting when primisulfuron was applied to corn rows that received 1/2 and 1x rates of terbufos. Ketchersid et al. (14) and Holshouser et al. (11) have also reported injury to corn resulting from the primisulfuron and terbufos interaction. No difference in corn injury was observed between the two herbicides at either rate applied to rows treated with terbufos. Corn height reduction in 1992 was observed only when nicosulfuron was applied at 1x rate to corn plants that received a prior soil application of 1x rate of terbufos (Table 1). Similar reductions were observed when primisulfuron was applied at 1/2x rate to corn treated with 1x of terbufos, but not when primisulfuron was applied at 1x rate to corn treated with 1/4 or 1x of terbufos. In 1993 corn height reduction was observed when nicosulfuron was applied at 1/2 or 1x to corn that received 1/2 and 1x rates of terbufos, respectively. For the primisulfuron treatments, corn height reduction was observed only when it was applied at 1/2x to corn plants treated with 1/2 or 1x rates of terbufos. The correlation coefficients for plant height with the terbufos level were -0.64 for 1992 and -0.66 for 1993, respectively (Figure 2).

Correlation coefficients obtained with the pesticide detector ticket kit of 0.90 in 1992 and 0.94 in 1993 between visual corn injury and terbufos levels in the corn plants at the time of herbicide application. The regression analysis indicates that the terbufos levels in the corn plants should be 3 ppm or less to assure visual corn injury of 10% or less (Figure 3).

These results show that a rapid detection kit can be used by growers to detect terbufos levels present in the corn plants at the time of herbicide application. This will allow growers

to make a knowledge-based decision as to when or whether sulfonylurea herbicides can be safely applied to corn fields that were treated with terbufos earlier in the season.

Table 1. The effect of terbufos interaction with acetolactate synthase inhibiting herbicides on corn

Insecticide ^a	Rate	Herbicide ^b		Injury 14 DAT	Plant height 21 DAT ^c	
		treatments	Rate		1992	1993
				%	----- cm -----	
Terbufos	0			0	83	144
Terbufos	1/4			0	88	130
Terbufos	1/2			3	75	140
Terbufos	1 x			3	79	135
Terbufos	0	Nicosulfuron	1/2	7	77	149
Terbufos	1/4	Nicosulfuron	1/2	21	79	133
Terbufos	1/2	Nicosulfuron	1/2	42	70	87
Terbufos	1 x	Nicosulfuron	1/2	43	66	81
Terbufos	0	Nicosulfuron	1 x	13	80	144
Terbufos	1/4	Nicosulfuron	1 x	28	72	143
Terbufos	1/2	Nicosulfuron	1 x	42	63	101
Terbufos	1 x	Nicosulfuron	1 x	49	59	98
Terbufos	0	Primisulfuron	1/2	13	79	137
Terbufos	1/4	Primisulfuron	1/2	23	73	140
Terbufos	1/2	Primisulfuron	1/2	43	67	117
Terbufos	1 x	Primisulfuron	1/2	49	59	128
Terbufos	0	Primisulfuron	1 x	13	79	129
Terbufos	1/4	Primisulfuron	1 x	20	74	116
Terbufos	1/2	Primisulfuron	1 x	43	58	101
Terbufos	1 x	Primisulfuron	1 x	53	65	100
LSD _{0.05}				7.6	15.6	

^aTerbufos was applied in furrow in rows of 1000 meters long at rates of 0, 186, 375, and 750 gr, respectively.

^bHerbicide treatments included 35 (1x) and 70 (2x) g ai/ha nicosulfuron and 40 (1x) and 80 (1x) g ai/ha primisulfuron.

^cPlant height values are the means of five plants per row.

Figure 1. Standard curve for Enzytec Pesticide Detector Kit

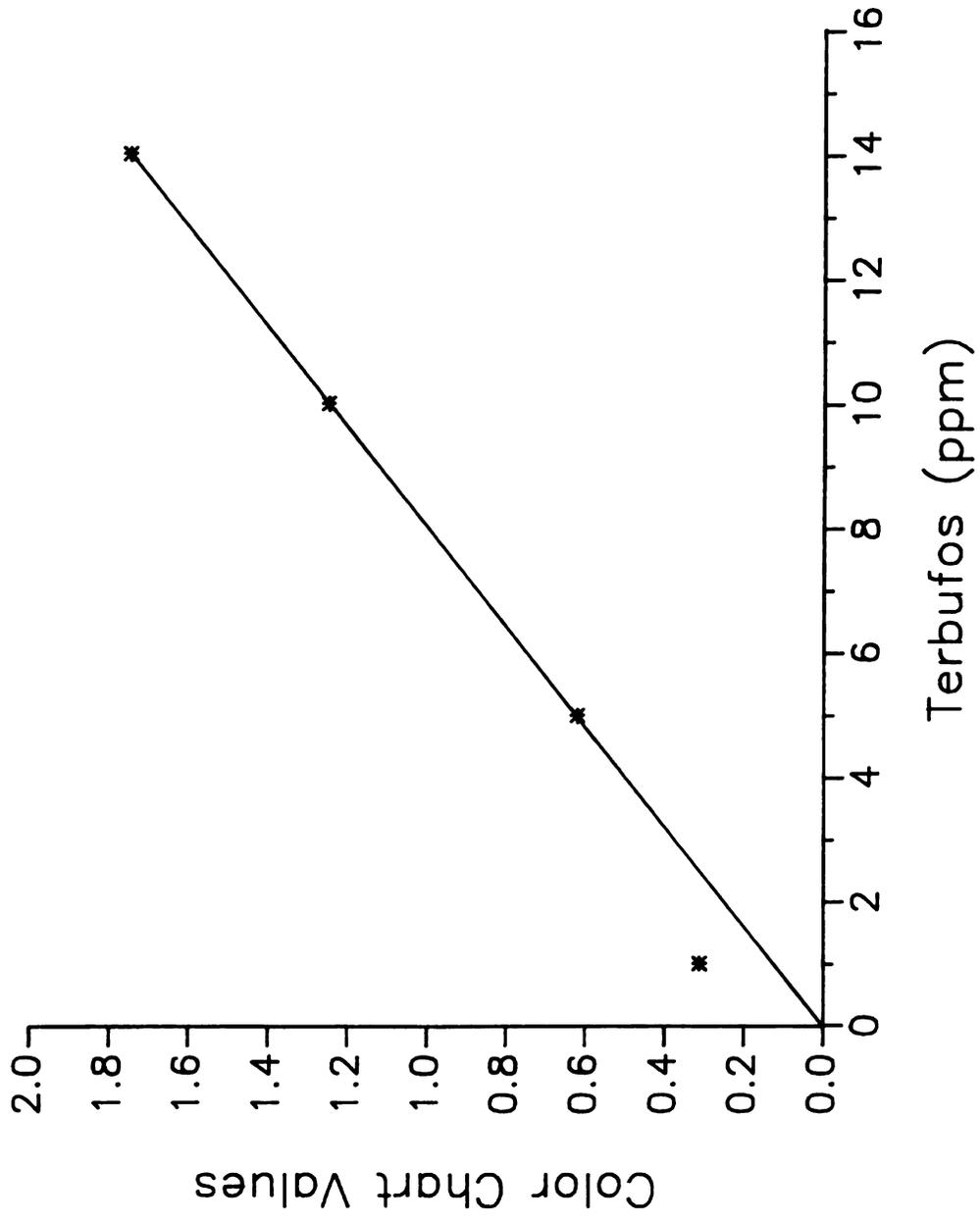


Figure 1.

Figure 2. Correlation of corn height from postemergence application of two rates of nicosulfuron and primisulfuron to various levels of

terbufos applied as in-furrow soil application. Enzytec Tector Ticket Kits were used for the detection of terbufos present in corn tissue at time of herbicide application.

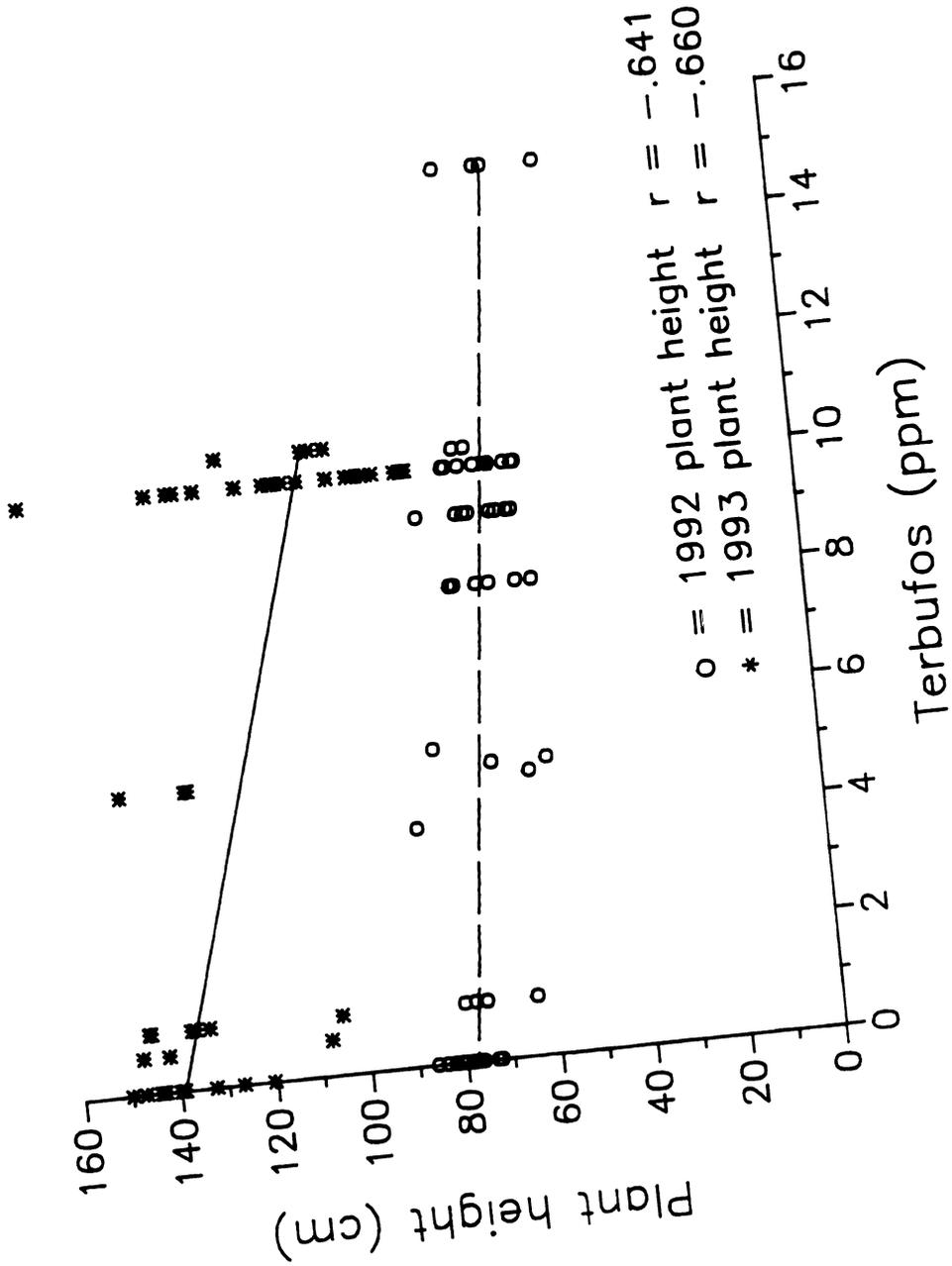


Figure 2.

Figure 3. Correlation of corn injury from postemergence application of two rates of nicosulfuron to various levels of terbufos applied as in-furrow soil application. Enzytec Pesticide Detector Ticket Kits were used for the detection of terbufos present in corn tissue at time of herbicide application.

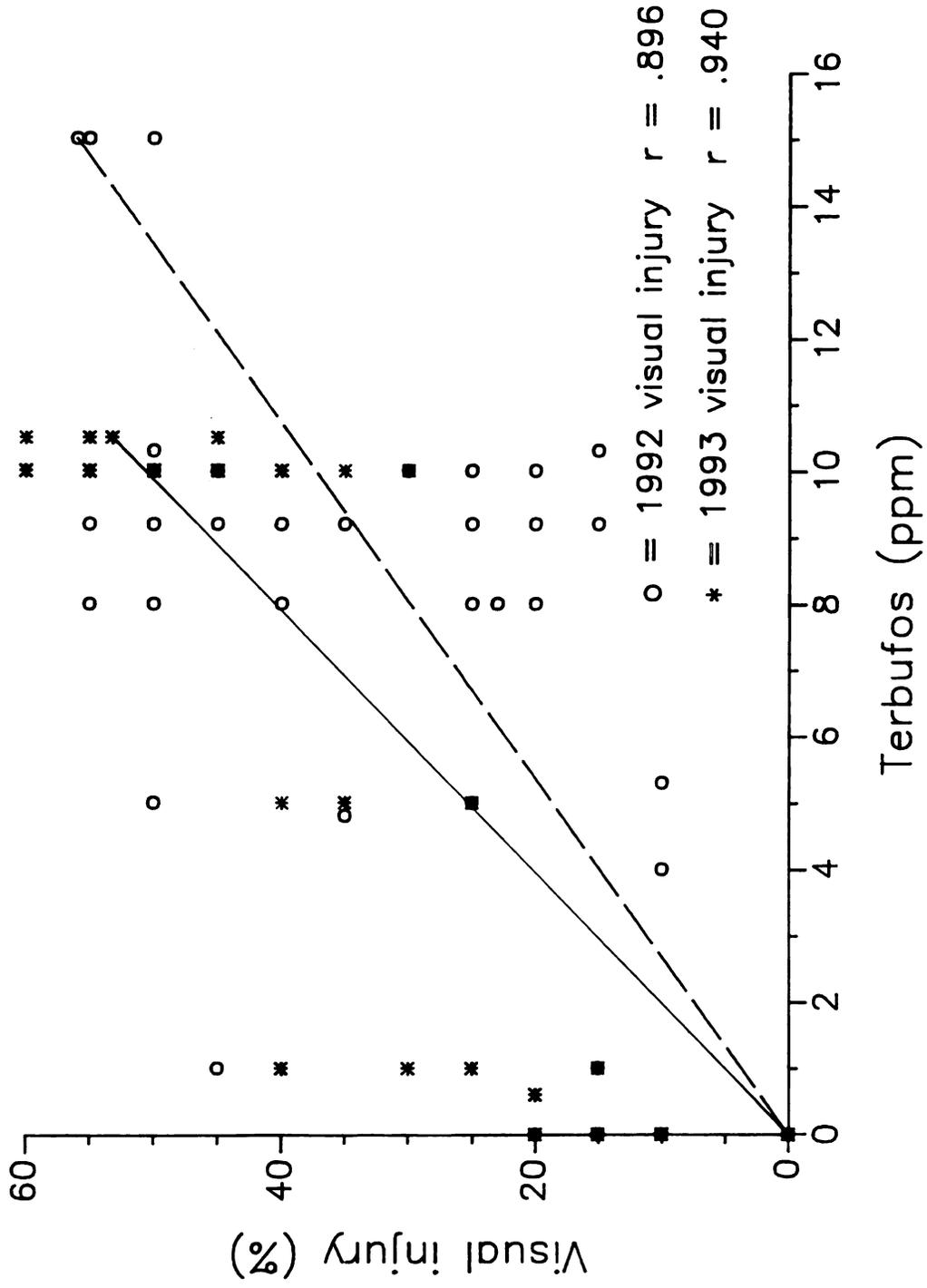


Figure 3.

LITERATURE CITED

1. Anonymous. 1988. Pesticide Detection Program. EnzyTec, Inc. Kansas City, MO. 41 p.
2. Ahrens, W. H. 1990. Corn variety response to DPX-9360 and CGA-136872 with soil-applied insecticides. *Proc. North Cent. Weed Sci. Soc.* 45:34.
3. Baerg, R. J. and M. Barrett. 1993. Insecticide modifications of cytochrome P450 mediated herbicide metabolism. *Proc. North Cent. Weed Sci. Soc.* 48:70.
4. Blewett, T. C. and R. I. Krieger. 1990. Field leaf-test kit for rapid determination of dislodgeable foliar residues of organophosphate and N-methyl carbamate insecticides. *Bull. Environ. Contam. Toxicol.* 45:12-124.
5. Diehl, K. E. and E. W. Stoller. 1991. Effect of soil organic matter on the interaction between terbufos and nicosulfuron in corn. *Proc. North Cent. Weed Sci. Soc.* 46:6.
6. Diehl, K. E. and E. W. Stoller. 1990. Interaction of organophosphate insecticides with nicosulfuron and primisulfuron in corn. *Proc. North Cent. Weed Sci. Soc.* 45:31.
7. Gunther, F. A., W. G. Westlake, and J. H. Barkley. 1993. Establishing dislodgeable pesticide residues on leaf surfaces. *Bull. Environ. Contam. Toxicol.* 9(4):243-249.
8. Hageman, L. H., J. D. Michael, and W. R. Scott. 1991. Update on the interactions between nicosulfuron and organophosphate insecticides. *Proc. North Cent. Weed Sci.* 46:46.
9. Harvey, R. G. 1992. MON-13900 for reducing field and sweet corn injury from nicosulfuron and terbufos. *Proc. North Cent. Weed Sci. Soc.* 47:11.
10. Hatzios, K. K. and D. Penner. 1985. Interaction of herbicides with other agrichemicals in higher plants. *Rev. Weed Sci.* 1:1-63.

11. Holshouser, D. L., J. M. Chandler, and H. R. Smith. 1991. The influence of terbufos on the response of five corn (*Zea mays*) hybrids to CGA-136872. *Weed Technol.* 5:165-168.
12. Iwata, Y., J. B. Knaak, R. C. Spear, and R. J. Foster. 1977. Worker reentry into pesticide treated crops. I. Procedure for the determination of dislodgeable pesticide residues on foliage. *Bull. Environ. Contam. Toxicol.* 18(6):649-655.
13. Kapusta, G. and R. F. Krausz. 1992. Interaction of terbufos and nicosulfuron on corn (*Zea mays*). *Weed Technol.* 6:999-1003.
14. Ketchersid, M. L., J. M. Chandler, and M. G. Merkle. 1989. Factors affecting the phytotoxicity of CGA-136872 to corn. *Proc. South Weed Sci. Soc.* 42:271.
15. Kwon, C. S. and D. Penner. 1992. The potential of piperonyl butoxide to enhance weed control with postemergence application of sulfonylurea herbicides in corn. *Weed Sci. Soc. Abstr.* 47:26.
16. Morton, C. A., R. G. Harvey, J. J. Kells, W. E. Lueschen, and V. A. Fritz. 1991. Effect of DPX-V9360 and terbufos on field and sweet corn (*Zea mays*) under three environments. *Weed Technol.* 5:130-136.
17. Owen, M.D.K. 1991. Interaction of herbicides and insecticides used for corn production. *Proc. North Cent. Weed Sci. Soc.* 46:44.
18. Peters, J. T., J. D. Mayonado, D. F. Loussaert, and R. E. Bulehler. 1991. MON 12000: Investigating the potential sulfonylurea herbicide/organophosphate soil insecticide interaction. *Proc. North Cent. Weed Sci. Soc.* 46:34.
19. Pike, D. R. and E. L. Knake. 1991. Interaction between DPX-V9360 and terbufos applied to corn. *Proc. North Cent. Weed Sci. Soc.* 45:51.
20. Rahman, A. and K. J. Trevor. 1993. Enhanced activity of nicosulfuron in combination with soil applied insecticides in corn (*Zea mays*). *Weed Technol.* 7:824-829.

21. Simarmata, M. and D. Penner. 1993. Protection from primisulfuron injury to corn (*Zea mays*) and sorghum (*Sorghum bicolor*) with herbicide safeners. *Weed Technol.* 7:174-179.
22. Smart, J. R., D. A. Mortensen, and L. J. Meinke. 1991. Method and timing of insecticide application with nicosulfuron and primisulfuron. *Proc. North Cent. Weed Sci. Soc.* 46:33.
23. Spener, W. F., Y. Iwata, W. W. Kilgore, and J. B. Knaak. 197. Worker reentry into pesticide-treated crops. II. Procedures for the determination of pesticide residues on soil surface. *Bull. Environ. Contam. Toxicol.* 18(6):656-662.
24. Taylor, S. L., K. E. Diehl, D. M. Simpson, and E. W. Stoller. 1993. The effect of nicosulfuron plus terbufos in vivo ALS activity in corn. *Proc. North Cent. Weed Sci. Soc.* 48:71.
25. Williams, J. B. and R. G. Harvey. 1992. Influence of application timing, adjuvants, rootworm insecticides, and hybrid on nicosulfuron injury to sweet corn. *Proc. North Cent. Weed Sci. Soc.* 47:11.

SUMMARY

Greenhouse and field research was conducted to study the interaction of ALS inhibitors with graminicides and insecticides. An antagonistic interaction between the imidazolinone herbicide, imazethapyr, and several graminicides on annual grass control was observed. The addition of ammonium sulfate to the spray solution helped to overcome the antagonistic interaction.

The role of excess 2-ketobutyrate on the interaction of imazethapyr and fluazifop-butyl was also evaluated. In sand cultivar studies, the addition of 2-ketobutyrate in the nutrient culture solution caused reduction of annual grass fresh shoot weight confirming suggested phytotoxicity of 2-ketobutyrate. Foliar application of fluazifop-butyl to plants provided with 2-ketobutyrate resulted in a significant interaction. Metabolic intermediates combined with imazethapyr foliarly applied increased injury to annual grass indicating that they may play a modulating role in imazethapyr injury.

In a separate study, an antagonistic interaction between tank-mixture of imazethapyr and fluazifop-butyl on giant foxtail control was observed. Foliar application of 2-ketobutyrate alone did not injure giant foxtail or soybean. However, if both 2-ketobutyrate and imazethapyr were applied, injury to giant foxtail was enhanced, but no injury to soybean was observed. The injury to giant foxtail from the application of imazethapyr, fluazifop-butyl, and 2-ketobutyrate was similar to that obtained with imazethapyr plus 2-ketobutyrate, but slightly less than with fluazifop-butyl with or without 2-ketobutyrate. These results raise the question whether the interaction between the ALS and ACCase inhibiting herbicides involve potential modulators of imazethapyr activity.

Field studies conducted to obtain the interaction of two sulfonylurea herbicides and terbufos were conducted. The correlation coefficient between corn injury and terbufos levels was greater than obtained with shoot height. A rapid pesticide detection kit was an efficient method to detect terbufos levels present in the corn tissue at the time of herbicide application.

APPENDIX

APPENDIX

ANOVA Tables for Data

Chapter 1, Table 1

Imazethapyr x Fluazifop-butyl

Factor A Experiments 2
 Factor B Imazethapyr, 0, 1/4X, 1/2X
 Factor C Fluazifop-butyl, 0, 1/4X, 1/2X

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	1953.13	1953.125	154.82	.000
4	B	2	19752.08	9876.042	782.83	.000
6	AB	2	168.75	84.375	6.69	.002
8	C	2	2027.08	1013.542	80.34	.000
10	AC	2	1064.58	532.292	42.19	.000
12	BC	4	22583.33	5645.833	447.52	.000
14	ABC	4	316.67	79.167	6.28	.000
-15	Error	54	681.25	12.616		
Coefficient of Variation = 6.02%						

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	974.61	974.611	35.90	.000
4	B	2	9254.72	4627.359	170.44	.000
6	AB	2	99.52	49.758	1.83	.169
8	C	2	2039.99	1019.995	37.57	.000
10	AC	2	587.55	293.75	10.82	.000
12	BC	4	5576.23	1394.059	51.35	.000
14	ABC	4	77.19	19.298	0.71	
-15	Error	54	1466.08	27.150		
Coefficient of Variation = 19.37%						

Chapter 1, Table 1

Imazethapyr x Sethoxydim

Factor A Imazethapyr, 0, 1/4X, 1/2X

Factor B Sethoxydim, 0, 1/4X, 1/2X

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	2	250.52	125.260	247.82	.000
4	B	2	6.77	3.385	6.70	.002
6	AB	4	258.33	64.583	127.77	.000
-7	Error	63	31.84	0.505		
Coefficient of Variation = 11.04%						

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	2	11159.41	5579.707	104.50	.000
4	B	2	1153.72	576.858	10.80	.000
6	AB	4	6968.93	1742.233	32.63	.000
-7	Error	63	3363.77	53.393		
Coefficient of Variation = 30.32%						

Chapter 1, Table 1

Imazethapyr x Quizalofop-ethyl

Factor A Experiments 2

Factor B Imazethapyr, 0, 1/4X, 1/2X

Factor C Quizalofop-ethyl, 0, 1/4X, 1/2X

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	12.50	12.500	1.10	.298
4	B	2	29675.69	14837.847	1308.15	.000
6	AB	2	577.08	288.542	25.44	.000
8	C	2	254.86	127.431	11.23	.000
10	AC	2	14.58	7.292	0.64	
12	BC	4	26574.31	6643.576	585.72	.000
14	ABC	4	289.58	72.396	6.38	.000
-15	Error	54	612.50	11.343		

Coefficient of Variation = 5.07%

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	120.12	120.125	13.08	.000
4	B	2	15805.34	7902.670	860.59	.000
6	AB	2	778.52	389.260	42.39	.000
8	C	2	2026.38	1013.191	110.33	.000
10	AC	2	29.15	14.573	1.59	.213
12	BC	4	5220.45	1305.113	142.12	.000
14	ABC	4	208.27	52.068	5.67	.000
-15	Error	54	495.87	9.183		

Coefficient of Variation = 15.26%

Chapter 1, Table 1

Imazethapyr x UBI-C4874

Factor A Experiments 2
 Factor B Imazethapyr, 0, 1/4X, 1/2X
 Factor C UBI-C4874, 0, 1/4X, 1/2X

Visual Injury ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	0.68	0.681	0.05	
4	B	2	35141.08	17570.542	1396.85	.000
6	AB	2	563.03	281.514	22.38	.000
8	C	2	13521.08	6760.542	537.46	.000
10	AC	2	55.53	27.764	2.21	.119
12	BC	4	7111.33	1777.833	141.34	.000
14	ABC	4	246.89	61.722	4.91	.001
-15	Error	54	679.25	12.579		

Coefficient of Variation = 5.49%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	7.67	7.670	0.36	
4	B	2	16251.34	8125.670	386.45	.000
6	AB	2	743.26	371.628	17.67	.000
8	C	2	2102.03	1051.014	49.98	.000
10	AC	2	32.53	16.264	0.77	
12	BC	4	5361.81	1340.451	63.75	.000
14	ABC	4	433.26	108.316	5.15	.001
-15	Error	54	1135.44	21.027		

Coefficient of Variation = 22.15%

Chapter 1, Table 2**Imazethapyr Formulations x Sethoxydim**

Factor A Experiments 2

Factor B Imazethapyr formulations, 0, 1/4X, 1/2X

Factor C Sethoxydim, 0, 1/4X, 1/2X

Visual Injury ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	10219.56	10219.560	20.22	.000
4	B	2	22864.93	11432.463	22.62	.000
6	AB	2	1903.32	951.658	1.88	.156
8	C	6	7806.45	1301.075	2.57	.021
10	AC	12	32637.78	2719.815	5.38	.000
12	BC	12	32637.78	2719.815	5.38	.000
14	ABC	12	6797.09	566.425	1.12	.349
-15	Error	126	63691.64	505.489		

Coefficient of Variation = 73.56%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	8542.88	8542.881	956.80	.000
4	B	2	56310.46	27155.232	3153.39	.000
6	AB	2	508.08	254.042	28.45	.000
8	C	6	5318.12	886.353	99.27	.000
10	AC	6	2077.29	346.214	38.78	.000
12	BC	12	53661.95	4471.829	500.84	.000
14	ABC	12	1408.50	117.375	13.15	.000
-15	Error	126	1125.00	8.929		

Coefficient of Variation = 5.40%

Chapter 1, Table 2

Imazethapyr Formulations x Fluazifop-butyl

Factor A Experiments 2

Factor B Imazethapyr formulations, 0, 1/4X, 1/2X

Factor C Fluazifop-butyl, 0, 1/4X, 1/2X

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	54.86	54.857	2.84	.094
4	B	2	63819.48	31909.738	1650.50	.000
6	AB	2	910.43	455.214	23.55	.000
8	C	6	13447.56	2241.260	115.93	.000
10	AC	6	3009.23	501.538	25.94	.000
12	BC	12	46832.44	3902.703	201.86	.000
14	ABC	12	596.49	49.707	2.57	.004
-15	Error	126	2436.00	19.333		
Coefficient of Variation = 9.61%						

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	6.48	6.482	0.17	
4	B	2	24022.90	12011.448	310.62	.000
6	AB	2	38.48	19.242	0.50	
8	C	6	5717.93	952.989	24.64	.000
10	AC	6	580.88	96.814	2.50	.025
12	BC	12	11607.97	967.331	25.02	.000
14	ABC	12	465.64	38.803	1.00	.449
-15	Error	126	487235	38.669		
Coefficient of Variation = 21.37%						

Chapter 1, Table 2**Imazethapyr Formulations x UBI-C4874**

Factor A Experiments 2

Factor B Imazethapyr formulations, 0, 1/4X, 1/2X

Factor C UBI-C4874, 0, 1/4X, 1/2X

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	1178.72	1178.720	402.76	.000
4	B	2	134106.25	67053.125	22911.71	.000
6	AB	2	2937.80	1468.899	501.92	.000
8	C	6	1628.87	271.478	92.76	.000
10	AC	6	647.32	107.887	36.86	.000
12	BC	12	32954.17	2746.181	938.36	.000
14	ABC	12	626.79	52.232	17.85	.000
-15	Error	126	368.75	2.927		

Coefficient of Variation = 2.91%

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	74.53	74.533	2.45	.120
4	B	2	44189.97	22094.987	725.26	.000
6	AB	2	59.45	29.725	.098	
8	C	6	346.52	57.753	1.90	.086
10	AC	6	46.22	7.704	0.25	
12	BC	12	5561.89	463.491	15.21	.000
14	ABC	12	445.90	37.158	1.22	.276
-15	Error	126	838.60	30.465		

Coefficient of Variation = 23.78%

Chapter 1, Table 3

Imazethapyr x UBI-C4874 x Ammonium sulfate

Factor A Experiments 2

Factor B Imazethapyr, 0, 1/4X, 1/2X

Factor C UBI-C4874, 0, 1/4X, 1/2X

Factor D Ammonium sulfate, 0, 1135 gr/ha

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	6.25	6.250	0.30	
4	B	1	7367.36	7367.361	349.75	.000
6	AB	1	850.69	850.694	40.38	.000
8	C	2	61626.04	30813.021	1462.77	.000
10	AC	2	594.79	297.396	14.12	.000
12	BC	2	1335.76	667.882	31.71	.000
14	ABC	2	308.68	154.340	7.33	.001
16	D	2	2879.17	1439.583	68.34	.000
18	AD	2	1266.67	633.333	30.07	.000
20	BD	2	5272.22	2636.111	125.14	.000
22	ABC	2	301.39	150.694	7.15	.001
24	CD	4	40422.92	10105.729	479.74	.000
26	ACD	4	366.67	91.667	4.35	.002
28	BCD	4	971.53	242.882	11.53	.000
30	ABCD	4	948.61	237.153	11.26	.000
-31	Error	108	2275.00	21.065		

Coefficient of Variation = 6.91%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	59.16	59.162	2.24	.137
4	B	1	1910.42	1910.418	72.45	.000
6	AB	1	12.31	12.308	0.47	
8	C	2	16013.99	8006.995	303.68	.000
10	AC	2	7.53	3.765	0.14	
12	BC	2	1700.67	850.335	32.25	.000
14	ABC	2	220.37	110.184	4.18	.017
16	D	2	1889.12	944.562	35.82	.000
18	AD	2	4.77	2.384	0.09	
20	BC	2	877.32	438.661	16.64	.000
22	ABD	2	22.07	11.033	0.42	
24	CD	4	8514.87	2128.717	80.73	.000
26	ACD	4	70.10	17.526	0.66	
28	BCD	4	890.71	222.677	8.45	.000
30	ABCD	4	269.69	67.422	2.56	.042
-31	Error	108	2847.63	26.367		

Coefficient of Variation = 27.60%

Chapter 1, Table 3

Imazethapyr x Fluazifop-butyl x Ammonium sulfate

Factor A Experiments 2

Factor B Imazethapyr, 0, 1/4X, 1/2X

Factor C Fluazifop-butyl, 0, 1/4X, 1/2X

Factor D Ammonium sulfate, 0, 1135 gr/ha

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	291.84	291.840	62.26	.000
4	B	1	16362.67	16362.674	3490.70	.000
6	AB	1	291.84	291.840	62.26	.000
8	C	2	30064.93	15032.465	3206.93	.000
10	AC	2	287.85	143.924	30.70	.000
12	BC	2	60.76	30.382	6.48	.002
14	ABC	2	208.68	104.340	22.26	.000
16	D	2	7325.35	3662.674	781.37	.000
18	AD	2	887.85	443.924	94.70	.000
20	BD	2	13069.10	6434.549	1394.04	.000
22	ABD	2	906.60	453.299	96.70	.000
24	CD	4	35664.24	8916.059	1902.09	.000
26	ACD	4	2274.65	568.663	121.31	.000
28	BCD	4	1199.65	299.913	63.98	.000
30	ABCD	4	847.57	211.892	45.20	.000
-31	Error	108	506.25	4.687		

Coefficient of Variation = 3.77%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	2.75	2.750	0.10	
4	B	1	5714.10	5714.100	199.60	.000
6	AB	1	8.95	8.950	0.31	
8	C	2	9819.59	4909.793	171.51	.000
10	AC	2	45.00	22.502	0.79	
12	BC	2	383.82	191.909	6.70	.001
14	ABC	2	149.63	74.813	2.61	.077
16	D	2	1368.10	484.052	23.89	.000
18	AD	2	305.16	152.581	5.33	.006
20	BD	2	3074.98	1537.489	53.71	.000
22	ABD	2	62.11	31.055	1.08	.341
24	CD	4	11182.31	2795.579	97.65	.000
26	ACD	4	411.39	102.847	3.59	.008
28	BCD	4	320.46	80.115	2.80	.029
30	ABCD	4	196.12	49.029	1.71	.152
-31	Error	108	3091.78	28.628		

Coefficient of Variation = 24.51%

Chapter 1, Table 3

Imazethapyr x Sethoxydim x Ammonium sulfate
 Factor A Experiments 2
 Factor B Imazethapyr, 0, 1/4X, 1/2X
 Factor C Sethoxydim, 0, 1/4X, 1/2X
 Factor D Ammonium sulfate, 0, 1135 gr/ha

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	3500.69	3500.694	311.81	.000
4	B	1	26136.11	26136.111	2328.00	.000
6	AB	1	506.25	506.250	45.09	.000
8	C	2	27638.54	13819.271	1230.91	.000
10	AC	2	264.93	132.465	11.80	.000
12	BC	2	114.93	57.465	5.12	.007
14	ABC	2	9.37	4.687	0.42	
16	D	2	9129.17	4564.583	406.58	.000
18	AD	2	372.22	186.111	16.58	.000
20	BD	2	9343.06	4671.528	416.10	.000
22	ABD	2	54.17	27.083	2.41	.094
24	CD	4	44954.17	11238.542	1001.04	.000
26	ACD	4	202.78	50.694	4.52	.002
28	BCD	4	234.03	58.507	5.21	.000
30	ABCD	4	452.08	113.021	10.07	.000
-31	Error	108	1212.50	11.227		

Coefficient of Variation = 5.12%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	926.70	926.695	41.16	.000
4	B	1	11687.41	11687.412	519.12	.000
6	AB	1	724.96	724.956	32.20	.000
8	C	2	11213.95	5606.976	249.04	.000
10	AC	2	80.75	40.377	1.79	.171
12	BC	2	144.17	72.083	3.20	.044
14	ABC	2	14.63	7.313	0.32	
16	D	2	1198.26	599.130	26.61	.000
18	AD	2	63.22	31.608	1.40	.250
20	BD	2	6197.73	3098.864	137.64	.000
22	ABD	2	108.95	54.474	2.42	.093
24	CD	4	16542.41	4135.602	183.69	.000
26	ACD	4	478.78	119.696	5.32	.000
28	BCD	4	107.71	26.928	1.20	.316
30	ABCD	4	360.13	90.031	4.00	.004
-31	Error	108	2431.51	22.514		

Coefficient of Variation = 21.51%

Chapter 2, Table 4

Fluazifop-butyl x 2-ketobutyrate

Factor A Experiments 2

Factor B 2-ketobutyrate, 0, 10^{-2} , 10^{-3} , 10^{-4} molar

Factor C Fluazifop-butyl, 0, 1/4X, 1/2X

Fresh Weight ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	0.14	0.145	9.29	.003
4	B	3	0.86	0.288	18.44	.000
6	AB	3	0.63	0.210	13.45	.000
8	C	2	1.84	0.919	58.92	.000
10	AC	2	0.34	0.169	10.86	.000
12	BC	6	0.98	0.164	10.52	.000
14	ABC	6	1.03	0.172	11.00	.000
-15	Error	72	1.12	0.016		

Coefficient of Variation = 48.84%

Chapter 2, Table 5

Imazethapyr x Pyruvate x 2-ketobutyrate x 2-aminobutyrate

Factor A Experiments 2

Factor B Imazethapyr, 0, 1/4X, 1/2X

Factor C Modulators, 0, 10^{-2} , 10^{-3} , 10^{-4} molar**Visual Injury ANOVA**

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	0.39	0.391	0.02	
4	B	1	67925.39	67925.391	3548.76	.000
6	AB	1	0.39	0.391	0.02	
8	C	3	2138.67	712.891	37.24	.000
10	AC	3	863.67	287.891	15.04	.000
12	BC	3	2138.67	712.891	37.24	.000
14	ABC	3	863.67	287.891	15.04	.000
-15	Error	48	918.75	19.141		

Coefficient of Variation = 13.43%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	712.22	712.223	29.84	.000
4	B	1	26875.50	26875.504	1125.98	.000
6	AB	1	133.69	133.691	5.60	.022
8	C	3	2400.48	800.160	33.52	.000
10	AC	3	360.04	120.014	5.03	.004
12	BC	3	1470.01	490.004	20.53	.000
14	ABC	3	665.95	221.983	9.30	.000
-15	Error	48	1145.69	23.868		

Coefficient of Variation = 11.95%

Chapter 2, Table 6

Imazethapyr x Quizalofop-ethyl

Factor A Experiments 2
 Factor B Fluazifop-butyl, 0, 1/4X, 1/2X
 Factor C Imazethapyr, 0, 1/4X, 1/2X
 Factor D 2-ketobutyrate, 0, 10^{-3} molar

Visual Injury ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	58653.13	58653.125	1025.43	.000
4	B	1	13819.53	13819.531	241.61	.000
6	AB	1	41328.13	41328.125	722.54	.000
8	C	1	9625.78	9625.781	168.29	.000
10	AC	1	450.00	450.000	7.87	.005
12	BC	1	8613.28	8613.281	150.59	.000
14	ABC	1	703.13	703.125	12.29	.000
16	D	1	413.28	413.281	7.23	.008
18	AD	1	28.13	28.125	0.49	
20	BD	1	282.03	282.031	4.93	.028
22	ABD	1	78.13	78.125	1.37	.245
24	CD	1	225.78	225.781	3.95	.049
26	ACD	1	112.50	112.500	1.97	.163
28	BCD	1	175.78	175.781	3.07	.082
30	ABCD	1	78.13	78.125	1.37	.245
-31	Error	112	6406.25	57.199		

Coefficient of Variation = 15.20%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	18540.16	18540.158	687.73	.000
4	B	1	11829.14	11829.143	438.79	.000
6	AB	1	14185.60	14185.596	526.20	.000
8	C	1	379.85	379.846	14.09	.000
10	AC	1	104.22	104.221	3.87	.051
12	BC	1	196.27	196.268	7.28	.008
14	ABC	1	14.78	14.783	0.55	
16	D	1	115.33	115.330	4.28	.040
18	AD	1	167.67	167.674	6.22	.014
20	BD	1	34.55	34.549	1.28	.260
22	ABD	1	0.56	0.564	0.02	
24	CD	1	95.39	95.93	3.54	.062
26	ACD	1	184.08	184.080	6.83	.010
28	BCD	1	68.30	68.299	2.53	.114
30	ABCD	1	4.69	4.689	0.17	
-31	Error	112	3019.34	26.958		

Coefficient of Variation = 18.76%

Chapter 2, Table 7

Imazethapyr x 2-ketobutyrate

Factor A Experiments 2

Factor B Imazethapyr, 0, 1X

Factor C 2-ketobutyrate, 0, 10^{-3} molar

Shoot Height ANOVA						
Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	3064.01	3064.005	438.17	.000
4	B	1	78.80	78.797	11.27	.001
6	AB	1	24.80	24.797	3.55	.066
8	C	1	2.76	2.755	0.39	
10	AC	1	1.17	1.172	0.17	
12	BC	1	29.30	29.297	4.19	.047
14	ABC	1	2.30	2.297	0.33	
-15	Error	40	279.71	6.993		
Coefficient of Variation = 5.50%						

Chapter 3, Table 1**Terbufos x Herbicides**

Factor A Years, 2
 Factor B Terbufos, 0, 1/4X, 1/2X, 1X
 Factor C Herbicides, 0, 1/4X, 1/2X, 1X

Visual Injury ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	603.01	603.008	13.77	.000
4	B	3	18462.43	6154.142	140.50	.000
6	AB	3	333.09	111.031	2.53	.062
8	C	4	17290.87	4322.717	98.68	.000
10	AC	4	1999.53	499.883	11.41	.000
12	BC	12	3812.20	317.683	7.25	.000
14	ABC	12	808.20	67.350	1.54	.128
-15	Error	78	3416.65	43.803		

Coefficient of Variation = 26.03%

Shoot Height ANOVA

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	A	1	603.01	603.008	13.58	.000
4	B	3	18462.43	6154.142	138.61	.000
6	AB	3	333.09	111.031	2.50	.065
10	AC	4	1999.53	4322.717	97.36	.000
12	BC	12	3812.20	317.683	7.16	.000
14	ABC	12	808.20	67.350	1.52	.135
-15	Error	80	3552.00	44.400		

Coefficient of Variation = 26.21%

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