INVESTIGATIONS OF THE ACTIVITY, PERSISTENCE, AND MODE OF ACTION OF SEVERAL SULFONYLUREA HERBICIDES

By

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INTRODUCTION

Each of the parts of this thesis is a separate manuscript to be submitted for publication; Part I in *Crop Science*, a Crop Science Society of America publication, Part II in *Weed Technology*, a Weed Science Society of America publication, and Part III in *Weed Science*, a Weed Science Society of America publication.

PART I

THE MODE OF ACTION OF CGA 131036

THE MODE OF ACTION OF CGA 131036

Abstract. Investigations were conducted in the laboratory to determine whether CGA 131036 (N-[[(6-methoxy-4-methyl-1,3,5,-triazin-2yl-amino] carbonyl-2-(2-chloroethoxy)]-benzenesulfonamide), a new sulfonylurea herbicide, inhibits the enzyme acetolactate synthase which is required for the biosynthesis of branched chain amino acids. Chlorsulfuron (2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] benzenesulfonamide), another herbicide in this family, has been found to inhibit this enzyme. A bean (*Phaseolus vulgaris*) root assay conducted under sterile conditions was used to determine whether 100 μ M isoleucine plus 100 M valine would alleviate the growth inhibition caused by 10 to 300 nM concentrations of these herbicides. The results revealed that the growth inhibition caused by either herbicide could be alleviated by the addition of valine and isoleucine. The length of roots grown in herbicide solution supplemented with the amino acids was less than the length of the roots grown in the absence of herbicide. The protective effects associated with the amino acids were not explained on the basis of a general stimulation of growth since the controls grown with and without supplemented amino acids were not different. These results indicate that CGA 131036, like chlorsulfuron, inhibits acetolactate synthase.

Additional index words. Bioassay, beans, chlorsulfuron, sulfonylurea.

INTRODUCTION

Sulfonylurea herbicides are a relatively new family of highly active and highly selective herbicides used mainly in cereal grains; however, soybeans (Glycine max (L.) Merr.) are tolerant to other members of this family. Ray (6) reported that the sulfonylurea herbicide chlorsulfuron inhibits the biosynthesis of the amino acids valine and isoleucine by the inhibition of acetolactate synthase in peas (Pisum sativum L.). This enzyme, which catalyzes the first step of the biosynthetic pathway for branched chain amino acids, is highly sensitive to inhibition by chlorsulfuron in both tolerant and susceptible species. As the result cell division is inhibited (5). Differences in metabolism have been reported to be the basis of selectivity for chlorsulfuron (3,7). Similar information is not available for the new sulfonylurea herbicide CGA 131036. Since CGA 131036 has a 2-ethoxychloro substitution on the benzene ring whereas chlorsulfuron has a chloro substitution and since CGA 131036 does not control the same species that chlorsulfuron does (4), this research was initiated to determine whether CGA 131036 has a similar mode of action similar to chlorsulfuron.

MATERIALS AND METHODS

Beans (Phaseolus vulgaris, var. 'Kentucky Wonder') were surface sterilized by two 3 min baths with gentle agitation in commercial bleach (5.25 % NaOCl) then planted in autoclave sterilized vermiculite moistened with one-half strength Hoagland's nutrient medium (1) and incubated under sterile conditions for 72 h at 25 C. The resulting seedlings were used to obtain 10 mm excised root tips. Using procedures adopted from Ray (6), eight root tips were placed in 250 ml Erlenmeyer flasks containing 50 ml of White's medium (9) along with the herbicide and amino acid treatments. Chlorsulfuron or CGA 131036 were added at 0, 10, 30, 100, or 300 nM in the presence and absence of 100 μM concentrations of valine and isoleucine. The White's medium was supplemented with 20 g/l of sucrose as described by Van't Hof (8) to provide a carbohydrate source. All culture manipulations were performed in a sterile environment under a laminar air flow hood.

Stock solutions of 75 % DF chlorsulfuron, technical grade CGA 131036[1] and the reagent grade amino acids were prepared in White's nutrient medium and filter sterilized through 0.2 μm filter systems. Aliquots of the appropriate volumes of these solutions were transferred to flasks containing autoclave sterilized nutrient medium to obtain the desired concentrations and 50 ml volumes as discussed previously. After the flasks were prepared, the roots were grown in the dark at 32 C with agitation. After 60 h the roots were removed from the flasks and the

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1. Technical grade CGA 131036 from the Ciba-Geigy Corporation, Greensboro, NC 27419.

net root growth determined to the nearest mm with a ruler. This procedure was replicated over time with eight observations per replication.

RESULTS AND DISCUSION

As reported by Ray (6), growth inhibition caused by chlorsulfuron was alleviated by the addition of valine and isoleucine (Figure 1.). The same amino acids alleviated growth inhibition caused by CGA 131036. At herbicide concentrations of 10 to 300 nM, the length of the roots in the medium supplemented with the amino acids was significantly greater than those grown without added amino acids. This information suggests that the mode of action of CGA 131036 is the same as that reported for chlorsulfuron (6). Although the lengths of the roots grown in medium supplemented with amino acids were significantly lower than the control root lengths, the herbicide concentrations used in this experiment are much higher than rates required to significantly reduce root growth (6). Furthermore, the protective effects associated with the amino acids cannot be explained on the basis of a general stimulation of growth since the controls grown with and without these materials were not significantly different. Similar variations among systems in the effectiveness of exogenous amino acids in circumventing a block in their biosynthesis are also known from studies on aromatic amino acids and glyphosate [N-(phosphonomethyl) glycine] (2).

Since growth inhibition did not increase with increasing concentrations of both herbicides in the absence of supplemented isoleucine and valine, both chlorsulfuron and CGA 131036 effectively blocked the biosynthesis of the amino acids at 10 μ M concentrations. The lack of growth that occurred in the absence of the supplemented amino acids resulted from depletion of the amino acid pool that was available when the root tips were excised.

Since chlorsulfuron and CGA 131036 appear to have a common site of action, the differences in species that are tolerant to these herbicides must be the result of other properties. Chlorsulfuron selectivity has been reported to be the result of differential metabolism (3,7). Similar differences must exist between different plant species for CGA 131036.

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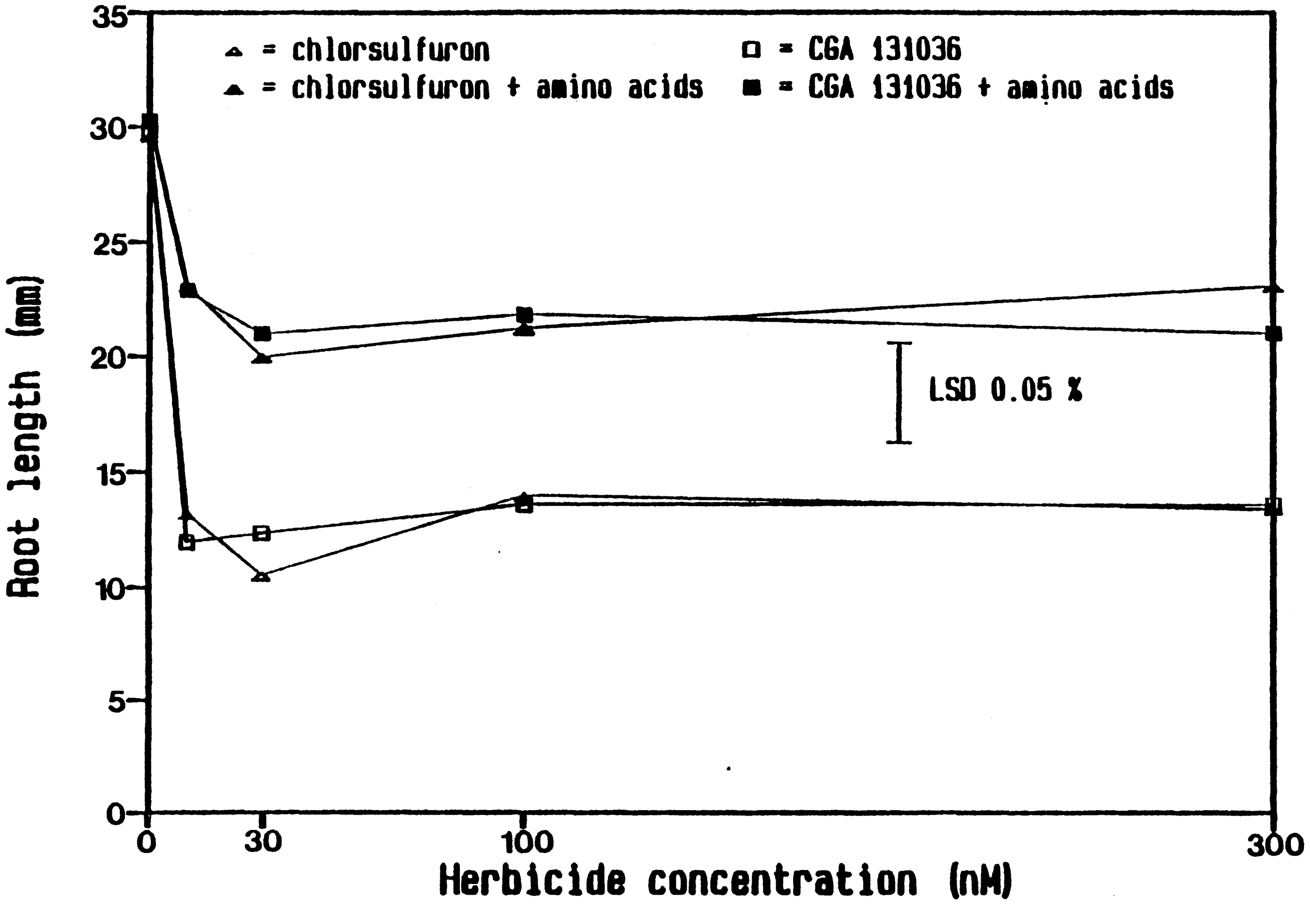

Figure 1. The effect of 100 µM each of valine and isoleucine on the growth of bean roots in the presence of various concentrations of chlorsulfuron and CGA 131036.

PART II

WEED CONTROL IN WINTER WHEAT (TRITICUM AESTIVUM) WITH CGA 131036 AND CHLORSULFURON

WEED CONTROL IN WINTER WHEAT (TRITICUM AESTIVUM)
WITH CGA 131036 AND CHLORSULFURON

Abstract. In field experiments neither preemergence or postemergence applications of chlorsulfuron (2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] benzenesulfonamide) or CGA 131036 (N-[[(6-methoxy-4-methyl-1,3,5,-triazin-2yl-amino]carbonyl-2-(2-chloroethoxy)]-benzenesulfonamide) caused injury to winter wheat (*Triticum aestivum* L.). Both herbicides applied postemergence at 9 and 10 g/ha controlled flixweed (*Descurainia sophia* (L.) Webb. ex Prantl #[1] DESSO) 100 %. CGA 131036 was 10 percent less effective than chlorsulfuron on cutleaf eveningprimrose (*Oenothera laciniata* Hill. # OEOLA). CGA 131036 was also 42 % less effective than chlorsulfuron on henbit (*Lamium amplexicaule* L. # LAMAM) at 10 and 9 g/ha respectively. Both controlled wild buckwheat (*Polygonum convolvulus* L. # POLCO) 77 % when applied postemergence at 18 g/ha. Chlorsulfuron controlled more Italian ryegrass (*Lolium multiflorum* Lam. # LOLMU) when applied preemergence than CGA 131036 at equal rates. However, 91 % control was obtained 53 g/ha of CGA 131036. At equal rates, chlorsulfuron was more effective in controlling woolly croton (*Croton capitatus* Michx. # CVNCP) than CGA 131036, but neither was as effective as 1120 g/ha of 2,4-D [(2,4-dichlorophenoxy) acetic acid] butyl ester. Neither of the sulfonylurea

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[1] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weeds, Weed Sci. 32, Supp. 2.

herbicides were as effective in controlling prostrate spurge (Euphorbia humistrata Engelm. # EPHHT) as 2,4-D.

Additional index words. Sulfonylurea, 2,4-D, LAMAM, DESSO, OEALA, POLCO, LOLMU, CVNCP, EPHHT.

INTRODUCTION

Chlorsulfuron is used for broadleaf weed control and the suppression of some grass species in winter wheat (7). It controls many 2,4-D resistant broadleaf weeds (3) and provides residual weed control at relatively low use rates (4). These attributes combined with the excellent tolerance of winter wheat to foliar and soil applications (1,5) permit great flexibility in the use of this herbicide on wheat. However, a limitation in the use of chlorsulfuron is the possibility of residues injuring rotational crops, particularly on neutral to alkaline soils (1). CGA 131036 differs chemically from chlorsulfuron only in that it contains a 2-ethoxychloro substitution on the benzene ring where chlorsulfuron has a 2-chloro substitution. However, this structural change increases the pK from 3.8 to 4.5[2] which should permit more rapid acid hydrolysis of the parent molecule at given pH's (6,9). If CGA 131036 is as effective on common weeds as chlorsulfuron, its potentially shorter persistence would reduce the probability of rotational crop injury. Therefore, the objectives of this research was to evaluate the

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[2] McMahan, A. Ciba-Geigy Corporation. Personal communication.

efficacy of this herbicide in winter wheat and in fallow periods between continuous wheat production.

METHODS AND MATERIALS

Weed control in winter wheat. During the 1985-86 and 1986-87 growing seasons, 15 field experiments were conducted at sites selected to provide a wide range of soil types to evaluate the performance of CGA 131036 for the control of henbit, flixweed, cutleaf eveningprimrose, wild buckwheat, or Italian ryegrass in winter wheat. The design for each experiment was a randomized complete block with 3 or 4 replications. Plot sizes in the experiments varied from 2.1 by 6.1 m to 2.1 by 8.1 m. The weed species, application dates, weed and crop growth stages, and weed densities are detailed in Table 1. The soil type, soil pH, and the legal description of the soils used are in Table 2.

CGA 131036 and chlorsulfuron were applied postemergence at rates of 9 to 20 g ai/ha to winter wheat and all weeds except Italian ryegrass. The growth stage of the wheat at the time of herbicide application varied from 5 tillers (Zadoks 25) to the first joint (Zadoks 31) (8). In the Italian ryegrass control experiments chlorsulfuron was applied preemergence at the labeled rate of 26 g/ha and CGA 131036 was applied preemergence at 26 and 53 g/ha.

All herbicide treatments were applied with a compressed gas plot sprayer at a carrier volume of 281 L/ha except for one cutleaf eveningprimrose control experiment where the carrier volume was 94 L/ha. All of the postemergence treatments were applied with 1/4% v/v nonionic surfactant[3] Visual ratings 1 to 2 months after treatment were used to evaluate wheat injury and weed control.

Analysis of variance was initially performed on the control data from individual experiments. However, pooling the data for each weed species over locations revealed no significant location effects or location by treatment interactions. Therefore, data for each species was pooled over locations in which the given weed species was present. Henbit control was pooled over locations A-1 and A-2, flixweed control was pooled over locations B-1 and B-2, Italian ryegrass control was pooled over locations C-1, C-2, and C-3, wild buckwheat control was pooled over locations D-1, D-2, and D-3, and cutleaf eveningprimrose control was pooled over locations E-1 through E-5.

Summer weed control. Four field experiments were established to evaluate control of woolly croton or prostrate spurge in small grains stubble with chlorsulfuron and CGA 131036. The weed species, application dates, weed growth stages, and weed densities are detailed in Table 1. The soil types, soil pH's, and the legal descriptions for these experiments are presented at locations F-1, F-2, E-1 and E-2 in Table 2. The experimental designs used were randomized complete block designs with a minimum plot size of 1.5 by 3 m and 3 or 4 replications.

Chlorsulfuron and CGA 131036 were applied postemergence to the weeds at 18 and 35 g/ha with a compressed gas plot sprayer in a carrier volume of 281 L/ha with 1/2% v/v nonionic surfactant. A treatment containing 560 or 1120 g ae/ha of 2,4-D butyl ester was included in the

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[3] The surfactant was Triton AG-98, containing alkylarypolyoxyethylene glycols, produced by Rohm and Haas Co., Philadelphia, PA 19105.

prostrate spurge and woolly croton control experiments, respectively, as a standard treatment. Plant fresh weight and moisture content were used to evaluate woolly croton weed control whereas these parameters were combined with visual ratings for the prostrate spurge control evaluations. Moisture content was determined from the combined fresh weight and oven dry weight of 5 and 3 plants per plot from the woolly croton control experiments in 1985 and 1986 respectively and 3 plants per plot in the prostrate spurge experiments each year. Woolly croton fresh weights and moisture content was determined 24 and 50 days after treatment in 1985 and 50 days after treatment in 1986. Prostrate spurge fresh weight and control data were collected 28 days after application both years. Analysis of variance was performed on each experiment and the 0.05 least significant difference was used for mean separation.

RESULTS AND DISCUSSION

Weed control in winter wheat. Neither chlorsulfuron or CGA 131036 at rates as high as 26 and 53 g/ha respectively caused any wheat injury when applied preemergence to winter wheat. There was also no wheat injury observed when the herbicides were applied postemergence at rates from 9 to 20 g/ha to wheat with growth stages from 5 tillers to the first joint. However, there were differences in the efficacy on the various weed species. Pooled over location A-1 and A-2, 10 g/ha CGA 131036 only controlled henbit 42 % whereas chlorsulfuron applied at 9 g/ha controlled henbit 84 % (Table 3). In contrast, both herbicides controlled almost all of the flixweed present in locations B-1 and B-2 at rates as low as 9 g/ha (Table 3).

Differences in control of cutleaf eveningprimrose were also observed between these herbicides (Table 4). Although the differences were not as great as those observed in the control of henbit, the results of pooling the data from experiments F-1 through F-5 revealed that CGA 131036 at 9 g/ha provided significantly less control than chlorsulfuron at the same rate.

Pooling the wild buckwheat control data from D-1, D-2 and D-3 revealed no significant differences in control between the herbicides at rates of 9 or 18 g/ha (Table 4). Although these treatments only provided fair control, 18 g/ha is only two-thirds the recommended chlorsulfuron rate for wild buckwheat control (2).

Pooling the control data from locations C-1, C-2, and C-3 revealed a distinct rate response in Italian ryegrass control with CGA 131036. CGA 131036 at 26 and 53 g/ha controlled 77 and 91 percent of the Italian ryegrass respectively. In contrast, chlorsulfuron applied at 26 g/ha controlled 90 percent of the Italian ryegrass. Thus, at equal rates, CGA 131036 was not as effective for Italian ryegrass control as chlorsulfuron.

The lack of treatment by location interactions is an indication of consistent performance of these herbicides across the range of soils utilized. Although CGA 131036 was not as effective for the control of henbit and cutleaf eveningprimrose as chlorsulfuron at the rates investigated, it is possible that similar control of these species could be achieved with CGA 131036 by increasing the application rate. An example of this was discussed previously for preemergence Italian ryegrass control. However, increasing application rates would increase the possibility of extended residual carryover.

Weed control in stubble. Due to year by treatment interactions, the data from woolly croton control experiments were not pooled. In 1985 all of the treatments reduced woolly croton fresh weight 24 days after treatment (Table 5). However, the plant moisture content was reduced only by the high rate of chlorsulfuron and by 2,4-D. The differences between fresh weight and moisture content indicate that the sulfonylurea herbicide treatments inhibited plant growth without causing an appreciable decrease in moisture content. The same parameters measured 50 days after treatment revealed that woolly croton did not recover from the 2,4-D treatment, but the weeds were not killed by the sulfonylurea treatments.

The woolly croton plants were much larger when the herbicides were applied in 1986 (Table 1). All of the treatments continued to provide fresh weight reductions 50 days after treatment (Table 5). However, the 2,4-D treatment and chlorsulfuron at 35 g/ha were the only treatments that significantly reduced moisture content. Thus, as occurred 24 days after treatment in 1985, the sulfonylurea treatments inhibited woolly croton growth without actually killing them. Visual observations of chlorsulfuron and CGA 131036 treated plants 50 days after treatment revealed desiccated leaf material and semi-succulent stems whereas the 2,4-D treated plants were totally desiccated. The larger plant size in 1986 can be attributed to higher summer rainfall, higher soil fertility and 4 C cooler mean maximum daily temperature during the 1986 experiment. However, the woolly croton plants were actively growing during both experiments.

The results from the prostrate spurge experiments were also different in 1985 and 1986. In 1985 only chlorsulfuron provided appreciable visual control of prostrate spurge (Table 6). Both chlorsulfuron treatments and 2,4-D reduced moisture content. However, reductions in plant fresh weight were not observed due to substantial variation in weed size. During 1986 visual prostrate spurge control was very poor with all treatments except 2,4-D. Likewise, only the 2,4-D treatment reduced plant moisture content. The plant fresh weight revealed no significant differences between 2,4-D and the high rates of CGA 131036 and chlorsulfuron. Thus, as occurred in the woolly croton experiments, the sulfonylurea herbicide treatments caused growth inhibition without killing the weeds.

This data indicates that neither woolly croton or prostrate spurge was consistently controlled with postemergence applications of these two sulfonylurea herbicides at rates up to 35 g/ha. However, growth suppression and some control were observed. The 2,4-D treatments were consistently more effective for controlling these weeds than the chlorsulfuron and CGA 131036 treatments.

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Table 1. Weed species, application dates, growth stages, and weed densities of the 15 experimental locations established on winter wheat and the 4 experimental locations established on small grain stubble.

			Growth stage		
Location	Weed	Application date	Weed	Wheat	Weed population
					----(No./m^2)---
A-1	LAMAM	Feb. 25, 1986	3-10 cm tall	8 to 10 tillers	86 to 129
A-2	LAMAM	Feb. 26, 1986	3-10 cm tall	8 to 10 tillers	22 to 32
B-1	DESSO	Mar. 4, 1986	7-8 cm tall	8 to 12 tillers	10 to 22
B-2	DESSO	Mar. 4, 1986	10-18 cm tall	7 to 12 tillers	22 to 32
C-1	LOLMU	Sept. 11, 1985	preemergence	preemergence	334
C-2	LOLMU	Sept. 14, 1986	preemergence	preemergence	539 to 646
C-3	LOLMU	Sept. 9, 1986	preemergence	preemergence	539
D-1	POLCO	Mar. 26, 1986	3-4 leaves	6 to 8 tillers	108 to 323
D-2	POLCO	Mar. 27, 1986	2-4 leaves	1st joint	108
D-3	POLCO	Mar. 26, 1986	1-2 leaves	1st joint	10 to 97
F-1	OEOLA	Feb. 25, 1986	3-15 cm rosette	8 to 10 tillers	22 to 43

Table 1. Weed species, application dates, growth stages, and weed densities of the 15 experimental locations established on winter wheat and the 4 experimental locations established on small grain stubble, cont.

			Growth stage		
Location	Weed	Application date	Weed	Wheat	Weed population
					----(No./m^2)---
F-2	OEOLA	Feb. 28, 1986	8 cm rosettes	5 to 7 tillers	5
F-3	OEOLA	Feb. 26, 1986	3-8 cm rosettes	9 to 12 tillers	43 to 65
E-4	OEOLA	Feb. 26, 1986	3-8 cm rosettes	8 to 10 tillers	22 to 43
E-5	OEOLA	Feb. 26, 1986	3-8 cm rosettes	8 to 12 tillers	10 to 43
F-1	CVNCP	July 29, 1985	30-46 cm tall	postharvest	6 to 10
F-2	CVNCP	Aug. 12, 1986	20-25 cm tall	postharvest	1 to 2
G-1	EPHHT	Aug. 7, 1985	15-46 cm stems	postharvest	2 to 6
G-2	EPHHT	Aug. 12, 1986	25-38 cm stems	postharvest	2 to 3

Table 2. Soil types, soil pHs and legal decriptions of the 15 experimental locations on winter wheat and the 4 experimental locations established on small grain stubble.

Location	Soil type	pH	Legal description
A-1	Teller sandy loam	5.8	NW 1/4 Sec. 36, T18N; R2E
A-2	Teller sandy loam	5.9	NW 1/4 Sec. 36, T18N; R2E
B-1	Vernon clay loam	6.5	NW 1/4 Sec. 20, T27N; R14W
B-2	Norge silt loam	5.8	NW 1/4 Sec. 3, T22N; R6W
C-1	Teller sandy loam	6.1	NW 1/4 Sec. 36, T18N; R2E
C-2	Teller loam	5.9	NW 1/4 Sec. 36, T18N; R2E
C-3	Norge loam	6.1	SW 1/4 Sec. 16, T19N; R2E
D-1	Kirkland silt loam	5.4	SE 1/4 Sec. 25, T28N; R2E
D-2	Agra sandy clay loam	5.1	SE 1/4 Sec. 4, T17N; R4E
D-3	Kirkland clay	5.3	SE 1/4 Sec. 11, T19N; R4W
E-1	Teller sandy loam	5.8	NW 1/4 Sec. 36, T18N; R2E
E-2	Zaneis sandy clay loam	5.5	SE 1/4 Sec. 7 T19N; R2E
E-3	Teller sandy loam	5.3	NW 1/4 Sec. 36, T18N; R2E
E-4	Teller sandy loam	5.9	NW 1/4 Sec. 36, T18N; R2E
E-5	Teller sandy loam	5.4	NW 1/4 Sec. 36, T18N; R2E
F-1	Norge loam	6.1	SE 1/4 Sec. 8, T19N; R2E
F-2	Norge sandy loam	6.0	SE 1/4 Sec. 8, T19N; R2E
G-1	Norge loam	5.8	SE 1/4 Sec. 16, T19N; R2E
G-2	Norge sandy clay loam	6.2	SE 1/4 Sec. 8, T19N; R2E

Table 3. Control of flixweed and henbit pooled over two locations with postemergence applications of CGA 131036 and chlorsulfuron.

		Control	
Herbicide[1]	Rate	# DESSO	# LAMAM
	(g/ha)	---------(%)----------	
CGA 131036	10	99	42
" "	20	99	-----
Chlorsulfuron	9	100	84
" "	20	99	-----
Check	---	0	0
LSD .05		2	15

[1] All treatments were applied with 1/4 % v/v surfactant.

Table 4. Control of cutleaf eveningprimrose and wild buckwheat pooled over five locations and three locations respectively, with postemergence applications of CGA 131036 and chlorsulfuron.

		Control	
Herbicide[1]	Rate	# OEOLA	# POLCO
	(g/ha)	-----------(%)--------	
CGA 131036	9	81	75
" "	18	----	77
Chlorsulfuron	9	91	67
" "	18	----	77
Check	----	0	0
LSD 0.05		8	15

[1] All treatments were applied with 1/4 % v/v surfactant.

Table 5. The effect of CGA 131036, chlorsulfuron and 2,4-D treatments on woolly croton moisture content and fresh weight during 1985 and 1986.

		1985				1986	
		24 DAT		50 DAT		50 DAT	
Herbicide[1]	Rate	Fresh wt.	Moisture	Fresh wt.	Moisture	Fresh wt.	Moisture
	(g/ha)	--(g/pl)-	--(%)---	--(g/pl)-	--(%)---	--(g/pl)-	--(%)---
CGA 131036	18	4.3	60	10.6	63	42.7	58
" "	35	5.0	63	6.8	65	37.0	54
Chlorsulfuron	18	3.3	54	8.0	65	17.6	39
" "	35	3.4	42	4.4	62	22.0	33
2,4-D	1120	1.6	14	1.2	28	17.3	16
Check	----	8.4	66	7.8	60	123.0	58
LSD .05		2.3	14	NSD	9	63.3	22

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[1] All treatments except 2,4-D were applied with 1/2 % v/v surfactant.

Table 6. Control, moisture, and fresh weight of prostrate spurge with CGA 131036 and chlorsulfuron 28 days after application in 1985 and 1986.

		1985			1986		
Herbicide[1]	Rate	Control	Fresh wt.	Moisture	Control	Fresh wt.	Moisture
	(g/ha)	--(%)--	--(g/pl)-	--(%)---	--(%)--	--(g/pl)-	--(%)---
CGA 131036	18	12	134	52	0	162	68
" "	35	12	215	55	0	99	67
Chlorsulfuron	18	45	54	32	0	165	68
" "	35	78	68	33	17	62	66
2,4-D	560	25	75	33	93	2	54
Check	----	0	154	55	0	235	66
LSD .05		26	102	17	18	110	4

[1] All treatments except 2,4-D were applied with 1/2 % v/v surfactant.

PART III

PERSISTENCE OF BIOLOGICAL ACTIVITY OF CGA 131036, CHLORSULFURON, AND DPX M 6316 IN SOUTHWESTERN OKLAHOMA SOILS

PERSISTENCE OF BIOLOGICAL ACTIVITY OF CGA 131036, CHLORSULFURON, AND DPX M 6316 IN SOUTHWESTERN OKLAHOMA

Abstract. The persistence of the biological activity of CGA 131036 (N-[[(6-methoxy-4-methyl-1,3,5,-triazin-2yl-amino]carbonyl-2-(2-chloroethoxy)]-benzenesulfonamide), chlorsulfuron (2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] benzenesulfonamide), and DPX M 6316 (methyl 3-[[(4-methoxy-6-methyl-1,3,5-triazin-2 yl aminocarbonyl]aminosulfonyl]-2-thiophenecarboxylate) were compared in three soils in southwestern Oklahoma by using a corn (Zea mays L.) root bioassay. The predicted concentrations of chlorsulfuron and CGA 131036 were below detectable levels (0.25 ppb) in a Meno loamy fine sand with pH 6.6 after 6 months. After 4 months the concentration of these herbicides were below detectable levels in a Hollister clay loam with pH 6.1. However, in a Tillman silt loam with pH 7.1 the predicted concentration of chlorsulfuron was 2 ppb 8 months after application whereas CGA 131036 had degraded below detectable levels by the sixth month after application. The faster loss of biological activity associated with CGA 131036 is consistent with the acid hydrolysis theory of sulfonylurea degradation since CGA 131036 has a higher pKa. At all locations DPX M 6316 was below detectable levels (2ppb) by 1 month after application.

Additional index words. Bioassay, corn, residue analysis.

INTRODUCTION

The sulfonylurea herbicide, chlorsulfuron has become widely used for weed control in winter wheat (*Triticum aestivum* L.) in Oklahoma. This herbicide provides excellent control of a broad spectrum of broadleaf weeds and a few grasses at use rates of 10 to 40 g ai/ha. However, due to the persistence of this herbicide and the susceptibility of many rotational crops, restrictions on seeding rotational crops are necessary. Persistence is longer in soils with neutral or alkaline pH's because the major means of chlorsulfuron degradation is acid hydrolysis (13). Chlorsulfuron is an acid with a pKa of approximately 3.8 (15). Therefore, ionization increases with soil pH which limits acid hydrolysis (10).

Fredrickson and Shea (3) found only 20% degradation of chlorsulfuron 6 weeks after application to a silty clay loam soil with an adjusted pH of 7.5 verses 90% degradation when the pH was adjusted to 5.6. In other research the half-life of chlorsulfuron has been reported to vary from 1 to 2.5 months (8, 12, 13) depending on soil pH, texture and temperature. Although other herbicides have similar residual properties, few can cause crop injury at such low soil concentrations. Injury to rotational crops has occurred up to 2 yr after chlorsulfuron application (5, 7, 9, 11). Because of the susceptibility of some crops to extremely low chlorsulfuron concentrations an understanding of the persistence of the newer sulfonylurea herbicides CGA 131036 and DPX M 6316 is needed.

While residual characteristics and degradation of chlorsulfuron have been documented, there is very little information available on CGA 131036 and DPX M 6316. The objectives of this research were to compare the degradation of biological activity of these two sulfonylurea herbicides with chlorsulfuron.

METHODS AND MATERIALS

Field Experiments. Field experiments were established in southwestern Oklahoma on a Meno loamy fine sand (Aquic Arenic Haplustalfs), a Tillman silt loam (Typic Paleustolls) and a Hollister clay loam (Pachic Paleustolls). The physical and chemical characteristics of these soils are detailed in Table 1. The experimental designs were randomized complete blocks with four replications with 3 by 7.6 m plots on the Hollister and Tillman soils and three replications and 3 by 6 m plots on the Meno loamy fine sand. Chlorsulfuron, DPX M 6316 and CGA 131036 were applied at 40 g ai/ha (approximately 27 ppb w/w based on a 10 cm depth) in March, 1986, with a bicycle sprayer. This date is consistent with normal herbicide application dates for broadleaf weed control in winter wheat. The treatments were applied to winter wheat in the first joint growth stage (Zadok's 31) (14) at the experiments established on the Meno and Hollister soils whereas the Tillman soil was tilled and free of vegetation.

Two 10 cm diameter soil cores 10 cm deep were taken from each plot immediately after application and at 0.5, 1, 2, 3, 4, 6 and 8 months after application. After the experiments established on wheat were harvested in June, 1986, and throughout the entire sampling period for the experiment on the Tillman silt loam, periodic applications of glyphosate (N-[phosphonomethyl] glycine) were used to control weed growth without tillage.

Bioassay Procedure. The soil samples described previously were screened with a 4 mm mesh screen and stored at -5 C until bioassayed with a corn root bioassay procedure slightly modified from that described by Groves and Foster (4). A standard curve for each herbicide was prepared by adding 20 ml of herbicide solution to 2 kg of soil from untreated plots to obtain concentrations of 0.25 to 16 ppb (w/w on a dry soil weight basis) for chlorsulfuron and CGA 131036 and 2 to 32 ppb (w/w) for DPX M 6316 in the Meno soil and 2 to 16 ppb the Hollister and Tillman soil. To avoid drying the soil used for the standard curves, extra samples were dried at 105 C for 8 h to determine the percent dry weight of the soil. After adding the herbicide solution to the soil, the standards were thoroughly mixed and allowed to equilibrate at room temperature for 12 h before corn seedlings were planted.

The concentrations below 2 ppb were not used during the initial sampling dates for the chlorsulfuron and CGA 131036 standard curves. The plot soils taken during the initial sampling dates for these herbicides were mixed 1:1 with untreated soil to reduce the concentration below 16 ppb to be within the concentration range of the standards. This was also done for the DPX M 6316 treatments in the Hollister clay loam and Tillman silt loam soils during the first sampling date.

The prepared standards and plot soils were placed in 237 ml polystyrene pots. Due to differences in the bulk density of the soils used, 220 g of soil per pot were used for the Tillman silt loam and

Hollister clay loam soils whereas 250 g were used for the Meno loamy fine sand. Three corn seedlings (Funk F1 hybrid G 4673A) that had been incubated for 72 h at 28 C and had 5 to 10 mm long roots were transplanted into the pots which where then subirrigated to capacity with one-half strength Hoagland's (6) solution. Each pot was returned to 80 % of the calculated holding capacity by weight with distilled water on a daily basis. The pots of both the standards and plot soils were placed on a light table under 300 uE/m/s of continuous light for 7 days at 30 ± 2 C. The soil was then washed from the roots and length of the primary root was determined. This process was repeated for each location and sampling date. The soil collected after 2, 3, and 4 months was bioassayed simultaneously with the use on one set of standards as was the soil collected 6 and 8 months after application.

The root length data from each soil collection date were analyzed using a randomized complete block design with replications corresponding to replications in the field experiments. The experiments established on the Tillman silt loam and Hollister clay loam soils had four replications with two pots/replication and three plants/pot for a total of 24 observations/treatment. The experiment established on the Meno loamy fine sand soil had three replications with three pots/replication and three plants/pot for a total of 27 observations/treatment. The treatment means were used in conjunction with the standards to estimate the herbicide concentration. The standards were replicated eight times. Each replication consisted of one pot with three corn plants. The standards for each location were grown simultaneously with the plot soils.

Statistical Analysis. Regression analysis was used to plot root length against the natural log of herbicide concentration. However, unlike Groves and Foster (4), the regression was performed on each individual data point instead of the means. As the result of pooling the standards from the different sampling dates, the DPX M 6316 standards for the Tillman and Hollister soils consisted of a total of 192 observations. The DPX M 6316 standard for the Meno soil consisted of 240 observations at five concentrations. The CGA 131036 and chlorsulfuron standards each had 432 and 528 observations respectively for the Hollister and Meno soils. In the Tillman soil the CGA 131036 standard had 528 total observations whereas the chlorsulfuron standard had 672.

RESULTS AND DISCUSION

Herbicide standards. The corn root bioassay described by Groves and Foster (4) for quantifying chlorsulfuron residues was found to be effective for quantifying CGA 131036 concentrations as low as 0.25 ppb and 2 ppb of DPX M 6316. The r^2 values for these standards varied from 0.98 to 0.90 with the exception of the DPX M 6316 standard in the Tillman soil which had a r^2 value of 0.67. Although the regression was performed on the individual data points, the r^2 values are based on regression of the means. The standard error of the mean for the regression lines were used as a statistical parameter to estimate the upper and lower values for the predicted concentration. However, because the independent variable of the standards is the natural logarithm of herbicide concentration, the accuracy associated with the predicted concentration decreases with increasing herbicide concentration (Tables 2, 3, and 4). But, the accuracy of this technique at the lower concentrations was considered the most important feature.

The regression lines for each of the herbicide standards are presented by location in Figure 1, 2, and 3 using the mean intercept. Pooling revealed that within standards for each herbicide and each location the standards had common slopes. However, the intercepts for each of the sampling dates were slightly different and thus could not be pooled. Differences in the intercepts could have resulted from slight variation in root lengths of transplanted seedlings, and minor differences in environmental conditions during the growing period. Since the intercepts could not be pooled, the predicted herbicide concentrations were estimated with the use of the common slope and the intercept associated with the corresponding sampling date. The unit activity of chlorsulfuron was much greater than CGA 131036 at the lower concentrations as indicated by the predicted corn root length at concentrations below 8 ppb (ln 2.08, Figure 1, 2, and 3). Due to the greater negative slope of the CGA 131036 standards, the biological activity was nearly as great as chlorsulfuron at the higher concentrations. Both chlorsulfuron and CGA 131036 exhibited greater unit activity than DPX M 6316 (Figure 1, 2, and 3). The slopes and mean intercepts associated with the chlorsulfuron and CGA 131036 standards were relatively consistent for each herbicide on all three soil types. In contrast, the DPX M 6316 regression equations resulted in substantially different slopes between soil types. The activity of this herbicide was considerably lower in the Tillman silt loam.

Degradation of biological activity. The disappearance of the biological activity of DPX M 6316 was very rapid in the pH 6.6 Meno loamy fine sand (Table 2). The predicted concentration of this herbicide was 31.3 ppb initially, 2.1 ppb 2 weeks later and below detectable levels (< 2 ppb) 1 month after application. Chlorsulfuron concentrations did not decrease appreciably until 2 months after the treatment had been applied at which time the predicted concentration was approximately 3 ppb (Table 2). The concentration of CGA 131036 decreased gradually until 2 months after application. However, the estimated concentrations 2, 3, and 4 months after application were relatively constant at approximately 3 to 4 ppb. The predicted concentration of both of these herbicides had reached undetectable concentrations (< 0.25 ppb) 6 months after the treatment.

In the Hollister clay loam (pH 6.1), the predicted concentration of chlorsulfuron decreased from 20.9 ppb at 0.5 months after treatment to 2.3 ppb at 3 months after treatment (Table 3). The greatest decrease in the concentration of this herbicide occurred between 1 and 2 months after treatment. The CGA 131036 concentrations had increased to 35.9 ppb 0.5 month after treatment and then decreased to 0.9 ppb 2.5 months later (Table 3). The lower concentration associated with the initial sampling date was attributed to herbicide interception by the wheat canopy during application. Adequate rainfall (1.4 cm) occurred during the next 2 week period to activated the herbicide before the next sampling data. As occurred in the Meno loamy fine sand, both chlorsulfuron and CGA 131036 had reached undetectable levels

(< 0.25 ppb) within the same sampling period. The faster disappearance of the biological activity of these herbicides in the Hollister clay loam (pH 6.1) relative to the Meno loamy fine sand (pH 6.6) is consistent with the acid hydrolysis theory for the degradation of chlorsulfuron (13). DPX M 6316 concentrations had reached undetectable levels by one month after treatment (Table 3.) as was the case in the Meno soil.

In the Tillman silt loam (pH 7.1), approximately 1.4 ppb of chlorsulfuron was detectable 8 months after herbicide application (Table 4). The CGA 131036 concentration had reached undetectable levels by 6 months after treatment. The 3 month sampling date was not included in this experiment due to inadvertent contamination of the samples collected. The differences in the persistence of these herbicides in the Tillman silt loam support the hypothesis that CGA 131036 degrades by acid hydrolysis in the same manner as chlorsulfuron. Since the pKa of CGA 131036 is 4.5[1] whereas the pKa of chlorsulfuron is 3.8 (15), more CGA 131036 would be in a nonionic state and subject to acid hydrolysis than chlorsulfuron at a given soil pH. Therefore, at a given soil pH CGA 131036 should degrade somewhat faster than chlorsulfuron. The concentration of DPX M 6316 , as in the Meno and Hollister soils, had reached undetectable levels by 1 month after herbicide application (Table 4). Recent investigations have revealed that the rapid degradation of DPX M 6316 in soil is mainly a function of microbial activity (2).

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[1] McMahan, A. Ciba-Geigy Corporation. Personal communication

The use of the corn root bioassay for detecting concentrations of these herbicides revealed that this technique can be used as an effective tool at lower herbicide concentrations. The results of these investigation revealed that soil pH appeared to be the most important parameter affecting the degradation of chlorsulfuron and CGA 131036 in the soils studied. Other researchers have found similar results for chlorsulfuron (3, 8, 12, 13). Although chlorsulfuron persistence has also been shown to be affected by soil texture (1), in this research soil pH appeared more important in controlling the rate of disappearance of biological activity of chlorsulfuron and CGA 131036. The persistence of the biological activity of DPX M 6316 was consistent with other recent research (2). Since the persistence of this herbicide was less than one month its use in winter wheat should not restrict the seeding of rotational crops. There is no infromation available on the persistence of CGA 131036. However, these results suggest that the mechanism of degradation is very similar to chlorsulfuron.

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Table 1. Physical and chemical characteristics of soils.

Soil	pH	Physical composition			
		Sand	Silt	Clay	Organic matter
		(%)			
Hollister clay loam	6.1	32	36	32	1.5
Meno loamy fine sand	6.6	84	10	6	0.7
Tillman silt loam	7.1	24	51	25	1.5

Table 2. Estimated herbicide concentrations in Meno loamy fine sand.

		Time (months)							
Herbicide	Estimate	0	0.5	1	2	3	4	6	8
		(ppb)							
Chlorsulfuron	Upper	25.5	19.5	28.5	3.6	0.8	0.8		
	Predicted	16.4	12.8	18.8	2.9	0.6	0.5	<0.25	<0.25
	Lower	11.1	8.5	12.4	1.6	0.4	0.4		
CGA 131036	Upper	43.0	17.9	8.5	5.6	5.1	5.5		
	Predicted	28.7	11.8	5.6	3.7	3.3	3.6	<0.25	<0.25
	Lower	18.9	7.7	3.7	2.4	2.4	2.4		
DPX M 6316	Upper	50.8	3.4						
	Predicted	31.3	2.1	< 2.0	< 2.0	----	----	----	----
	Lower	19.3	1.3						

Table 3. Estimated herbicide concentrations in Hollister clay loam.

		Time (months)							
Herbicide	Estimate	0	0.5	1	2	3	4	6	8
		(ppb)							
Chlorsulfuron	Upper	40.1	43.4	25.2	8.0	4.8			
	Predicted	19.4	20.9	12.1	3.9	2.3	<0.25	<0.25	<0.25
	Lower	9.3	10.1	5.8	1.1	0.9			
CGA 131036	Upper	20.1	50.2	33.8	3.1	1.3			
	Predicted	14.4	35.9	24.1	2.2	0.9	<0.25	<0.25	<0.25
	Lower	10.3	25.6	17.2	1.6	0.6			
DPX M 6316	Upper	43.0	9.5						
	Predicted	31.5	6.8	< 2.0	< 2.0	----	----	----	----
	Lower	22.1	4.9						

Table 4. Estimated herbicide concentrations in Tillman silt loam.

		Time (months)						
Herbicide	Estimate	0	0.5	1	2	4	6	8
		(ppb)						
Chlorsulfuron	Upper	43.1	42.6	24.9	21.8	17.1	2.4	2.8
	Predicted	21.1	20.9	12.2	11.1	8.4	1.2	1.4
	Lower	10.4	10.3	6.0	5.2	4.1	0.6	0.7
CGA 131036	Upper	42.3	47.0	18.0	22.5	5.1		
	Predicted	26.3	29.2	11.2	14.2	3.1	<0.25	<0.25
	Lower	16.4	18.2	7.0	8.9	2.0		
DPX M 6316	Upper	52.1	12.5					
	Predicted	28.2	6.8	< 2.0	< 2.0	----	----	----
	Lower	15.3	3.7					

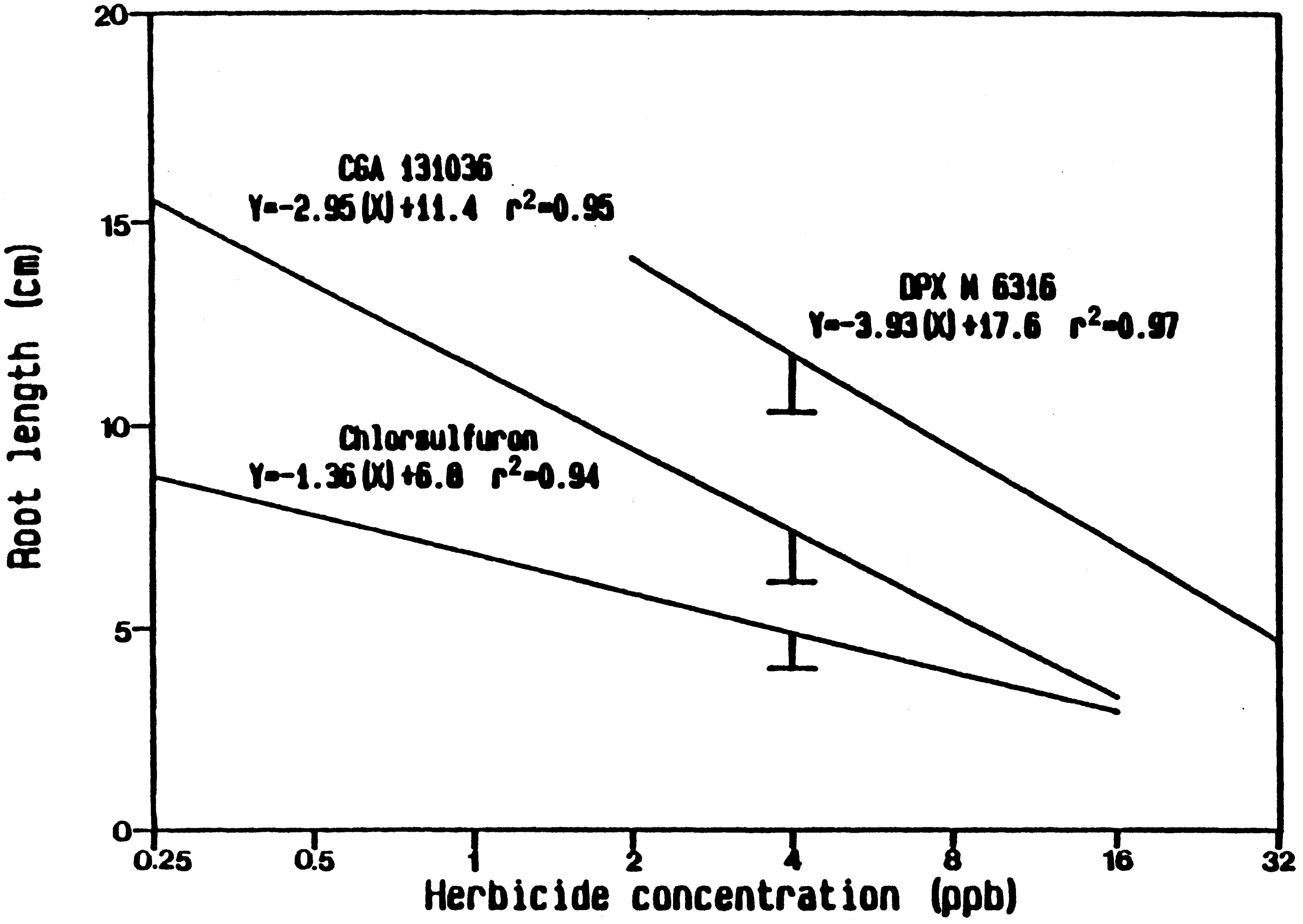

Figure 1. Response of corn roots to chlorsulfuron, CGA 131036, and DPX M 6316 in a Meno loamy fine sand. The regression lines are based on the common slope and the mean intercept. The vertical lines are standard deviations.

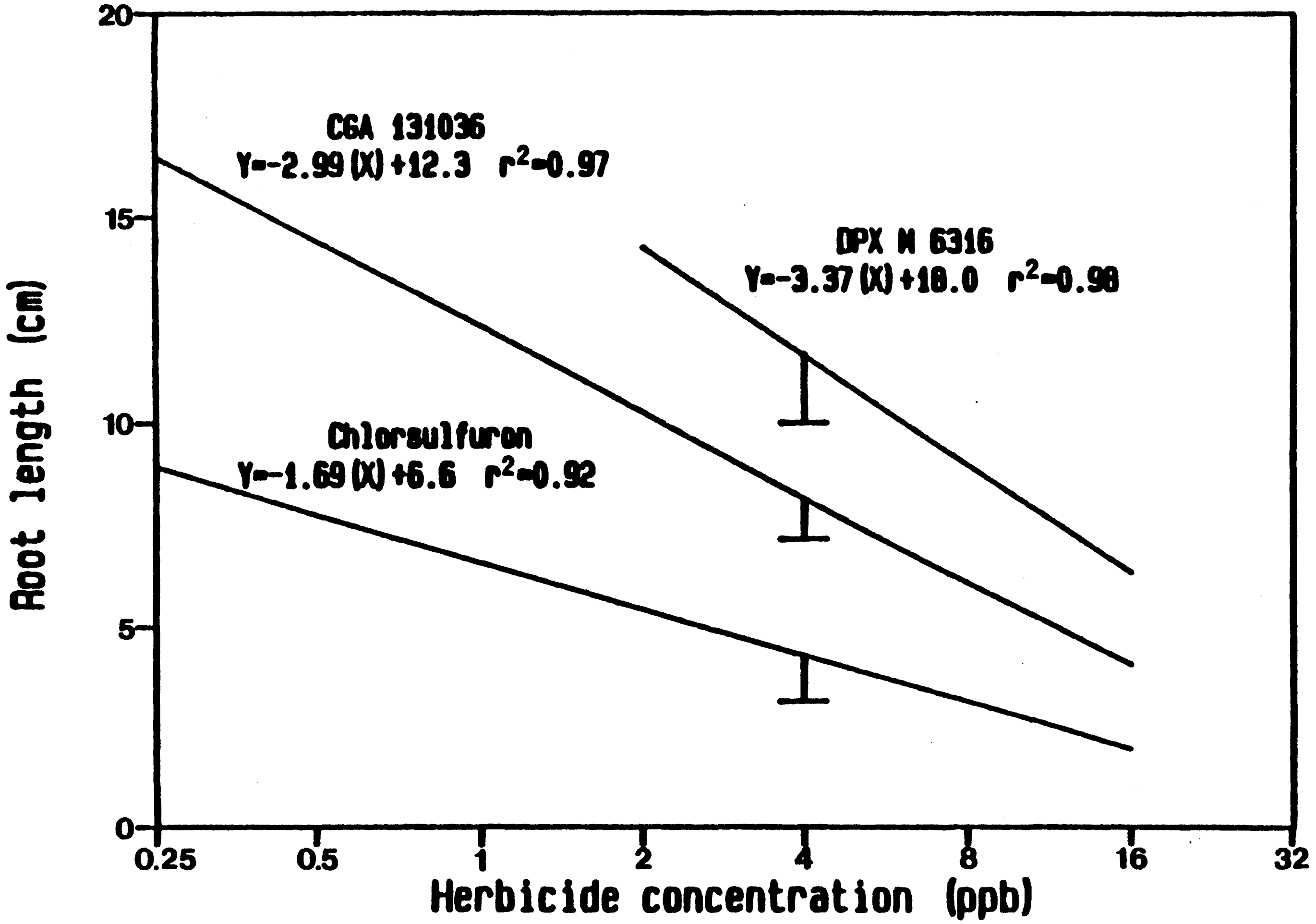

Figure 2. Response of corn roots to chlorsulfuron, CGA 131036, and DPX M 6316 in a Hollister clay loam. The regression lines are based on the common slope and the mean intercept. The vertical lines are standard deviations.

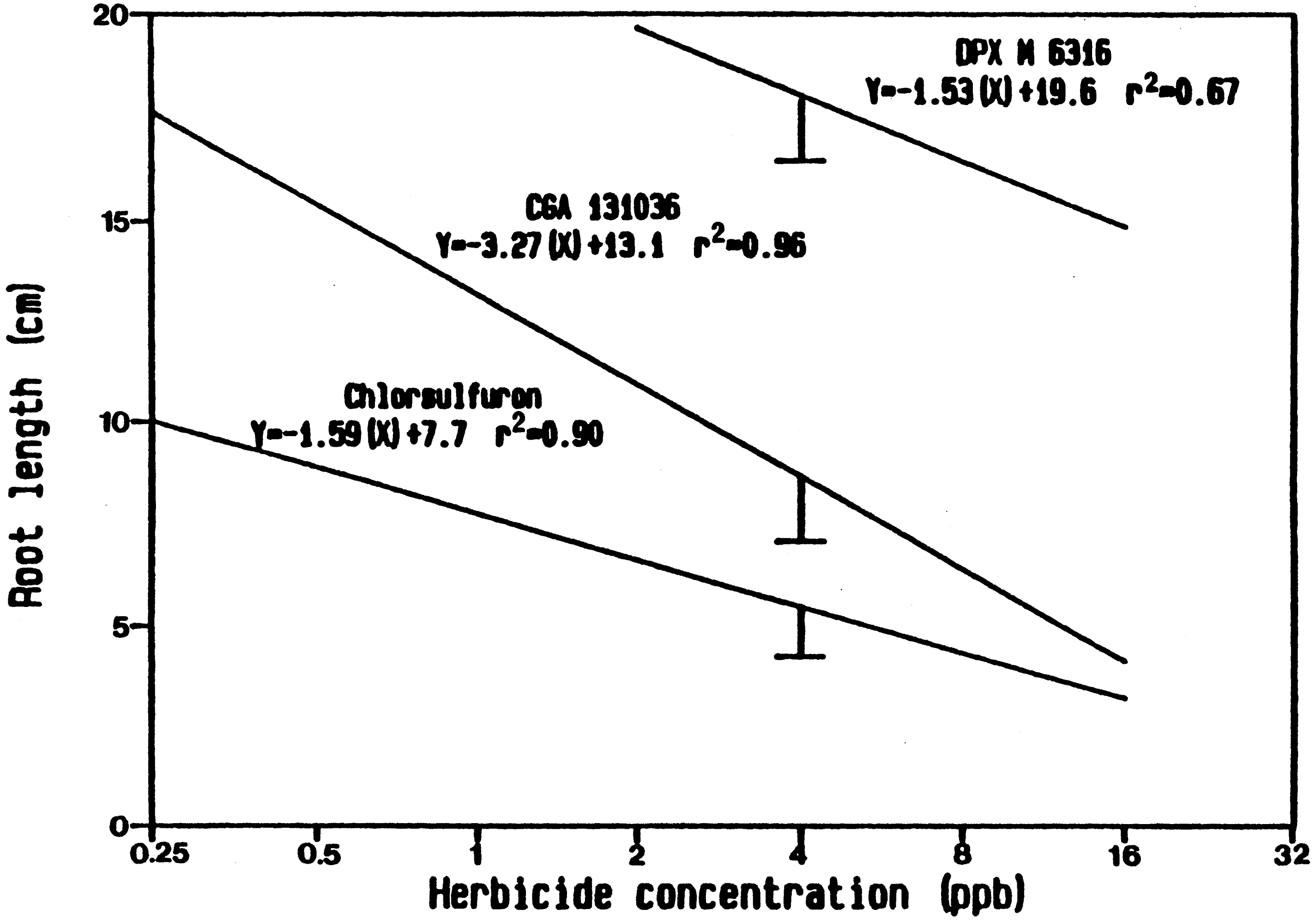

Figure 3. Response of corn roots to chlorsulfuron, CGA 131036, and DPX M 6316 in a Tillman silt loam. The regression lines are based on the common slope and the mean intercept. The vertical lines are standard deviations.

VITA

2

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