Uptake, Translocation, and Metabolism of Oxabetrinil and CGA-133205 in Grain Sorghum (*Sorghum bicolor*) and Their Influence on Metolachlor Metabolism†

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The uptake, translocation, and metabolism of the oxime ether safeners oxabetrinil and CGA-133205 in grain sorghum [*Sorghum bicolor* (L.) Moench, var. Funk G-522-DR] were investigated. Following application of [^{14}C]oxabetrinil and [^{14}C]CGA-133205 to imbibed seeds, the uptake of both safeners by grain sorghum increased for the 72-h duration of this experiment with a greater percentage of the applied oxabetrinil being taken up than that of CGA-133205. Very little translocation of either safener to the roots occurred. Total extractable oxabetrinil and CGA-133205 from coleoptiles of grain sorghum increased with time. The water-soluble portion of the extracted radioactivity increased with time, indicating that the two safeners were metabolized by grain sorghum seedlings. TLC analysis of the water-soluble extracts revealed that a small portion of the safener oxabetrinil may be conjugating with glutathione in sorghum. The safener CGA-133205 did not conjugate with glutathione. Two unidentified metabolites of [^{14}C]-oxabetrinil and one unidentified metabolite of [^{14}C]CGA-133205 were detected. Treatment of grain sorghum with oxabetrinil (1.25 g of ai/kg of seeds) increased [^{14}C]metolachlor uptake, but did not affect metolachlor translocation. CGA-133205 at 0.4 g of ai/kg of seeds did not influence [^{14}C]metolachlor uptake, but it increased the amount of radiolabel from [^{14}C]metolachlor that translocated to the roots. Both oxabetrinil and CGA-133205 increased the rate of [^{14}C]metolachlor metabolism to a water-soluble metabolite during the initial 4 h after [^{14}C]metolachlor application, but not at 8 h. TLC analysis of water-soluble extracts from grain sorghum coleoptiles 4 h after application of [^{14}C]metolachlor revealed that both safeners increased the formation of the glutathione conjugate of metolachlor and concurrently decreased the amount of parent metolachlor in the extract. It appears that oxabetrinil and CGA-133205 are conferring protection to grain sorghum by increasing the rate of metolachlor metabolism.

INTRODUCTION

Cyometrinil was the first oxime ether safener introduced by Ciba-Geigy to protect the marginally tolerant grain sorghum from injury caused by metolachlor. Cyometrinil was later replaced by oxabetrinil, a more effective safener of grain sorghum against metolachlor. Another oxime ether analogue that is being evaluated as a safener for metolachlor on grain sorghum is CGA-133205. All of the currently available oxime ether safeners of grain sorghum are applied as seed treatments; thus, they are readily available for uptake by the germinating grain sorghum seed. Early research with cyometrinil and oxabetrinil has shown that these safeners do not reduce the amount of metolachlor taken up by grain sorghum seedlings (LeBaron et al., 1988); therefore, the protection conferred by the application of these safeners to grain sorghum must be due to a safener-induced increase in the rate or pattern of metolachlor metabolism or due to effects at the site of action. Gronwald et al. (1987) reported that the degree of protection provided by safeners to grass crops against chloroacetanilide herbicides correlates rather strongly with the ability of safeners to enhance glutathione *S*-transferase (GST; EC 2.5.1.18) activity.

Currently, information on the uptake and metabolism of the safeners oxabetrinil and CGA-133205 in grain sorghum is not available. Similar to metolachlor, cyometrinil is taken up through the coleoptile of sorghum seedlings, although some uptake has also been shown to occur through the seed coat of grain sorghum (LeBaron et al., 1988). Breaux et al. (1989) demonstrated that flurazole, a thiazolecarboxylate safener that is seed-applied to grain sorghum, was rapidly absorbed by 3-5-day-old etiolated shoots of corn and grain sorghum. In both crops, flurazole was metabolized rapidly and the major metabolite detected was the glutathione conjugate of flurazole (Breaux et al., 1989).

In the preceding paper (Yenne and Hatzios, 1990b) we reported that the chemical structures of oxime ether safeners and metolachlor are quite similar at the molecular level. In addition, we have reported (Yenne and Hatzios, 1989) that the interactive influence of oxime ethers and metolachlor on lipid synthesis and acetyl-CoA carboxylase activity were not sufficient to explain the mechanism of action of these safeners. Thus, the structural similarity of these safeners to metolachlor did not appear to support the "competitive antagonism" theory proposed to explain the safening action of oxime ethers on grain sorghum against metolachlor. Another proposed mechanism for the action of herbicide safeners suggests a safener-induced enhancement of herbicide detoxication in safened plants (Hatzios, 1989). Therefore, the objectives of this research were to determine (a) the uptake, translocation, and metabolism of the oxime ether safeners oxabetrinil and CGA-133205 in grain sorghum and (b) the influence of oxabetrinil and CGA-133205 on the uptake,

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translocation, and metabolism of the herbicide metolachlor in grain sorghum.

MATERIALS AND METHODS

Chemicals. Analytical grade (>95% purity) and radiolabeled oxabetrinil, CGA-133205, and metolachlor were provided by Ciba-Geigy Corp., Greensboro, NC.

Plant Material. Grain sorghum seed variety Funk G-522 DR was obtained from Ciba-Geigy. The seed was either untreated or coated with the recommended use rates of the safeners oxabetrinil (1.25 g of ai/kg of seeds) or CGA-133205 (0.4 g of ai/kg of seeds). Approximately 40 seeds of each treatment were placed on moistened filter paper in petri dishes and incubated at 30 °C for varying lengths of time depending on the experiment.

Uptake and Translocation of Oxabetrinil and CGA-133205. Untreated seed, prepared as described above, was incubated for 12 h at 30 °C. After the initial 12-h incubation, 2 μL of 1 mM [^{14}C]oxabetrinil (phenyl labeled; sp act. 12.2 mCi/mmol) or 1 mM [^{14}C]CGA-133205 (phenyl labeled; sp act. 9.8 mCi/mmol) was added to 40 untreated seeds of grain sorghum. At 0, 0.5, 1, 2, 4, 24, 48, and 72 h after ^{14}C-labeled safener application, seeds or seedlings were rinsed in 80% methanol to determine the amount of ^{14}C-labeled safener that remained on the exterior of the seeds. At 0, 0.5, 1, 2, and 4 h the seeds were oxidized (Packard sample oxidizer Model B306), trapping $^{14}CO_2$ that is a byproduct of the combustion of the plant material and the ^{14}C-labeled safener. At 24 h, the root was removed and oxidized to evaluate the amount of translocation from the seed to the root. At 48 and 72 h, the coleoptile and the root were removed from the seed, and all three parts were oxidized to determine the amount of translocation of radiolabeled into the respective tissues.

Safener and Metolachlor Metabolism. Seeds were prepared as described under Plant Material and incubated for 3 days. After incubation, 2 μL of 1 mM [^{14}C]metolachlor (carbonyl labeled; sp act. 59.5 mCi/mmol), [^{14}C]oxabetrinil (phenyl labeled; sp act. 12.2 mCi/mmol), or [^{14}C]CGA-133205 (phenyl labeled; sp act. 9.8 mCi/mmol) was applied to coleoptiles of untreated and safener-treated grain sorghum seedlings. At 0, 1, 2, 4, and 8 h after application of radiolabeled safener or herbicide, the coleoptiles were removed and rinsed in 80% methanol. After the rinse, coleoptiles were ground in 80% methanol with a mortar and pestle. The methanol extract was centrifuged for 5 min at 13000*g*. The supernatant was decanted and saved for further analysis. The pellet was oxidized to determine the amount of unextracted radiolabel originating from [^{14}C]metolachlor or ^{14}C-labeled safener.

A subsample of the methanol extract was radioassayed for total radiolabel uptake originating from [^{14}C]metolachlor or ^{14}C-labeled safener. To determine the amount of water-soluble metabolites, a 60-μL subsample of the total extract was diluted with 60 μL of distilled water and mixed vigorously with 1 mL of dichloromethane. This solution was centrifuged for 3 min at 13000*g* for thorough mixing. The aqueous phase was then subsampled and radioassayed.

To further characterize the metabolites, the extract from the 4-h time period was dried under nitrogen gas. The residue was dissolved in 50 μL of methanol, and 20 μL was spotted onto 150 A silica gel thin-layer chromatography (TLC) plates. Plates were developed with a butanol/acetic acid/water (12:3:5 v/v/v) solvent mixture. After development, the plates were scraped in 1-cm increments to quantify the amount of radiolabel in each increment. [^{14}C]Metolachlor, [^{14}C]oxabetrinil, [^{14}C]CGA-133205, and synthetic conjugates of GS–metolachlor or GS–safener, prepared by a nonenzymatic procedure as described by Yenne and Hatzios (1990a), were cochromatographed with the plant extracts to aid in the identification of the detected metabolites.

Uptake and Translocation of Metolachlor. Seeds prepared as described under Plant Material were incubated for 5 days, after which time, 2 μL of 1 mM [^{14}C]metolachlor (carbonyl labeled; sp act. 59.5 mCi/mmol) was placed on the coleoptile of each untreated and safener-treated grain sorghum seedling. At 0, 0.5, 1, 2, and 4 h after [^{14}C]metolachlor application, the coleoptiles were removed from the seed, rinsed in 80% methanol, and oxidized for total radiolabel uptake. The seed and root were also oxidized to analyze for basipetal translocation of the radiolabel from [^{14}C]metolachlor.

Table I. Uptake of Radioactivity by Germinating Seeds and Seedlings of Grain Sorghum, following Application of [^{14}C]Oxabetrinil and [^{14}C]CGA-133205[a]

time, h	% of appld [^{14}C]oxabetrinil	% of appld [^{14}C]CGA-133205
0.5	54.2^{D}	11.1^{E}
1	55.4^{DB}	20.0^{D}
2	66.7^{CB}	20.2^{D}
4	72.5^{BA}	27.6^{C}
24	78.0^{A}	35.9^{B}
48	81.5^{A}	41.9^{A}
72	84.1^{A}	46.2^{A}

[a] Means within a column followed by the same letter are not significantly different as determined by Fishers' protected LSD$_{(0.05)}$.

Table II. Distribution of Radioactivity in Grain Sorghum Seedlings, following Seed Application of [^{14}C]Oxabetrinil and [^{14}C]CGA-133205[a]

time, h	% of appld [^{14}C]oxabetrinil			% of appld [^{14}C]CGA-133205		
	coleoptile	root	seed	coleoptile	root	seed
48	64.3^{A}	10.9^{A}	6.3^{A}	31.3^{A}	5.6^{A}	5.0^{A}
72	68.3^{A}	12.2^{A}	3.6^{B}	34.6^{A}	5.5^{A}	6.1^{A}

[a] Means within a column followed by the same letter are not significantly different as determined by Fishers' protected LSD$_{(0.05)}$.

RESULTS AND DISCUSSION

Uptake and Translocation of Oxabetrinil and CGA-133205. Safener uptake by germinating seeds of grain sorghum increased for the 72-h duration of this experiment (Table I). Uptake of radioactivity from [^{14}C]oxabetrinil by grain sorghum seed was greater and more rapid than that of CGA-133205 (Table I). Within the first 0.5 h, over 50% of the applied radiolabel from [^{14}C]oxabetrinil had been taken up by sorghum seeds compared to only 20% of the [^{14}C]CGA-133205 (Table I). Following seed application of either ^{14}C-labeled safener, most of the radioactivity was concentrated in the coleoptile of the germinating seedlings of the grain sorghum at 48 and 72 h after application (Table II). With both safeners a small amount of the applied radioactivity was localized in the roots and seeds of the germinated seedlings of grain sorghum at 48 and 72 h (Table II). With this application method, it appears that either the radiolabel from either the intact or metabolized ^{14}C-labeled safener is taken up by the coleoptile as it emerges from the seed or the safener is translocated to the coleoptile after uptake of the safener through the seed. Treatment of sorghum seeds with either safener did not cause any adverse effects on seed germination or seedling growth.

Metabolism of Oxabetrinil and CGA-133205 in Grain Sorghum. Following the application of [^{14}C]oxabetrinil, total extractable radioactivity from coleoptiles of untreated and oxabetrinil-treated seedlings of grain sorghum increased with time for the 8-h duration of this experiment (Table III). The amount of extractable radioactivity was greater in the untreated grain sorghum at 1, 2, and 4 h, but not significantly different from that extracted from the oxabetrinil-treated grain sorghum at 8 h (Table III, B/A ratio). The water-soluble portion of the radiolabel from [^{14}C]oxabetrinil contained in the total extract also increased with time in the untreated and oxabetrinil-treated grain sorghum, indicating that oxabetrinil is metabolized into a water-soluble compound (Table III, D/C ratio).

Table III. Metabolism of [^{14}C]Oxabetrinil in Coleoptiles of 3-Day-Old Untreated and Oxabetrinil-Treated Grain Sorghum Seedlings[a]

time, h	untreated		oxabetrinil[b]		ratios	
	% of appld radioact (A)[c]	% of extrd radioact (C)[d]	% of appld radioact (B)	% of extrd radioact (D)	B/A	D/C
1	22.8^{B}	8.8^{B}	14.4^{BC}	3.5^{B}	0.63	0.40
2	23.9^{B}	14.6^{B}	13.8^{BC}	15.2^{B}	0.58	1.04
4	33.0^{BA}	24.5^{B}	25.3^{B}	26.9^{B}	0.76	1.10
8	51.4^{A}	55.1^{A}	52.0^{A}	58.7^{A}	1.01	1.07

[a] Means within a column followed by the same letter are not significantly different as determined by Fishers' protected LSD$_{(0.05)}$. [b] Sorghum seed was treated with nonradioactive oxabetrinil at 1.25 g of ai/kg of seeds. [c] Total extractable radioactivity following application of [^{14}C]oxabetrinil. [d] Water-soluble portion of extractable radioactivity.

Table IV. TLC Analysis of Water-Soluble Metabolites Extracted from 3-Day-Old Sorghum Coleoptiles 4 h after Application of [^{14}C]Oxabetrinil

metabolite	R_f	% of recovd radioact[c]	
		untreated[a]	oxabetrinil[b]
unknown 1	0.33	2.4 ± 0.8	25.6 ± 4.5
GS–oxabetrinil	0.53	11.8 ± 2.9	ND[d]
unknown 2	0.69	ND	24.2 ± 2.9
oxabetrinil	0.88	81.0 ± 3.3	52.6 ± 4.7

[a] Coleoptiles were derived from untreated grain sorghum seed. [b] Sorghum seed was treated with nonradioactive oxabetrinil at 1.25 g of ai/kg of seeds. [c] Mean ± SE. [d] ND, not detected.

TLC analysis of the total extract for oxabetrinil metabolites revealed that the majority of the extracted radioactivity originating from [^{14}C]oxabetrinil was still in the parent form 4 h after application to the untreated grain sorghum and the oxabetrinil-treated grain sorghum (Table IV). Analysis of the water-soluble extract of coleoptiles derived from untreated grain sorghum showed that 11.8% of the extracted radioactivity chromatographed as the GS–oxabetrinil conjugate. However, no GS–oxabetrinil conjugate was detected in extracts from coleoptiles derived from oxabetrinil-treated grain sorghum (Table IV), even though 58.7% of the total extract was a water-soluble metabolite (Table III). An unidentified metabolite (R_f = 0.33) was detected in extracts from both untreated and oxabetrinil-treated grain sorghum, and the amount of this metabolite was 10 times higher in the extract from the oxabetrinil-treated than from the untreated grain sorghum (Table IV). These results indicate that pretreatment with the safener oxabetrinil influences the pattern of its metabolism in sorghum tissues.

Like oxabetrinil, total extractable radioactivity following application of [^{14}C]CGA-133205 increased with time for the duration of the experiment with no differences between untreated and CGA-133205-treated grain sorghum at 1, 2, and 4 h after application (Table V, B/A ratio). At 8 h after [^{14}C]CGA-133205 application, the amount of total extractable radioactivity originating from [^{14}C]CGA-133205 was greater from the CGA-133205-treated grain sorghum than from the untreated grain sorghum (Table V, B/A ratio). The amount of the radiolabel from [^{14}C]CGA 133205 in the water-soluble portion of the total extract does not appear to be different between treatments (Table V, D/C ratio).

TLC analysis of water-soluble extracts from sorghum tissues treated with [^{14}C]CGA-133205 revealed that the majority of the extracted radioactivity from coleoptiles derived from untreated and CGA-133205-treated grain sorghum chromatographed as the parent compound (R_f = 0.93; Table VI). One other major metabolite (R_f = 0.68) was detected in the extract from untreated and CGA-133205-treated grain sorghum (Table VI). These results indicate that pretreatment of grain sorghum with CGA-133205 increased the rate of CGA-133205 metabolism. The resulting metabolite is currently unidentified.

Table V. Metabolism of [^{14}C]CGA-133205 in Coleoptiles of 3-Day-Old Untreated and CGA-133205-Treated Grain Sorghum Seedlings[a]

time, h	untreated		CGA-133205[b]		ratios	
	% of appld radioact (A)[c]	% of extrd radioact (C)[d]	% of appld radioact (B)	% of extrd radioact (D)	B/A	D/C
1	14.4^{BA}	20.8^{B}	10.6^{B}	25.5^{B}	0.74	1.23
2	11.7^{A}	17.9^{B}	13.3^{B}	23.0^{B}	1.14	1.28
4	19.1^{BA}	16.2^{B}	18.5^{B}	23.8^{B}	0.97	1.47
8	22.3^{A}	41.7^{A}	39.5^{A}	50.1^{A}	1.77	1.20

[a] Means with a column followed by the same letter are not significantly different as determined by Fishers' protected LSD$_{(0.05)}$. [b] Sorghum seed was treated with nonradioactive CGA-133205 at 0.4 g of ai/kg of seeds. [c] Total extractable radioactivity following application of [^{14}C]CGA-133205. [d] Water-soluble portion of extractable radioactivity.

Table VI. TLC Analysis of Water-Soluble Metabolites Extracted from 3-Day-Old Sorghum Coleoptiles Grown from Untreated or CGA-133205-Treated Grain Sorghum Seeds 4 h after Application of [^{14}C]CGA-133205

metabolite	R_f	% of recovd radioact[b]	
		untreated	CGA-133205[a]
unknown 1	0.68	27.0 ± 2.8	54.5 ± 9.2
CGA-133205	0.93	63.9 ± 2.3	44.3 ± 11.2

[a] Sorghum seed was treated with nonradioactive CGA-133205 at 0.4 g of ai/kg of seeds. [b] Mean ± SE.

Uptake and Translocation of Metolachlor. Uptake of radiolabel following [^{14}C]metolachlor application by grain sorghum coleoptiles significantly increased over time in both untreated and safener-treated seedlings (Table VII). At 4 h after [^{14}C]metolachlor application, the degree of metolachlor uptake by coleoptiles of oxabetrinil-treated grain sorghum seedlings was greater than that taken up by untreated or CGA-133205-treated grain sorghum coleoptiles (Table VII). An increase in metolachlor uptake resulting from pretreatment with oxabetrinil has been demonstrated by Zama and Hatzios (1986, 1987) using either isolated leaf protoplasts or hydroponically grown seedlings of grain sorghum. Fuerst and Gronwald (1986) have also reported an enhancement of metolachlor uptake by excised leaves of grain sorghum following treatment with oxabetrinil.

At 2 and 4 h after metolachlor application, the amount of radioactivity translocated to the roots and seeds of grain sorghum seedlings was enhanced by the safener CGA-133205 (Table VII). Oxabetrinil caused a slight, but not significant, increase in the amount of radioactivity translocated to roots and seeds of grain sorghum seedlings at 4 h after metolachlor application(Table VII).

Metolachlor Metabolism in Safened or Unsafened Sorghum. Total extractable [^{14}C]metolachlor from grain sorghum coleoptiles was decreased by treatment with the safener CGA-133205, at 1 h, and with either safener at 2 and 4 h after metolachlor application (Table VIII). At 8 h after [^{14}C]metolachlor application, greater than 94% of the applied radioactivity was recovered from the coleoptiles of untreated and safener-treated grain sorghum (Table VIII). The water-soluble portion of radioactivity originating from [^{14}C]metolachlor extracted from oxabetri-

Table VII. Metolachlor Uptake by Grain Sorghum Coleoptiles and Translocation to Roots and Seeds As Influenced by Seed-Applied Oxabetrinil and CGA-133205

	% of appld[c]									
	untreated		oxabetrinil[a]		CGA-133205[b]		ratios			
time, h	coleoptile (*A*)	root and seed (*B*)	coleoptile (*C*)	root and seed (*D*)	coleoptile (*E*)	root and seed (*F*)	*C/A*	*D/B*	*E/A*	*F/B*
0.5	14.8^{BC}	1.9^{A}	14.4^{C}	3.0^{A}	13.6^{CB}	3.1^{BC}	0.97	1.58	0.92	1.63
1	29.2^{BA}	2.1^{A}	21.2^{BC}	2.2^{A}	24.2^{BC}	3.2^{BC}	0.72	1.05	0.83	1.52
2	27.8^{BA}	3.0^{A}	21.2^{B}	3.1^{A}	25.5^{BC}	6.2^{BA}	0.76	1.03	0.92	2.07
4	34.0^{A}	3.0^{A}	58.2^{A}	4.1^{A}	38.5^{AB}	8.5^{A}	1.71	1.37	1.12	2.83

[a] Sorghum seed was treated with oxabetrinil at 1.25 g of ai/kg of seeds. [b] Sorghum was treated with CGA-133205 at 0.4 g of ai/kg of seeds. [c] Means within a column followed by the same letter are not significantly different as determined by Fishers' protected $LSD_{(0.05)}$.

Table VIII. Metabolism of [^{14}C]Metolachlor in Coleoptiles of 3-Day-Old Untreated, Oxabetrinil-Treated, and CGA-133205-Treated Grain Sorghum Seedlings[a]

	untreated		oxabetrinil[b]		CGA-133205[c]					
							ratios			
time, h	% appld radioact (*A*)[d]	% extrd radioact (*B*)[e]	% appld radioact (*C*)	% extrd radioact (*D*)	% appld radioact (*E*)	% extrd radioact (*F*)	*C/A*	*D/B*	*E/A*	*F/B*
1	18.0^{B}	7.2^{B}	19.3^{B}	12.4^{C}	11.8^{B}	29.4^{C}	1.07	1.72	0.66	4.08
2	30.8^{B}	14.9^{B}	20.5^{B}	41.5^{BA}	14.8^{B}	66.4^{BA}	0.67	2.79	0.48	4.46
4	41.8^{B}	15.1^{B}	24.4^{B}	36.5^{B}	20.9^{B}	41.6^{B}	0.58	2.41	0.50	2.75
8	107.3^{A}	71.2^{A}	94.1^{A}	64.6^{A}	94.7^{A}	77.4^{A}	0.88	0.90	0.88	1.09

[a] Means within a column followed by the same letter are not significantly different as determined by Fishers' protected $LSD_{(0.05)}$. [b] Sorghum seed was treated with oxabetrinil at 1.25 g of ai/kg of seeds. [c] Sorghum seed was treated with CGA-133205 at 0.4 g of ai/kg of seeds. [d] Total extractable radioactivity following application of [^{14}C]metolachlor. [e] Water-soluble portion of extractable radioactivity.

nil- and CGA-133205-treated plant material was higher than that extracted from untreated grain sorghum at 1, 2, and 4 h after [^{14}C]metolachlor application (Table VIII). At 8 h after [^{14}C]metolachlor application there were no significant differences in the amount of the water-soluble portion of the total radiolabel from [^{14}C]metolachlor extracted from untreated or safener-treated coleoptiles of grain sorghum (Table VIII). The water-soluble portion of the total extractable radioactivity contains the metolachlor–glutathione conjugate and possibly other polar metabolites that may have been formed; thus, it appears that the safeners are increasing the rate of metolachlor metabolism for the first 4 h after metolachlor application.

The first step in the metabolism of chloroacetamide herbicides such as metolachlor in tolerant or moderately tolerant plants is conjugation with reduced glutathione (GSH) (LeBaron et al., 1988; Gronwald, 1989; Breaux, 1986). In some legumes such as soybeans, conjugation of metolachlor with homoglutathione (hGSH) is known to occur (Gronwald, 1989). The conjugation of metolachlor with GSH is mainly an enzymatic reaction catalyzed by specific glutathione *S*-transferase isozymes (GSTs). After formation of the GS–metolachlor conjugate, peptidases remove the glycine and glutamic acid residues from the glutathione molecule forming the cysteine conjugate of metolachlor. The cysteine conjugate is further catabolized by deamination to thiolactic acid conjugates or by N-acylation with malonic acid (Gronwald, 1989). In tolerant plants such as corn, thiolactic acid conjugates are considered terminal metabolites of metolachlor (Gronwald, 1989). The conjugation of metolachlor with GSH can also proceed nonenzymatically, but at a much lower rate than the enzymatic reaction (Gronwald et al., 1987).

TLC analysis of the water-soluble extract revealed that 46–49% of the radioactivity from [^{14}C]metolachlor in the total extract from the coleoptiles of oxabetrinil- and CGA-133205-treated material chromatographed as the glutathione conjugate (R_f = 0.5), while only 22.5% of extractable radioactivity from the untreated grain sorghum chromatographed as the glutathione conjugate (Table IX). The amount of total extractable radioactivity that chromatographed as the parent metolachlor (R_f = 0.89) decreased in the extracts from oxabetrinil- and CGA-133205-treated grain sorghum when compared to the amount extracted from the untreated grain sorghum (Table IX). Two additional unidentified metabolites of [^{14}C]metolachlor were present in the extracts from the untreated and oxabetrinil-treated grain sorghum, but not in the CGA-133205-treated grain sorghum (Table IX).

Table IX. TLC Analysis of Water-Soluble Extracts of 3-Day-Old Sorghum Coleoptiles 4 h after Application of [^{14}C]Metolachlor

		% of recovd radioact[d]		
metabolite	R_f	untreated[a]	oxabetrinil[b]	CGA-133205[c]
GS–metolachlor	0.5	22.5 ± 9.9	45.9 ± 7.4	49.0 ± 11.9
unknown 1	0.56	ND[e]	14.5 ± 7.5	ND
unknown 2	0.75	21.6 ± 8.1	ND	ND
metolachlor	0.89	54.1 ± 9.3	40.4 ± 10.1	42.5 ± 10.1

[a] Coleoptiles were derived from untreated seed. [b] Coleoptiles derived from sorghum seed were treated with oxabetrinil at 1.25 g of ai/kg of seeds. [c] Coleoptiles derived from sorghum seed were treated with CGA-133205 at 0.4 g of ai/kg of seeds. [d] Mean ± SE. [e] ND, not detected.

These results indicate that pretreatment of grain sorghum with either safener increases the amount of metolachlor that is metabolized by conjugation to glutathione. Gronwald and Fuerst (1987) recently reported that a seed treatment of grain sorghum with oxabetrinil or flurazole increased the rate of formation of the metolachlor–glutathione conjugate and decreased the amount of unmetabolized parent metolachlor during the first 100 min after application of [^{14}C]metolachlor to etiolated grain sorghum shoots. Breaux (1986) reported that treatment with the safener flurazole increased the rate of acetochlor detoxication in corn and sorghum. Further analysis of the acetochlor detoxication revealed that flurazole increased the rate of acetochlor conjugation with glutathione and cysteine (Breaux et al., 1989). At present it is unclear as to whether the cysteine conjugate of acetochlor results from the catabolism of the glutathione–acetochlor conjugate or whether acetochlor conjugates directly with cysteine (Breaux et al., 1989).

In summary, uptake of both safeners and metolachlor by grain sorghum increases in a time-dependent manner. Uptake of oxabetrinil is almost 2 times greater than CGA-

133205 uptake, and little redistribution of either safener occurs. Treatment of grain sorghum with oxabetrinil increased metolachlor uptake, but treatment with CGA-133205 did not. Treatment of grain sorghum with oxabetrinil and CGA-133205 increased the metabolism of metolachlor to a water-soluble metabolite by 26.6 and 51.5%, respectively, compared to untreated grain sorghum at 2 h after metolachor application. However, by 8 h after application, no difference in metolachlor conjugation to GSH between safener-treated and untreated grain sorghum was evident.

Metabolism of the safeners was also time dependent. The rate of oxabetrinil metabolism was greater than the rate of CGA-133205 metabolism in coleoptiles of grain sorghum seedlings. TLC analysis of the oxabetrinil and CGA-133205 metabolites in the total extract indicated that metabolism of these safeners in grain sorghum does occur, but currently most of the safener metabolites are unidentified. It has been speculated that oxabetrinil and CGA-133205 are reactive enough to conjugate with glutathione (Breaux et al., 1989). The results of the present study show that this may be true for oxabetrinil, which appeared to conjugate with GSH in coleoptiles of grain sorghum seedlings. Nevertheless, further research with additional analytical techniques (e.g., mass spectrometry) is needed to conclusively identify the GS conjugates of oxime ether safeners or any other metabolites of these safeners.

Oxabetrinil and CGA-133205 confer protection to grain sorghum by increasing the rate of metolachlor metabolism by conjugation to glutathione. It is possible that the safeners and metolachlor are metabolized by the same mechanism and that pretreatment of grain sorghum with these safeners increases the activity of the associated metabolic pathway.

ABBREVIATIONS USED

Acetochlor, 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide; alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide; CGA-133205, 2,2,2-trifluoro-4′-chloroacetophenone *O*-(1,3-dioxolan-2-ylmethyl)oxime; cyometrinil, (Z)-α-[(cyanomethoxy)imino]benzeneacetonitrile; flurazole, phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylate; GSH, reduced glutathione; hGSH, homoglutathione; GST, glutathione *S*-transferase; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; oxabetrinil, α-[(1,3-dioxolan-2-ylmethoxy)imino]benzeneacetonitrile.

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