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Comparative Effects of CGA-92194, Cyometrinil, and Flurazole on Selected Metabolic Processes of Isolated Soybean Leaf Cells*

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Abstract. The potential adverse phytotoxic effects of the herbicide safeners CGA-92194 {α-[1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile}, cyometrinil [α-(cyanomethoxy)imino-benzeneacetonitrile] and flurazole [phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazole-carboxylate] on selected metabolic processes of enzymatically isolated leaf cells of soybean [*Glycine max* (L.) Merr.] were compared in time- and concentration-course studies. CO_2 fixation, protein synthesis, RNA synthesis, DNA synthesis, and lipid synthesis were assayed by the incorporation of $NaH^{14}CO_3$, [^{14}C]-leucine, [^{14}C]-uracil, [^{3}H]thymidine, and [^{14}C]-acetate, respectively, into the isolated cells. CGA-92194 and cyometrinil behaved similarly, and at low concentrations (0.1, 1, and 10 μM) they stimulated rather than inhibited the five metabolic processes assayed, following incubation periods of up to 2 h. At the highest concentration of 100 μM, both safeners inhibited all metabolic processes of the soybean leaf cells but neither compound exhibited rapid and distinct inhibitions as might be expected in the case of inhibition of a primary target site by a potent inhibitor. At low concentrations and early incubation periods (30 and 60 min), flurazole effects on all metabolic processes were also stimulatory rather than inhibitory. However, the stimulation of CO_2 fixation by 0.1 and 1.0 μM was highly significant. At 100 μM flurazole was extremely potent on all metabolic processes of soybean leaf cells examined. At the 2-h incubation period, flurazole also inhibited all metabolic processes at concentrations lower than 100 μM. The sensitivity of the five metabolic processes to flurazole decreased in the following order: photosynthesis = lipid synthesis > DNA synthesis > protein synthesis > RNA synthesis.

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The recent development and commercialization of chemicals conferring crop tolerance to nonselective herbicides is undoubtedly one of the major advances in the areas of plant growth regulation and herbicide technology. Five chemicals are presently marketed as herbicide safeners (also referred to as antidotes or protectants) and they have been the subject of several reviews (Blair et al. 1976, Gressel et al. 1982, Hatzios 1983, Hoffman 1978, Pallos and Casida 1978, and Parker 1983). Cyometrinil[1], CGA-92194, and flurazole[1], applied as seed treatments, protect grain sorghum from injury caused by chloroacetanilide herbicides such as alachlor and metolachlor (Davidson et al. 1978, Dill et al. 1982, Ellis et al. 1980, Nyffeler et al. 1980, Sacher et al. 1983, Schafer et al. 1980).

Low potential for phytotoxicity at recommended rates and reliability under field conditions are two important considerations for the selection and development of candidate chemicals as herbicide safeners. However, since all of the currently marketed herbicide safeners contain in their molecule functional chemical groups similar to those found in many herbicides, they have the potential to cause adverse phytotoxic effects under certain circumstances. Early or recent reports have documented the potential of the safener NA (naphthalene-1,8-dicarboxylic anhydride) to interfere with the growth of corn, grain sorghum, oats, rice, and beans even at the recommended rate of 0.5% by seed weight (Blair 1979, Eastin 1972, Guneyli 1971, Hatzios 1984a/1984b, Thiessen et al. 1980). Recently, Hatzios and Zama (1986) showed that changes in the chemical structure of NA could reduce the phytotoxic potential of this safener without any significant loss of its antidotal activity against thiocarbamate herbicide injury to corn. Adverse effects caused by the safener dichlormid (*N,N*-diallyl-2,2-dichloroacetamide) on the growth of corn or beans have been reported (Blair 1979, Ezra et al. 1982). Reduction of the viability of sweet and yellow-endosperm sorghum seeds treated with the safeners cyometrinil and CGA-92194 has also been reported (Davidson et al. 1978, Dill et al. 1982). In addition, cyometrinil used at rates higher than 1.88 g/kg of seed caused significant phytotoxic effects on grain sorghum (Davidson et al. 1978). Seed viability and growth of grain sorghum did not appear to be adversely affected by the safener flurazole (Schafer et al. 1980). However, measurable effects of flurazole as well as CGA-92194 on the growth and respiration of grain sorghum seeds have been observed by Ketchersid and Merkle (1983) during the early stages of germination of sorghum seeds. Under similar conditions, cyometrinil was more inhibitory than either CGA-92194 or flurazole (Ketchersid and Merkle 1983).

Studies on the potential phytotoxicity of cyometrinil, CGA-92194, and flurazole on certain aspects of plant metabolism at the cellular level are not currently available. Enzymatically isolated or cultured cells of selected plant species offer an attractive and reliable system for studying selected metabolic processes (Servaites and Ogren 1980) and screening the activity of natural or synthetic substances with phytotoxic potential (Ashton et al. 1977, Gressel 1984, Hatzios and Howe 1982).

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[1] Cyometrinil and flurazole are the same compounds as CGA-43089 and MON-4606, respectively, in other publications.

The studies in this report were conducted to determine and compare the potential adverse effects of the safeners cyometrinil, CGA-92194, and flurazole on CO_2 fixation, and precursor incorporation into proteins, RNA, DNA, and lipids in enzymatically isolated leaf cells of soybean in time- and concentration-course experiments. Soybeans were used in these studies because they are a very good source of enzymatically isolated, active mesophyll cells that have been useful for screening herbicides or other growth regulators. In addition, since soybeans may follow corn or grain sorghum in crop rotation systems, they could be exposed to soil residues of these safeners under field conditions.

Materials and Methods

Plant Material

Soybean [*Glycine max* (L.) Merr, cv. Essex] seeds were planted in plastic cups (473 ml) filled with a mixture of potting medium (Weblite Corporation, Blue Ridge, Virginia), vermiculite, and sphagnum peat moss in a 2:2:1 ratio. Limestone and a controlled release fertilizer (14-14-14) were added to the soil mixture to provide additional nutrients. After germination, the plants were transferred to a growth chamber with photon flux density of 50–60 W m^{-2} provided by a blend of incandescent and fluorescent lamps. A photoperiod cycle of 16 h of light at 30°C and 8 h of dark at 20°C was maintained during the first 2 weeks of soybean emergence and growth. Then, the plants were acclimated for 5 days to a 6-h photoperiod to reduce the amount of starch in the chloroplasts. A low starch content has been reported to increase the photosynthesis rates of soybean leaf cells isolated from plants exposed to a short day treatment (Servaites and Ogren 1980).

Cell Isolation

Mature trifoliate leaves were detached from plants at least 1 h after the initiation of the light period in the growth chamber, rinsed with distilled water, had their midribs removed, and were cut into small 1 mm × 1 cm strips with a sharp razor blade. Two to three grams of cut leaf tissue served as a source for the enzymatic isolation of mesophyll cells through a 3-step procedure. The procedure included infiltration of the leaf tissue with the maceroenzyme (macerase®, Calbiochem-Behring Corp., La Jolla, California) under vacuum, maceration of the infiltrated tissue through slow magnetic stirring, and repeated washings of the released cells through centrifugation. Detailed descriptions of these procedures have been given by Ashton et al. (1977), Hatzios and Howe (1982), and Servaites and Ogren (1980). The released cells were made up to the desired volume with an incubation medium containing 0.2 M sorbitol, 2 mM $Mg(NO_3)_2$, and 1 mM $CaCl_2$. The pH of the medium was maintained at 7.8 with HEPES buffer for the CO_2 fixation assays, or at 5.8 with MES buffer for the incorporation of the respective precursors into proteins, RNA, DNA, and lipids. Chlorophyll content was determined by the method of Arnon (1949).

The chlorophyll content of cell suspension used in this study ranged from 15 to 25 μg of chlorophyll per ml of the assay medium.

Time-Course and Concentration Studies with the Three Safeners

Analytical grade samples (>95% pure) of the safeners CGA-92194 and cyometrinil and of flurazole were kindly provided by CIBA-GEIGY Corp. Greensboro, North Carolina and Monsanto Chemical Co., St. Louis, Missouri, respectively. The three safeners were dissolved in methanol and made up to the desired volume with distilled water so that the final methanol concentration was less than 1%. Methanol was also used in the preparation of the control (safener-free) solutions. The assay methods for the five metabolic processes studied are available in the literature (Ashton et al. 1977, Hatzios and Howe 1982, Servaites and Ogren 1980). The assaying medium for all metabolic studies contained 2 ml of the cell preparation in a 25-ml Erlenmeyer flask, 0.1 ml of radioactive substrate containing 1 μCi of radioactivity, and 0.05 ml of the safener solution, making a final volume of 2.15 ml. The flasks with the assay mixtures were sealed and placed in a shaking water bath at 25°C and illuminated from above with a combination of incandescent and fluorescent lamps supplying 7.4 W m^{-2} photon flux density at the level of the flasks. Samples were removed at selected incubation periods and treated as previously described (Ashton et al. 1977, Hatzios and Howe 1982) prior to liquid scintillation counting. CO_2 fixation was assayed by incubating the cells with 1 μCi of $NaH^{14}CO_3$ (sp. act. 44.4 mCi/mmol) containing 5 mM of $NaH^{12}CO_3$. Incorporation into proteins was determined by measuring the incorporation of 1 μCi of L-[U-^{14}C] leucine (sp. act. 276 mCi/mmol) into protein. Incorporation into RNA was assayed by measuring the incorporation of 1 μCi of [2-^{14}C]uracil (sp. act. 55 mCi/mmol), while incorporation into DNA synthesis was assayed with [^{3}H]thymidine (sp. act. 25 Ci/mmol). Incorporation into lipids was determined by the incorporation of 1 μCi of [1,2-^{14}C] sodium acetate (sp. act. 56.2 mCi/mmol) into lipids. Radioactivity was determined by adding 10 ml of scintillation fluid (ACS, Amersham) and counting in a liquid scintillation spectrometer (Beckman LS-250) with a 92% counting efficiency for $^{14}CO_2$ and 70% efficiency for ^{3}H. CO_2 fixation was expressed as μmoles of $^{14}CO_2$ fixed per mg of chlorophyll. Incorporation of precursors into proteins, RNA, and lipids were expressed as dpm of the [^{14}C] from the respective radioactive substrate incorporated into the cells per 100 μg of chlorophyll. Incorporation into DNA was expressed as dpm of [^{3}H] incorporated into the cells per mg of chlorophyll. All of the assays were repeated 4 times, and the data were analyzed for variance in a completely randomized design. The standard errors of each mean were also calculated and used for separation of treatment means. In addition, the results were also calculated as percent inhibition caused by each concentration of the three safeners. Negative values indicate stimulation instead of inhibition.

Results

The effects of CGA-92194, cyometrinil, and flurazole on CO_2 fixation, protein, RNA, DNA, and lipid syntheses of isolated soybean leaf cells are presented in

Tables 1 through 5. Data in Table 1 show that CGA-92194 and cyometrinil are not inhibitors of CO_2 fixation of soybean leaf cells. Appreciable inhibition of this metabolic process was observed only with the two highest concentrations (10 and 100 μM) of CGA-92194 at the 60 and 120 min incubation periods. However, as revealed with the help of the standard errors of each treatment mean, these slight inhibitions of this process by CGA-92194 were significant only with the highest concentration of 100 μM. Appreciable stimulation of CO_2 fixation by the low concentrations of CGA-92194 was evident at 30 min or later incubation periods. Cyometrinil did not inhibit the fixation of $^{14}CO_2$ by soybean leaf cells at any concentration or at any incubation period examined (Table 1). On the contrary, this safener significantly stimulated this metabolic process at any incubation period examined, when used at 0.1, 1, and 10 μM concentrations. Flurazole applied at a non-physiological concentration of 100 μM caused a rapid and distinct inhibition of the CO_2 fixation of soybean leaf cells (Table 1). A slight inhibition of this process was also obtained with 10 μM of flurazole at the 120-min period. However, flurazole at low concentrations was stimulatory rather than inhibitory of this process. Stimulation percentages caused by 0.1 and 1 μM of this safener at the 30 or 60 min incubation periods were highly significant (Table 1).

A time-dependent inhibition of the incorporation of ^{14}C-leucine into protein of isolated soybean cells was caused by 100 μM of CGA-92194. The respective inhibition percentages were 23%, 33%, and 47% at 30, 60, and 120 min (Table 2). The effects of 0.1, 1, and 10 μM of CGA-92194 and of all concentrations of cyometrinil on this metabolic process were either stimulatory or slightly inhibitory at all incubation times. Comparisons of the treatments means with the help of their standard errors, indicate that most of these stimulatory or slight inhibitory effects of the two safeners on protein synthesis were not significant. Flurazole at 100 μM caused a rapid and strong inhibition of the incorporation of ^{14}C-leucine into the isolated soybean leaf cells (Table 2). Percent inhibitions at 30, 60, and 120 min were 64%, 79%, and 84% indicating a time-dependence of the inhibition caused by this concentration of flurazole. A slight but significant inhibition was also observed with the 10 μM concentration of flurazole. The two lowest concentrations of flurazole were stimulatory rather than inhibitory of this metabolic process.

RNA synthesis of isolated soybean cells was the least sensitive process to all three safeners. Significant inhibition of the incorporation of ^{14}C-uracil into soybean cells was obtained only with the two high concentrations (10 and 100 μM) of CGA-92194, cyometrinil, and flurazole at the 2 h incubation time (Table 3). At the highest concentration of 100 μM, flurazole was more inhibitory (52%) than either CGA-92194 (29%) or cyometrinil (32%) of this process. At 100 μM CGA-92194 and flurazole were inhibitory of this process also at the 60 min incubation period. At 30 min of incubation the three safeners at all concentrations were stimulatory.

In Table 4, data on the effects of the three safeners on DNA synthesis of isolated soybean leaf cells are presented only for the 120-min incubation period. This was done because the incorporation of ^{3}H-thymidine into the soybean cells was very limited at 30 or 60 min of incubation and because increases in incubation time did not correspond to linear increases in the incorporation of radioactivity into the soybean cells. Problems in studying DNA synthesis of

Table 1. The effect of CGA-92194, cyometrinil and flurazole on $^{14}CO_2$ fixation of isolated soybean cells

Incubation time (min)	Safener concentration (μM)	CGA-92194		Cyometrinil		Flurazole	
		$^{14}CO_2$ fixation[a] (μmol $^{14}CO_2$/mg chl)	Inhibition[b] (%)	$^{14}CO_2$ fixation[a] (μmol $^{14}CO_2$/mg chl)	Inhibition[b] (%)	$^{14}CO_2$ fixation[a] (μmol $^{14}CO_2$/mg chl)	Inhibition[b] (%)
30	0	12.99 ± 1.04	0	10.14 ± 0.24	0	16.04 ± 0.49	0
	0.1	16.14 ± 0.60	−24	11.43 ± 0.51	−12	29.68 ± 2.66	−85
	1	15.93 ± 0.32	−22	15.70 ± 1.73	−54	22.38 ± 2.81	−39
	10	13.20 ± 2.59	−1	14.55 ± 1.79	−43	14.95 ± 1.90	7
	100	11.45 ± 1.55	12	10.93 ± 0.68	−7	2.66 ± 0.14	84
60	0	26.42 ± 2.46	0	19.21 ± 0.75	0	28.67 ± 0.52	0
	0.1	28.98 ± 1.67	−9	21.76 ± 1.80	−13	48.77 ± 1.13	−70
	1	25.64 ± 0.57	−3	27.74 ± 0.06	−44	41.61 ± 2.13	−45
	10	22.81 ± 2.72	14	23.84 ± 2.89	−24	37.56 ± 2.91	−31
	100	19.59 ± 0.29	26	21.19 ± 1.79	−10	2.82 ± 0.44	91
120	0	115.21 ± 4.18	0	85.31 ± 4.15	0	110.70 ± 5.30	0
	0.1	114.41 ± 1.24	1	98.23 ± 3.04	−15	104.46 ± 5.36	6
	1	111.97 ± 4.67	3	126.63 ± 4.30	−48	96.19 ± 2.88	14
	10	95.44 ± 5.90	18	109.91 ± 7.47	−28	92.83 ± 5.60	17
	100	87.83 ± 4.50	24	96.89 ± 4.89	−13	3.08 ± 0.52	98

[a] Mean values from four replications ± standard errors of each mean.

[b] A minus (−) sign preceding a percentage value indicates stimulation instead of inhibition.

Table 2. The effect of CGA-92194, cyometrinil, and flurazole on protein synthesis of isolated soybean cells

Incubation time (min)	Safener concentration (μM)	CGA-92194 [^{14}C]-leucine incorporated[a] (dpm × 10^{-3}/100 μg chl)	CGA-92194 Inhibition[b] (%)	Cyometrinil [^{14}C]-leucine incorporated[a] (dpm × 10^{-3}/100 μg chl)	Cyometrinil Inhibition[b] (%)	Flurazole [^{14}C]-leucine incorporated[a] (dpm × 10^{-3}/100 μg chl)	Flurazole Inhibition[b] (%)
30	0	5.02 ± 0.95	0	4.87 ± 0.66	0	4.17 ± 0.37	0
	0.1	4.66 ± 0.55	8	5.17 ± 0.70	−6	4.72 ± 0.24	−13
	1	5.02 ± 0.59	0	5.84 ± 1.16	−19	4.81 ± 0.39	−15
	10	4.21 ± 0.94	17	5.49 ± 1.01	−12	3.56 ± 0.22	15
	100	3.90 ± 0.39	23	4.46 ± 0.71	9	1.51 ± 0.16	64
60	0	7.83 ± 1.17	0	7.93 ± 1.42	0	6.58 ± 0.33	0
	0.1	8.45 ± 0.77	−7	8.63 ± 0.83	−8	7.62 ± 1.69	−15
	1	7.15 ± 0.92	9	9.46 ± 0.95	−19	7.22 ± 1.31	−9
	10	7.63 ± 0.44	3	8.52 ± 1.19	−7	5.37 ± 0.58	19
	100	5.27 ± 0.67	33	7.73 ± 1.21	3	1.41 ± 0.07	79
120	0	13.60 ± 0.69	0	15.84 ± 0.86	0	12.12 ± 1.56	0
	0.1	14.57 ± 1.26	−7	16.96 ± 1.69	−7	15.03 ± 2.10	−24
	1	14.13 ± 2.20	−3	16.50 ± 0.69	−4	13.33 ± 1.26	−9
	10	13.29 ± 1.78	3	16.92 ± 1.20	−6	9.60 ± 1.47	21
	100	7.21 ± 1.48	47	12.87 ± 1.10	19	1.97 ± 0.16	84

[a] Mean values from four replications ± standard errors of each mean.

[b] A minus (−) sign preceding a percentage value indicates stimulation instead of inhibition.

Table 3. The effect of CGA-92194, cyometrinil, and flurazole on RNA synthesis of isolated soybean cells

Incubation time (min)	Safener concentration (μM)	CGA-92194		Cyometrinil		Flurazole	
		[^{14}C]-uracil incorporated[a] (dpm × 10^{-3}/100 μg chl)	Inhibition[b] (%)	[^{14}C]-uracil incorporated[a] (dpm × 10^{-3}/100 μg chl)	Inhibition[b] (%)	[^{14}C]-uracil incorporated[a] (dpm × 10^{-3}/100 μg chl)	Inhibition[b] (%)
30	0	0.76 ± 0.07	0	0.83 ± 0.01	0	0.83 ± 0.01	0
	0.1	0.94 ± 0.01	−23	1.00 ± 0.08	−9	0.94 ± 0.01	−13
	1	0.99 ± 0.01	−30	0.96 ± 0.10	−15	0.89 ± 0.01	−7
	10	0.77 ± 0.10	−1	0.91 ± 0.12	−9	0.80 ± 0.01	4
	100	0.81 ± 0.10	−6	0.91 ± 0.04	−9	0.77 ± 0.02	8
60	0	0.99 ± 0.07	0	0.99 ± 0.06	0	0.99 ± 0.06	0
	0.1	1.11 ± 0.11	−12	1.21 ± 0.13	−22	0.93 ± 0.03	7
	1	1.03 ± 0.02	−4	1.17 ± 0.04	−18	1.04 ± 0.03	−5
	10	0.97 ± 0.55	2	1.18 ± 0.19	−19	1.01 ± 0.06	−2
	100	0.85 ± 0.07	14	0.94 ± 0.06	6	0.71 ± 0.12	29
120	0	1.54 ± 0.61	0	1.73 ± 0.61	0	1.73 ± 0.61	0
	0.1	1.56 ± 0.57	−1	1.44 ± 0.38	17	1.39 ± 0.10	−20
	1	1.29 ± 0.20	17	1.35 ± 0.04	22	1.11 ± 0.01	36
	10	1.13 ± 0.03	27	1.26 ± 0.05	28	1.23 ± 0.01	29
	100	1.10 ± 0.06	29	1.18 ± 0.04	32	0.72 ± 0.01	59

[a] Mean values from four replications ± standard errors of each mean.

[b] A minus (−) sign preceding a percentage value indicates stimulation instead of inhibition.

Table 4. The effect of CGA-92194, cyometrinil, and flurazole on DNA synthesis of isolated soybean cells

Incubation time (min)	Safener concentration (μM)	CGA-92194		Cyometrinil		Flurazole	
		[^{3}H]-thymidine incorporated[a] (dpm × 10^{-3}/mg chl)	Inhibition[b] (%)	[^{3}H]-thymidine incorporated[a] (dpm × 10^{-3}/mg chl)	Inhibition[b] (%)	[^{3}H]-thymidine incorporated[a] (dpm × 10^{-3}/mg chl)	Inhibition[b] (%)
120	0	3.93 ± 0.47	0	3.93 ± 0.47	0	4.05 ± 0.79	0
	0.1	4.32 ± 0.38	−9	3.72 ± 0.02	6	4.96 ± 0.78	−22
	1	3.91 ± 0.55	1	3.61 ± 0.19	9	2.41 ± 0.73	41
	10	3.81 ± 0.34	4	3.34 ± 0.30	16	1.41 ± 0.14	66
	100	3.27 ± 0.30	17	3.07 ± 0.26	24	0.25 ± 0.07	94

[a] Mean values from four replications ± standard errors of each mean.
[b] A minus (−) sign preceding a percentage value indicates stimulation instead of inhibition.

Table 5. The effect of CGA-92194, cyometrinil, and flurazole on lipid synthesis of isolated soybean cells

Incubation time (min)	Safener concentration (μM)	CGA-92194 [^{14}C]-acetate incorporated[a] (dpm × 10^{-3}/100 μg chl)	CGA-92194 Inhibition[b] (%)	Cyometrinil [^{14}C]-acetate incorporated[a] (dpm × 10^{-3}/100 μg chl)	Cyometrinil Inhibition[b] (%)	Flurazole [^{14}C]-acetate incorporated[a] (dpm × 10^{-3}/100 μg chl)	Flurazole Inhibition[b] (%)
30	0	212.34 ± 4.81	0	204.82 ± 3.24	0	220.92 ± 0.08	0
	0.1	247.56 ± 0.23	−16	219.36 ± 4.55	−7	244.65 ± 4.03	−10
	1	261.10 ± 6.40	−23	241.14 ± 4.76	−17	265.37 ± 4.85	−20
	10	270.16 ± 7.69	−27	221.20 ± 5.55	−7	197.85 ± 4.11	11
	100	182.89 ± 0.04	14	179.80 ± 5.26	13	27.21 ± 1.65	88
60	0	278.62 ± 7.40	0	313.28 ± 7.25	0	317.96 ± 7.95	0
	0.1	363.99 ± 7.90	−30	288.56 ± 7.82	8	369.95 ± 8.40	−16
	1	391.20 ± 8.90	−40	370.16 ± 6.95	−18	391.52 ± 9.30	−23
	10	418.91 ± 10.50	−50	434.96 ± 3.80	−38	301.72 ± 6.85	6
	100	293.11 ± 9.20	−5	287.94 ± 6.75	9	31.01 ± 4.40	91
120	0	509.77 ± 2.34	0	494.57 ± 10.43	0	638.70 ± 13.55	0
	0.1	717.48 ± 10.45	−40	507.51 ± 6.10	−2	731.34 ± 16.68	−14
	1	687.42 ± 4.58	−34	666.83 ± 13.29	−34	717.80 ± 8.83	−12
	10	663.08 ± 13.56	−30	579.64 ± 12.10	−17	495.58 ± 9.31	23
	100	517.77 ± 9.38	−1	441.38 ± 5.57	11	37.43 ± 5.61	95

[a] Mean values from four replications ± standard errors of each mean.

[b] A minus (−) sign preceding a percentage value indicates stimulation instead of inhibition.

plant cell cultures by measuring the incorporation of ^{3}H-thymidine have been reported by other investigators (Gressel 1984, Zilberstein et al. 1973). The existence of high levels of thymidine phosphorylase in most plants has been offered as a possible explanation for these problems (Zilberstein et al. 1973). Under the conditions of measuring DNA synthesis in this study, flurazole appeared to be a more potent inhibitor of this process than either CGA-92194 or cyometrinil (Table 4). Percent inhibitions recorded for the 1, 10, and 100 μM concentrations of this safener were 41%, 66%, and 94%, respectively. Appreciable inhibition of DNA synthesis of soybean leaf cells by CGA-92194 or cyometrinil was observed only with the highest concentration (100 μM) of these safeners.

CGA-92194 and cyometrinil did not adversely interfere with the incorporation of ^{14}C-acetate into isolated soybean leaf cells at any concentration or any incubation period (Table 5). Significant stimulations of this process were observed following treatments of soybean cells with the lower concentrations of these safeners. A slight inhibition of lipid synthesis was observed only with the highest concentration of cyometrinil. A rapid and strong inhibition of the incorporation of ^{14}C-acetate into soybean cells was caused by 100 μM of flurazole. Percent inhibitions recorded at 30, 60, and 120 min were 88%, 91%, and 95%, respectively, indicating little time-dependence of the inhibition of lipid synthesis by 100 μM of flurazole. The effects of the lower concentrations of flurazole on this process were either stimulatory or slightly inhibitory (Table 5).

Discussion

The results of the present study revealed that with regard to their effects on soybean cell metabolism, the safeners CGA-92194 and cyometrinil behaved similarly. At low concentrations (0.1, 1, and 10 μM) these two safeners were stimulatory rather than inhibitory of the five metabolic processes assayed following incubation periods of up to 2 h. At the highest concentration of 100 μM, both safeners were inhibitory of all metabolic processes of the soybean leaf cells but neither compound exhibited a rapid and distinct inhibition of any process as might be expected in the case of a primary target site by a potent inhibitor (Ashton et al. 1977, Gressel 1984). These results are in concert with those reported by Zama and Hatzios (1985) on the effects of the safener CGA-92194 on the metabolism of isolated mesophyll protoplasts of grain sorghum. It is evident, therefore, that the potential phytotoxicity of these two safeners at the cellular level, even at the highest nonphysiological concentration of 100 μM, is very limited. However, the potential activity of these safeners on cellular processes other than those examined in the present study or on plant metabolism at organ or whole plant levels cannot be excluded. Ketchersid and Merkle (1983) demonstrated that CGA-92194 and cyometrinil could interfere with plant metabolic processes such as respiration during the early stages of seed germination of grain sorghum. A cyometrinil-induced reduction of grain sorghum seed germination has been reported also by Warmund et al. (1981).

The stimulatory effects caused by the low concentrations of CGA-92194 and cyometrinil on all metabolic processes of soybean cells are not unusual. Sub-

lethal concentrations of many herbicides or other plant growth regulations have been reported to cause stimulation of selected processes of plant metabolism (Ashton et al. 1977) and the subject has been reviewed in detail by Ries (1976).

The comparable activity of these two safeners on the metabolism of isolated soybean leaf cells was not unexpected. CGA-92194 is a chemical analog of cyometrinil and both of these oxime safeners would be expected to elecit the same biological responses on plants (Chang and Merkle 1983, Davidson et al. 1978, Dill et al. 1982).

Significant stimulations of the five metabolic processes of soybean cells by the low concentrations of the safener flurazole were also evident from data generated in this study. Stimulation of protein synthesis by the safeners cyometrinil and flurazole has been reported in a recent study by Mozer et al. (1983). They showed that both safeners enhanced the synthesis and activity of a glutathione *S*-transferase enzyme that is constitutively present in corn and conjugates chloroacetanilide herbicides with glutathione. In addition, the induction of the synthesis of a *de novo* glutathione *S*-transferase that conjugates chloroacetanilide herbicides with glutathione was observed as a result of seed pretreatment of corn with these safeners. However, at higher concentrations, and particularly at 100 μM, flurazole was extremely inhibitory of all metabolic processes of soybean cells examined. The sensitivity of the five metabolic processes to 100 μM of flurazole following 2 h of incubation period decreased in the following order: photosynthesis = lipid synthesis > DNA synthesis > protein synthesis > RNA synthesis. Interference of flurazole with the incorporation of ^{14}C-acetate into triglyceride lipids of grain sorghum seeds has been reported recently by Warmund et al. (1985). A slight effect of flurazole on respiration of germinating grain sorghum seeds has been also reported (Kerchersid and Merkle 1983, Warmund et al. 1985). In addition, the shikimic and phenylpropanoid acid pathways have been proposed as sites for the potential interference of flurazole with plant metabolism. Support for such a postulation comes from reports showing inhibition of anthocyanin and lignin biosynthesis in grain sorghum by 10 nM of flurazole (Dr. W. T. Molin, Monsanto Chemical Company, St. Louis, Missouri, personal communication).

Although at equimolar (100 μM) concentrations, flurazole was found to be a more potent inhibitor of all metabolic processes of soybean leaf cells examined in this study, the absence of rapid and marked inhibitions of any process, observed with concentrations of the three safeners lower than 100 μM suggests that use of CGA-92194, cyometrinil, and flurazole as seed protectants of grain sorghum against chloroacetanilide herbicides is not likely to cause any significant adverse effects on plant metabolism under field conditions.

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