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Absorption of Herbicides by Wheat as Influenced by the Phenoxy Compound

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Herbicide mixtures of the sodium salt of trichloroacetic acid (Cl_3CCOOH) and the amine salt of a phenoxy herbicide as post-emergence treatment in the greenhouse study improved wheat tolerance to Cl_3CCOOH. The physiological basis for tolerance, as measured by absorption, accumulation, and distribution of Cl_3CCOOH-^{14}C in intact wheat seedlings and excised root segments, was investigated. In a series of experiments wheat seedlings absorbed reduced amounts of ^{14}C-labeled Cl_3CCOOH and other herbicides from solutions containing one of five phenoxy compounds (10^{-4} to 10^{-6} M). Results also suggested that Cl_3CCOOH absorption by wheat roots behaved like an active process requiring a metabolic energy supply and that 2,4-dichlorophenoxyacetic acid (2,4-D) inhibited the stimulatory effect of adenosine 5′-triphosphate (ATP) on Cl_3CCOOH absorption and acted as a noncompetitive inhibitor for root entrance and movement of Cl_3CCOOH to the shoots of seedlings. Based on this investigation, improvement of wheat tolerance to Cl_3CCOOH with Cl_3CCOOH–phenoxy mixtures was mainly due to physiological restraint and noncompetitive inhibition of absorption and subsequent translocation of Cl_3CCOOH in the wheat plant by the phenoxy herbicide.

Herbicide mixtures are becoming more popular today allowing a reduction in the rate of a single herbicide, resulting in less environmental problems (Caseley, 1971), broadening the spectrum of weed control, and reducing the cost of some newly developed herbicides by partial substitute of a lower cost herbicide. The early developed herbicides, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) and [(4-chloro-*o*-tolyl)oxy]acetic acid (MCPA), have such characteristics as good broadleaf weed control and low cost. Thus, these two phenoxy herbicides in single application or in combination with other herbicides have been widely used in small grain production and ranked the highest in usage. In 1972 11 million pounds were applied on about 28 million acres in the Canadian Prairie provinces.

Through field and greenhouse experiments we observed (Chow and Dryden, 1973) that under some conditions Cl_3CCOOH in the phenoxy herbicide mixtures exhibited better weed control and higher crop tolerance as well. The studies described herein report that wheat treated with a phenoxy compound mixture accumulated less herbicide as compared with a herbicide applied alone at equal rates. The results offer a possible explanation why wheat tolerance was improved by applying a phenoxy herbicide mixture.

MATERIALS AND METHODS

Chemicals and Radioassay Instrument. In the greenhouse pot study, commercial herbicide products (73% sodium salt of Cl_3CCOOH, 50% amine salt of 2,4-D, and 50% amine salt of MCPA) were investigated. For other experiments, nonlabeled and ^{14}C-labeled herbicides and chemicals used are listed in Table I, along with common and chemical names, specific activities, purities, and sources.

Labeled compounds were dissolved in 50% ethanol as stock solutions. A certain amount of these stock solutions, alone or in combination with a nonlabeled compound, was diluted with distilled water to a desired concentration to form the treatment solution. The pH of the treatment solutions was adjusted prior to application.

Radioactivity in extracts was measured at ambient temperature by using a Liquimat 220 (Picker Nuclear) liquid scintillation spectrometer equipped with a ^{137}Cs external standard and photomultiplier tubes of a bialkali photocathode type. For toluene-^{14}C the instrument had a counting efficiency of 90% accompanied by about 20 cpm of background counting rate.

Greenhouse Pot Study; Wheat Tolerance to Herbicide Mixture. In the greenhouse pot tests with wheat (*Triticum aestivum* (L.) var. "Manitou"), 12 seeds were sown in pots containing 1500 g of clay loam soil (26.7% clay, 33.0% silt, 40.3% sand, 5.6% organic matter, and pH 7.5). Fluorescent light at about 16,500 lx for a 16-hr photoperiod was provided. After germination, seedlings were thinned to six plants per pot for treatment. The treatment solutions were sprayed on the foliage of seedlings as well as the soil surface at the rate of 262 l./ha of water at a pressure of 2.1 kg/cm^2 (30 psi) at the three-leaf stage of wheat. All rates of commercial products of herbicides were applied in terms of active ingredients in kilograms per hectare. Water was added to the surface of the soil in pots as required by the growth of seedlings. All treatments were arranged in a randomized block design with four replicates and pots on the bench were rearranged once a week in order to receive uniform light exposure and air circulation. The test was terminated at the end of 4 weeks with a visual rating of wheat tolerance to Cl_3CCOOH and the fresh weight yield of shoots.

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Table I. Labeled and Nonlabeled Herbicides and Chemicals Used

Common name	Chemical name	Sp act., mCi/ mmol	Purity, %	Supplier
Labeled herbicide				
Amitrole-*5*-^{14}C	3-Amino-*s*-triazole	4.95	98	New England Nuclear Corp.
Chloramben-*carboxyl*-^{14}C	3-Amino-2,5-dichlorobenzoic acid	2.19		Amchem Products Inc.
Dicamba-*carboxyl*-^{14}C	3,6-Dichloro-*o*-anisic acid	1.89	99.9	Velsico Chemical Corp.
Cl_3CCOOH-*1*-^{14}C	Trichloroacetic acid	6.4	98	Amersham/Searle Co.
Cl_3CCOOH-*1*-^{14}C	Trichloroacetic acid	4.0	98	New England Nuclear Corp.
Nonlabeled herbicide				
2,4-D sodium salt	Sodium (2,4-dichlorophenoxy)acetate		99	Fisher Scientific Co.
MCPA	[(4-Chloro-*o*-tolyl)oxy]acetic acid		99.5	Dow Chemical Co.
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid		98	Dow Chemical Co.
Silvex	2-(2,4,5-Trichlorophenoxy)propionic acid		98	Dow Chemical Co.
Chemical				
ATP disodium salt	Disodium adenosine 5′-triphosphate		99-100	Sigma Chemical Co.
DNP	2,4-Dinitrophenol		90-95	Matheson Coleman and Bell Co.

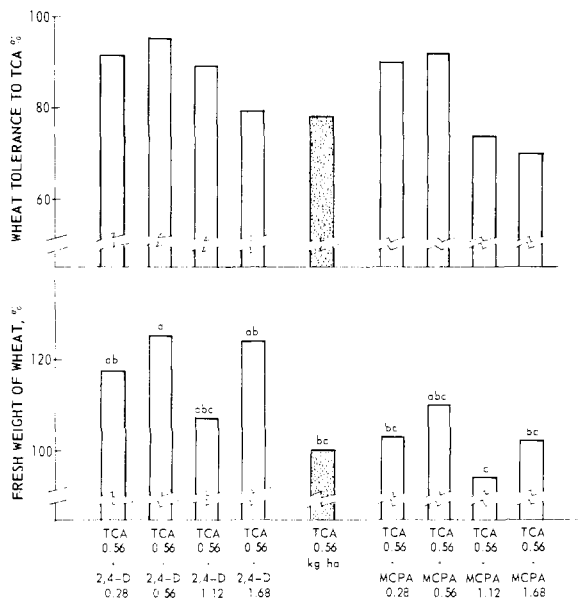

Figure 1. Effects of 2,4-D and MCPA in Cl_3CCOOH (throughout the figures TCA is used for trichloroacetic acid) mixtures on fresh weight yields and tolerance of "Manitou" wheat seedlings to Cl_3CCOOH. Fresh weight and tolerance (visual rating) were made 4 weeks after spraying. Typical Cl_3CCOOH-injured seedlings were arbitrarily defined by shorter stands, deeper green leaves, and some more small tillers (100% complete tolerance). Fresh weight yields of treatments followed by the same letter were not significantly different at the 5% of level, based on Duncan's Multiple Range Test (letters are listed on top of bars). Visual rating of tolerance was not subjected to analysis of variance and Duncan's Test.

Intact Seedlings and Analysis; Root Absorption Studies. In the root absorption studies with intact seedlings, seeds of wheat were germinated in thoroughly washed white quartz sand in the greenhouse and the seedlings allowed to grow for 10 to 14 days depending on the season. Three selected uniform seedlings for each treatment with three replicates were transferred and grown in continuously aerated, pH adjusted, daily fresh nutrient solution under about 16,500 lx for a 16-hr photoperiod for another 10 to 14 days. The temperature varied somewhat by season and usually ranged from 22 to 26° during daytime and 14 to 20° at night. At the appropriate seedling stage, a combination of a labeled and a nonlabeled compound in solution was applied to the roots. The solution of a corresponding labeled compound without an addition of a nonlabeled compound served as a control in each test and was applied at the same time. After the appropriate treatment periods (1 to 24 hr) the treated seedlings were harvested.

The roots were rinsed with tap water (45 sec) followed with distilled water (15 sec) to remove unabsorbed compounds. The rinsed roots were then blotted on a paper towel to remove free water. The seedlings were separated into roots and shoots. After weighing, the tissues were homogenized in a Waring Blendor with 50% ethanol in a ratio of 1 g fresh weight to 10 ml of ethanol for 1 min. The homogenates were filtered through Whatman No. 4 filter paper. The radioactivity of filtrates without cleanup was measured on three 0.2-ml aliquots in 10 ml of toluene scintillation solution each counted 10 min. Based on preliminary trials the 50% ethanol was chosen for extracting ^{14}C-labeled contents of all compounds used in this investigation from plant tissues due to its ease of handling and reasonably good percentage recovery. The overall average recovery of all applied radioactivity in crude ethanolic extracts was 85%. The radioactivity left in tissues and filter paper was not measured. The prepared scintillation solution contained 2 vol of toluene scintillation solution (0.4% 2,5-diphenyloxazole and 0.05% 1,4-bis[2-(5-phenyloxazolyl)]benzene in liquid scintillation counting grade toluene) and 1 vol of purified Triton X-100 (Patterson and Greene, 1965) plus 3.3% NCS solubilizer (Chow, 1974). Prior to counting, the samples were stored in vials in the dark overnight in order to eliminate any error due to chemiluminescence and, as a precaution, countings were often repeated. All counts of samples were corrected for background and for quenching by using the external standard channels ratio method. Radioactivity in samples was expressed in disintegrations per minute per gram of fresh weight of tissues (dpm/g). Data of a labeled compound distributed "in shoots" and retained "in roots" were referred to as "translocated" and "accumulated", respectively. The data obtained from sum of "in shoots" and "in roots" divided by the sum of fresh weight of shoots and roots in grams were referred to as a compound "absorbed" by roots of seedlings. This would not account for metabolites of herbicides in tissues with short treatment periods (less than 24 hr for all experiments).

Excised Root Segments and Analysis; Physiological Behavior Associated with Absorption. In studies with excised root segments in the laboratory, seeds were surface sterilized with 0.03% sodium hypochlorite for 2 hr. After rinsing with distilled water, the seeds were germinated over moist germination paper in an incubator at 22° for 6 days.

Table II. Effects of 2,4-D or MCPA on Cl_3CCOOH-^{14}C Absorption by Intact Seedlings of Three Wheat Varieties Treated for 24 hr

	Cl_3CCOOH-^{14}C, % of the control (Cl_3CCOOH-*1*-^{14}C alone)								
	"Neepawa"			"Manitou"			"Thatcher"		
Concn, *M*	Distributed in shoots	Accumulated in roots	Absorbed by roots	Distributed in shoots	Accumulated in roots	Absorbed by roots	Distributed in shoots	Accumulated in roots	Absorbed by roots
Cl_3CCOOH-^{14}C[a]									
+ 2,4-D, 10^{-6}	52.6	99.1	78.8	46.7	84.5	81.0	52.6	108.9	77.4
+ 2,4-D, 10^{-5}	20.2	50.7	36.6	19.4	67.1	36.6	19.7	56.3	42.5
+ 2,4-D, 10^{-4}	9.5	17.3	13.7	15.5	28.7	20.5	16.9	29.5	23.5
+ MCPA, 10^{-6}				32.5	116.9	62.9	45.0	64.2	53.7
+ MCPA, 10^{-5}				21.5	85.9	44.0	19.3	51.4	37.1
+ MCPA, 10^{-4}				10.5	39.4	20.8	16.9	24.2	20.3

[a] Concentration and radioactivity of Cl_3CCOOH-^{14}C fed to "Manitou" and "Thatcher" were 10^{-5} *M* (pH 5.50) and 22,267 dpm/ml. "Neepawa" was conducted in a separate test, in which 0.39×10^{-7} *M* (pH 5.00) and 11,830 dpm/ml were used.

Table III. Effects of Cl_3CCOOH-^{14}C Alone and in a Mixture with 2,4-D on Fresh Weight of Shoots and Roots of "Neepawa" Wheat Seedlings Treated from 1 to 24 hr

Treatment period, hr	Cl_3CCOOH-^{14}C[a]			Cl_3CCOOH-^{14}C[a] + 2,4-D[b]		
	Shoots,[c] g	Roots,[c] g	Sum, g	Shoots,[c] g	Roots,[c] g	Sum, g
1	1.21 ± 0.12	0.87 ± 0.04	2.08	1.36 ± 0.09	0.85 ± 0.04	2.21
2.5	1.41 ± 0.06	0.94 ± 0.05	2.35	1.15 ± 0.16	0.91 ± 0.13	2.06
5.5	1.24 ± 0.13	0.91 ± 0.06	2.15	1.19 ± 0.09	0.80 ± 0.08	1.99
11.5	1.46 ± 0.06	0.99 ± 0.03	2.45	1.32 ± 0.13	0.85 ± 0.04	2.17
24	1.31 ± 0.14	0.83 ± 0.05	2.14	1.44 ± 0.11	1.01 ± 0.15	2.45

[a] Concentration and radioactivity of Cl_3CCOOH-^{14}C were 0.39×10^{-7} *M* and 11,830 dpm/ml. [b] Concentration of 2,4-D in the mixture was 10^{-5} *M*. Solution (pH 5.00) was applied to roots at the three-leaf stage of wheat seedlings. [c] All values in each column were the mean of three replicates ± standard error.

The roots used were excised from the apical 0.5 cm. Three segments, 1.0 cm long, were cut from each root. Each treatment consisted of three replicates with 20 segments each. The segments were incubated in 150.0-ml solutions in the dark overnight. After incubation, the segments were rinsed with nonlabeled corresponding solutions at equal concentrations for 30 sec. The procedure for extraction of labeled compounds from the treated segments and the technique used for radioassay were similar to those described previously. The radioactivity in excised segments was simply referred to as "absorbed".

RESULTS

Wheat Tolerance to Herbicide Mixtures. In a 3-year field trial (1968–1970) in Brandon, Manitoba, the mixture of Cl_3CCOOH with 2,4-D or MCPA as a postemergence treatment substantially reduced the injury of "Manitou" wheat compared to Cl_3CCOOH applied alone at equal rates in 1968 and 1970. When the mixtures were compared, 2,4-D was found to be more effective than MCPA in reducing wheat injury (Chow and Dryden, 1973). A follow-up pot study in the greenhouse confirmed these findings (Figure 1). The tolerance of wheat to Cl_3CCOOH was increased more by 2,4-D amine than by MCPA amine. The best combination was Cl_3CCOOH at 0.56 kg/ha mixed with 2,4-D at 0.56 kg/ha. At the highest rate (1.68 kg/ha) of 2,4-D or MCPA, the mixtures with Cl_3CCOOH (0.56 kg/ha) were tolerated least by wheat seedlings. However, this detrimental effect was not reflected in fresh weight because the injured seedlings produced some small tillers.

Root Absorption Studies. Since there was less Cl_3CCOOH injury of wheat when mixtures were applied it seemed logical to measure Cl_3CCOOH absorption by intact wheat plants using radiotracer technique.

Effect of Phenoxy Compounds on Three Wheat Varieties. The absorption, accumulation, and distribution of Cl_3CCOOH-^{14}C by root systems of wheat seedlings were markedly reduced when the Cl_3CCOOH-^{14}C solution contained either phenoxy herbicide (Table II). All three varieties of wheat showed the same tendency in reduction of absorption. The effect of the phenoxy herbicide on Cl_3CCOOH-^{14}C absorption by wheat was a function of concentrations (10^{-4} to 10^{-6} *M*) of the phenoxy herbicide in the ambient media. The rate of root absorption decreased as the concentration of the phenoxy herbicide presented in Cl_3CCOOH-^{14}C solution increased. It was also noted that the inhibitory effect by a phenoxy compound was much greater on Cl_3CCOOH translocation to shoots than absorption by roots.

Timing Study with Effect of 2,4-D. Results indicated that neither Cl_3CCOOH-^{14}C (0.39×10^{-7} *M*) alone nor in a mixture with 2,4-D (10^{-5} *M*) distinctly affected fresh weight of shoots and roots of intact wheat seedlings treated at five intervals from 1 to 24 hr (Table III). Slight differences of fresh weight of shoots and roots between these two treatments at different intervals were mainly due to the variation of seedlings in spite of careful selection based on visual examination. Apparently both shoots and roots of wheat seedlings absorbed less Cl_3CCOOH-^{14}C when roots were fed with Cl_3CCOOH-^{14}C solution containing 10^{-5} *M*

Table IV. Effects of Phenoxy Compounds on Absorption of Cl_3CCOOH-^{14}C by "Neepawa" Wheat Seedlings Treated for 24 hr

Treatment	Distributed in shoots (*S*)		Accumulated in roots (*R*)		Absorption by roots (*S* + *R*)		
	Radioact.,[a] dpm/g fr wt	Inhibition, % of control 1	Radioact.,[a] dpm/g fr wt	Inhibition, % of control 1	Radioact.,[a] dpm/g fr wt	Inhibition, % of control 1	Ratio of dpm/g fr wt (*R*/*S*)
Cl_3CCOOH-^{14}C,[b] 0.62×10^{-7} *M*, 10,844 dpm/ml							
0[c] (control 1)	15,030 ± 3.2	0	28,984 ± 1.4	0	21,551	0	1.93
+ DNP,[c] 10^{-5} *M*	7,018 ± 3.1	53	20,764 ± 2.8	28	13,209	39	2.96
+ 2,4-D,[c] 10^{-5} *M*	4,754 ± 1.5	68	10,545 ± 9.7	64	7,517	65	2.22
+ MCPA,[c] 10^{-5} *M*	6,105 ± 4.2	59	17,332 ± 4.6	40	10,755	50	2.84
+ Silvex,[d] 10^{-5} *M*	4,806 ± 5.4	68	11,506 ± 2.1	60	7,943	63	2.39
+ 2,4,5-T,[c] 10^{-5} *M*	5,332 ± 4.4	64	17,191 ± 4.0	41	10,058	53	3.22
+ 0[d] (control 2)	13,146 ± 1.9	12	29,514 ± 1.6	+2	21,373	1	2.25

[a] All values in each column were the mean of three determinations (replicates) ± coefficient of variability (percent). [b] The individual solution after pipetting the stock solution of Cl_3CCOOH-^{14}C was adjusted to pH 4.80. [c] The amount of each compound for preparing 10^{-3} *M* was dissolved in 0.5 ml of 100% alcohol and then diluted to 10^{-5} *M* for testing. A corresponding concentration of alcohol was used for the control 1. [d] Silvex and the control 2 were prepared with 0.5 ml of acetone instead of alcohol in a similar manner as described previously.

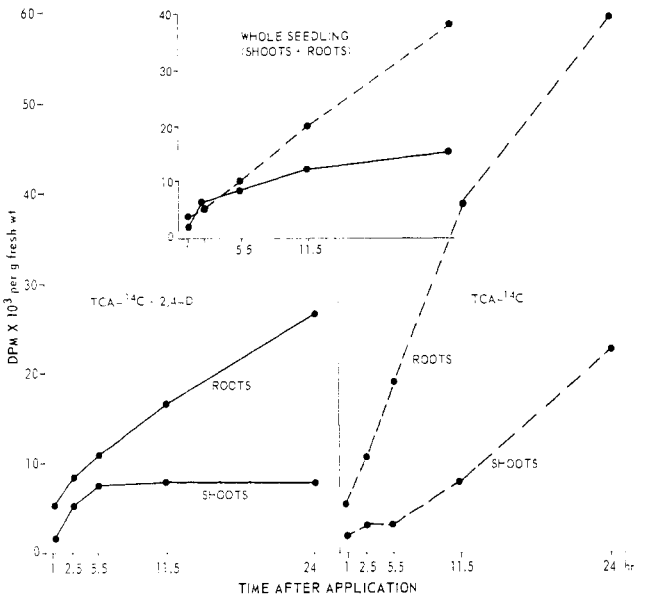

Figure 2. Timing study on root absorption and shoot translocation of Cl_3CCOOH-^{14}C (0.39×10^{-7} *M*, 11,830 dpm/ml) alone (●---●) and mixed with 2,4-D (10^{-5} *M*) (●—●) by "Neepawa" wheat seedlings from 1 to 24 hr. Solutions (pH 5.00) were applied to roots at the three-leaf stage of wheat seedlings.

Table V. Effect of 2,4-D on Root Absorption of Various Labeled Compounds by "Neepawa" Wheat Seedlings Treated for 24 hr

Treatment	Radioact., dpm/g fr wt (% of control)		
	Expt A[a]		
	Amitrole-^{14}C	Chloramben-^{14}C	Expt B,[a] dicamba-^{14}C
2,4-D, 10^{-6} *M*	84	66	83
2,4-D, 10^{-5} *M*	83	42	54

[a] Feeding solutions (pH 5.00) of amitrole-^{14}C (0.85×10^{-7} *M*, 11,410 dpm/ml), chloramben-^{14}C (1.85×10^{-7} *M*, 8110 dpm/ml), and dicamba-^{14}C (2.15×10^{-7} *M*, 7173 dpm/ml) were applied to roots of seedlings at four- and three-leaf stages of wheat in experiments A and B, respectively.

2,4-D in comparison to Cl_3CCOOH-^{14}C solution without addition (Figure 2). This differential absorption between the presence and absence of 2,4-D in Cl_3CCOOH-^{14}C solutions could be detected 5.5 hr after application. As time passed, the differential absorption of Cl_3CCOOH-^{14}C between these two treatments became greater, resulting in 56% less Cl_3CCOOH-^{14}C being accumulated in roots (27,438 dpm/g vs. 61,430) and a 68% lower content in shoots (7521 dpm/g vs. 23,191) as affected by 2,4-D over 24 hr. It seems that 2,4-D had a greater effect on Cl_3CCOOH translocation in shoots than accumulation in roots at later periods (11.5 and 24 hr after application).

Effect of Phenoxy Analogs. All four phenoxy herbicides at 10^{-5} *M* in a 24-hr period exhibited a strong inhibition, in relation to the controls, on the absorption, accumulation, and distribution of Cl_3CCOOH-^{14}C in wheat seedlings (Table IV). The inhibition of Cl_3CCOOH-^{14}C absorption by these herbicides ranged from 50 to 65%, exceeding the rate of inhibition (39%) caused by dinitrophenol (DNP), a classic uncoupler in oxidative phosphorylation. DNP is known to inhibit salt accumulation by plant tissues (Robertson et al., 1951) and to reduce 2,4-D accumulation in the roots and leaves of barley seedlings (Yamaguchi, 1965). The translocation of Cl_3CCOOH-^{14}C from roots to shoots appeared dependent on the amount absorbed by roots, as judged by the ratios of Cl_3CCOOH-^{14}C distribution and accumulation (*R*/*S*) of five Cl_3CCOOH-^{14}C phenoxy mixtures as compared with the Cl_3CCOOH-^{14}C controls (Table IV).

Effect of 2,4-D on Other Herbicides. It is important to know whether the effect of the phenoxy herbicide on Cl_3CCOOH absorption by wheat has similar effects on other herbicide compounds since these herbicide mixtures with 2,4-D are also used on some crops. A series of separate tests with wheat seedlings was conducted in a similar manner. The summary of results in absorption by roots expressed in percentage of the control is listed in Table V. It is clear that 2,4-D had a similar effect on absorption of amitrole-^{14}C, chloramben-^{14}C, and dicamba-^{14}C by wheat roots. However, the rate of absorption inhibited by 2,4-D varied among these three compounds. In relation to the

Table VI. Effects of ATP and 2,4-D on Cl_3CCOOH-^{14}C Absorption by Excised Wheat Root Segments Incubated in Various Solutions at 25° in the Darkness by Continuous Shaking for 21.5 hr

Treatment	Radioact., dpm/g fr wt, ± c.v., %	Inhibition (–) or stimulation (+) of absorption, % of control
Cl_3CCOOH-^{14}C, 1.23×10^{-5} *M* 21,731 dpm/ml		
+ 0 (control)	96,133 ± 1.6	0
+ ATP,[a] 10^{-6} *M*	101,176 ± 2.9	+5
+ ATP,[a] 10^{-4} *M*	151,270 ± 0.2	+57
+ 2,4-D,[a] 10^{-6} *M*	73,964 ± 1.9	–23
+ 2,4-D,[a] 10^{-4} *M*	12,762 ± 1.4	–87
+ ATP,[a] 10^{-6} *M*, + 2,4-D,[a] 10^{-6} *M*	73,855 ± 2.2	–23
+ ATP,[a] 10^{-4} *M*, + 2,4-D,[a] 10^{-4} *M*	13,798 ± 2.4	–86

[a] Disodium salt of ATP and sodium salt of 2,4-D were separately dissolved in distilled water as stock solutions, and diluted to desired concentrations alone or in mixtures. The same amount of Cl_3CCOOH-^{14}C stock solution was then added to each solution. Prior to incubation each treatment solution was adjusted to pH 5.00.

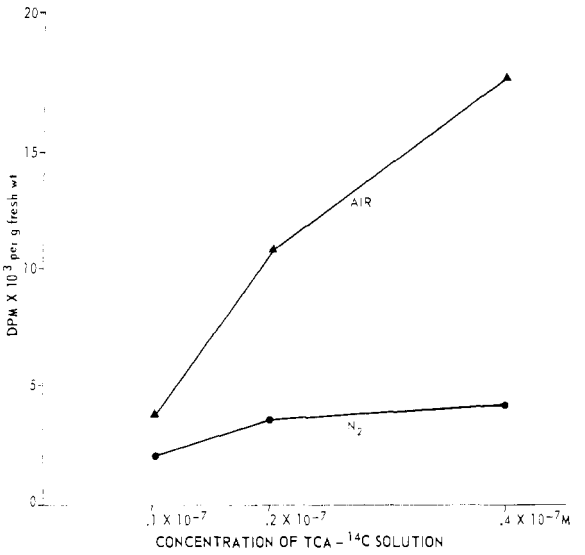

Figure 3. Cl_3CCOOH-^{14}C absorption by excised root segments of "Neepawa" wheat seedlings under oxygen (air) and oxygen restraint (N_2, 99.98% pure) conditions. The solutions were bubbled with air or N_2 for 0.5 hr prior to incubation and during the experimental period of 21.5 hr. The roots were incubated with solutions (pH 5.50) at 25° in the dark. Cl_3CCOOH-^{14}C concentrations and radioactivities used were 0.1×10^{-7} *M*, 1890 dpm/ml; 0.2×10^{-7} *M*, 3842 dpm/ml; and 0.4×10^{-7} *M*, 7482 dpm/ml, respectively.

control for a 24-hr period wheat roots absorbed much less chloramben-^{14}C from the solution than the other two herbicides to which 2,4-D at 10^{-6} or 10^{-5} *M* was added. On the other hand, the absorption of amitrole-^{14}C, a very mobile compound in plants (Crafts and Yamaguchi, 1960), by roots was not inhibited as strongly as chloramben. A similar observation was noted that DNP even at 10^{-4} *M* had no effect upon the movement of amitrole-^{14}C from roots of soybean (Foy and Yamaguchi, 1964). Dicamba absorption was affected very little by 2,4-D at lower concentration (10^{-6} *M*) but was affected more at higher concentration (10^{-5} *M*).

Physiological Behavior of Cl_3CCOOH Transport. *Effects of ATP and 2,4-D on Absorption.* In order to assess the effects of inhibition and stimulation on root absorption with the least possible linkage with shoot phloem translocation as in intact seedlings, this and the next experiments with excised root segments were conducted. Due to ATP being the energy source for many ion transport processes, an experiment with ATP as well as with 2,4-D simultaneously added to external Cl_3CCOOH-^{14}C incubation solutions may help understand the nature of Cl_3CCOOH absorption by roots and consequently the 2,4-D effect. Results revealed that an exogenous addition of ATP to Cl_3CCOOH-^{14}C solution promoted the absorption of Cl_3CCOOH-^{14}C by root segments ranging from 5% at the lower concentration (10^{-6} *M*) to 57% at the higher concentration (10^{-4} *M*) for a 21.5-hr incubation period (Table VI). In contrast, 2,4-D treatments at lower and higher concentrations inhibited Cl_3CCOOH-^{14}C absorption by 23 and 87%, respectively, giving results similar to those obtained from the tests using intact seedlings as described previously. It is interesting to note that the promotion of Cl_3CCOOH-^{14}C absorption by ATP was completely negated when both ATP and 2,4-D at equimolar concentrations were added to Cl_3CCOOH-^{14}C solutions at the same time. The inhibitory rates of Cl_3CCOOH-^{14}C absorption caused by these treatments were almost the same when 2,4-D was added alone. This indicated that the inhibitory effect of 2,4-D far exceeded the stimulatory effect of ATP as far as Cl_3CCOOH-^{14}C absorption by wheat root segments was concerned. The inhibition of Cl_3CCOOH-^{14}C uptake by DNP and 2,4-D, together with stimulation by ATP, is consistent with the suggestion of Cl_3CCOOH absorption by roots being a metabolic energy-requiring process.

Absorption of Cl_3CCOOH-^{14}C under Oxygen Restraint Condition. An experiment similar to the previous one utilizing excised root segments was performed to ascertain the physiological behavior of Cl_3CCOOH absorption under oxygen-deficient conditions by ventilating nitrogen gas (N_2) through treatment solutions. This experiment clearly showed the results on Cl_3CCOOH-^{14}C absorption as influenced by Cl_3CCOOH-^{14}C concentration and aeration (Figure 3). Indeed, under nitrogen ventilation, root segments absorbed substantially lower amounts of Cl_3CCOOH-^{14}C at all of the three concentrations measured as compared to aerated solutions. Over a period of 21.5 hr in nitrogen gas bubbling solutions the amount of Cl_3CCOOH-^{14}C absorbed by the roots was 58, 30, and 24% of that in air-ventilated solutions at 0.1×10^{-7}, 0.2×10^{-7}, and 0.4×10^{-7} *M* of Cl_3CCOOH-^{14}C, respectively. It is conceived that under oxygen restraint conditions, the respiration rate of roots was reduced and subsequently the generation of ATP in oxidative phosphorylation in mitochondria and other related metabolic activities might be also decreased (Crafts, 1961; Hopkins, 1956). This is a further suggestion that Cl_3CCOOH absorption by wheat roots behaved as one of the metabolic processes which physiologically needed a continuous metabolic energy supply.

Effects of Competition of 2,4-D on Absorption and Translocation. Another possibility dealing with 2,4-D action on absorption and translocation of Cl_3CCOOH was explored to ascertain whether the reduction in Cl_3CCOOH uptake by 2,4-D is linked to competition for binding sites on the same carrier in the cells. Both root absorption and shoot translocation of intact wheat seedlings from solutions of Cl_3CCOOH-^{14}C concentrations (2.5×10^{-6} to 20×10^{-6} *M*) over a period of 12 hr were measured in the presence and absence of 10×10^{-6} *M* 2,4-D. A Lineweaver–Burk plot of the data indicated (Figure 4) that 2,4-D inhibited

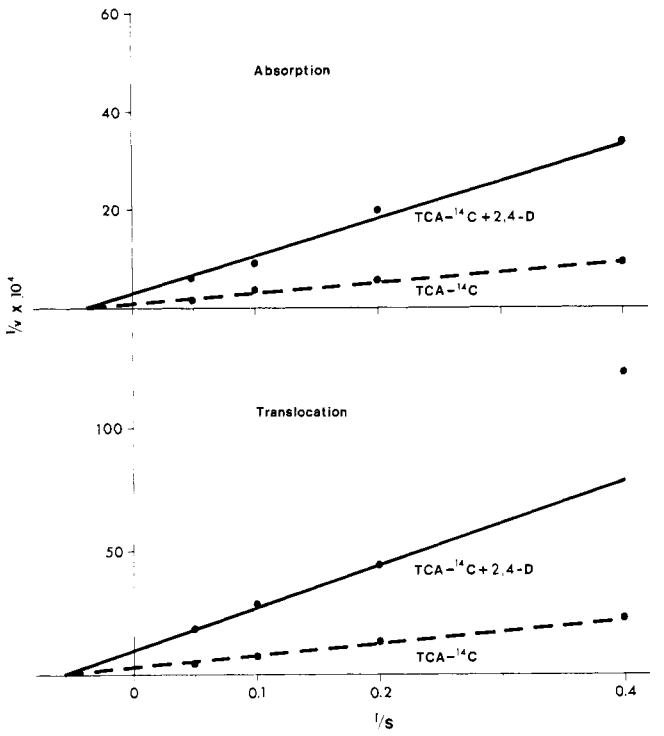

Figure 4. Effect of 2,4-D (10×10^{-6} *M*) on the absorption and translocation of Cl_3CCOOH-^{14}C (2.5×10^{-6} to 20×10^{-6} *M*, 4600 to 36,090 dpm/ml) by intact "Neepawa" wheat seedlings over a 12-hr period. All solutions used were adjusted to pH 5.00. Data were plotted by the method of Lineweaver–Burk as the reciprocal of the velocity of absorption and translocation of Cl_3CCOOH-^{14}C (*v*, expressed as disintegrations per minute per gram fresh weight per hour) against the reciprocal of Cl_3CCOOH-^{14}C concentrations (*S*).

the absorption of Cl_3CCOOH-^{14}C by roots and the subsequent translocation to shoots via the different carrier sites by a noncompetitive process. The repeated experiment with wheat seedlings treated for 21.5 hr showed similar results.

DISCUSSION

The chemical structures and properties of amitrole, chloramben, dicamba, and Cl_3CCOOH are different. However, the absorption of these compounds by wheat roots was reduced, though at different rates, by 2,4-D and its analogs. Obviously, the effect of 2,4-D on absorption of these compounds by wheat was not specific. This is in agreement with other observations that the regulatory effect on the accumulation of inorganic salts in excised wheat roots by 2,4-D was also nonspecific (Nance, 1949). Swenson and Burström (1960) made a similar observation with wheat seedlings and suggested that auxins including 2,4-D did not specifically affect ion uptake, or some specific ion, nor did they act by virtue of their growth inhibition, but that they affected some cell property making plants conducive to cation uptake and water absorption. A number of other workers also found that phenoxy compounds affected uptake, transport, and distribution of auxin, salt, and water (Cooke, 1957; Matlib and Kirkwood, 1970; Nance, 1949; Niedergang-Kamien and Leopold, 1959; Wildon et al., 1957). Results in Table IV also supported the ideas that these phenoxy compounds had similar effects, although to a different degree, on physiological actions of wheat related to uptake and transport.

There is no full understanding of why the phenoxy compounds modified the uptake and transport of other compounds by wheat. The multiple actions of the phenoxy compound in plant cells make interpretation difficult. However, several factors may explain the modified physiological phenomena in plants. Based on radioautographic study, Crafts and Yamaguchi (1964) suggested that there was a plugging phloem due to cell proliferation caused by 2,4-D. Results in Tables II and IV indicated that 2,4-D had a greater effect on the translocation of Cl_3CCOOH-^{14}C from roots to shoots than on the accumulation of Cl_3CCOOH in the roots. This suggests that partial plugging of phloem transport in intact seedlings exerted by the phenoxy herbicide might be involved.

Nance (1949) proposed that there was a relationship between the restraint of ion absorption induced by 2,4-D and the reduction of ATP generation. It seems reasonable to assume (Crafts, 1961) that metabolic energy is required for ion or compound transport across roots and subsequent translocation in the conductory tissues because considerable evidence links ion absorption to high-energy phosphate compounds. In this sense, the phenoxy compound may be recognized as an ATP uncoupler (Gruenhagen and Moreland, 1971; Lotlikar et al., 1968; Matlib and Kirkwood, 1970; Stenlid and Saddik, 1962; Switzer, 1957; Wedding and Black, 1961, 1962), similar to DNP (Slater, 1963). Results obtained from the studies with excised root segments (Table VI and Figure 3) also support this suggestion that the mechanism of Cl_3CCOOH absorption by wheat roots probably involves energy requirements as an active absorption process. Although there were no data available from this investigation, 2,4-D might have an inhibitory effect at some step(s) in oxidative phosphorylation on ATP action or synthesis in respiration (Gruenhagen and Moreland, 1971; Lotlikar et al., 1968). Similarities in the responses of root tissues to 2,4-D and DNP would also suggest a similar effect (Table IV). It is known that besides uncoupling the formation of ATP from respiration, DNP promoted the hydrolysis of ATP (Slater, 1963). It is possible that a similar action of 2,4-D may occur with DNP. The results of Lineweaver–Burk kinetics also suggested that a noncompetitive type of inhibition for Cl_3CCOOH-^{14}C absorption and translocation by 2,4-D through the different carrier sites might be involved (Figure 4).

Based on this investigation the phenoxy herbicides at appropriate concentrations under certain conditions are capable of inhibiting absorption, accumulation, and translocation of the other herbicides by wheat. Combinations of herbicides may have a significant effect in reducing herbicide contamination in food crops. This reduced absorption and translocation may also leave more of the applied herbicide available for better weed control.

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Absorption, Excretion, and Metabolism of Methoprene by a Guinea Pig, a Steer, and a Cow

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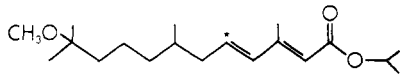
When the metabolic fate of methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) was studied in a guinea pig, a steer, and a cow, a rather large percentage of the radiolabel was incorporated in the tissues and respired by the animals. In the urine and feces, a small amount of radiolabel was metabolized into free primary metabolites, somewhat more was incorporated into simple glucuronides, and a considerable quantity of radiolabel was found in polar compounds, possibly complex conjugates or polar biochemicals. No methoprene was found in the urine, but approximately 40% of the radiolabel in feces was contributed by unmetabolized methoprene. The formation of conjugates and the metabolism of methoprene was more extensive in the steer than in the guinea pig.

Methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) (compound 1), a new insect



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growth regulator, has shown particular promise in the control of mosquitoes (Schaefer and Wilder, 1972, 1973) and horn flies (Harris et al., 1973, 1974). No toxicity to rats administered methoprene at 10,500 mg/kg was demonstrated (Siddall and Slade, 1974). Also, these workers observed no irritating effect on the conjunctiva of rabbits, and fish were not adversely affected by water containing 100 ppm. However, these insect growth regulators are so new as control agents that only a few studies have been made to determine the distribution or elimination of the materials or their metabolites in mammals. Hoffman et al. (1973) studied the metabolism (by rats) of 1-(4-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, and recently a study has been undertaken by Ivie (1974) on the metabolism of the same compound by a steer. Both these studies were conducted with the ^{14}C label within the phenoxy radical of the molecule. Also, a distribution and balance study was completed by Cline et al. (1975) with ^{3}H-labeled methoprene in white mice. Therefore, when methoprene was proposed for mosquito control in situations in which cattle might drink the treated water and when it was used successfully (at very low percentages) in mineral blocks as a feed-through-procedure for the control of horn flies (Harris et al., 1974), we undertook a study of ^{14}C-labeled methoprene (provided by Zoecon Corp.) in a guinea pig, a steer, and a cow.

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MATERIALS AND METHODS

The high-purity [^{14}C]methoprene (1) used in these studies was prepared by J. C. Leak, ICN Corp. (for details of radiosynthesis, see Schooley et al., 1975). Our most sensitive thin-layer chromatographic (TLC) system (2:1:1, benzene–pentane–methanol) indicated that the radiochemical purity of this product was 96.9%. The ^{14}C label was incorporated at the C-5 position which is important in the interpretation of the results.

In addition, Zoecon Corp. also supplied several known primary metabolites as nonradioactive standards. They were: **2,** isopropyl 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoate; **3,** 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoic acid; **4,** 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid; and **5,** 7-methoxycitronellic acid (7-methoxy-3,7-dimethyloctanoic acid). Also, a small sample of ^{14}C-labeled **4** was available as a radioactive standard, and purified methoprene was provided as a nonradioactive standard.

Treatment of Guinea Pig. The 1050-g male guinea pig was treated with 50.86 mg of the [5-^{14}C]methoprene, specific activity of 0.58 mCi/mmol, by adding the material to a no. 3 gelatin capsule conaining a small amount of powdered grain. The capsule was then held on the tip of a small rubber tube attached to a 1-ml syringe, and on compression of the syringe plunger, injected down the esophagus of the guinea pig. After treatment, the guinea pig was maintained for 24 hr in a metabolism cage (urine and feces automatically separated) and fed lettuce and fried pelleted food. The cage was completely covered with a plastic bag, and 400–500 ml of air/min was drawn through the cage and into an ethanolamine solution to remove CO_2. At the end of the test period, the guinea pig was killed and samples of muscle, fat, and blood were taken. The urine of the guinea pig was collected at irregular times during the 24 hr, and all the feces were collected at 24 hr.

Treatment of Steer. The 277-kg Hereford steer was treated with 2.0009 g (3.55 mCi) of the 5-^{14}C-labeled methoprene, specific activity of 0.58 mCi/mmol, by adding the