METABOLISM OF 2,4-DICHLOROPHENOXYACETIC ACID. I. $C^{14}O_2$ PRODUCTION BY BEAN PLANTS TREATED WITH LABELED 2,4-DICHLOROPHENOXYACETIC ACIDS

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(WITH FOUR FIGURES)

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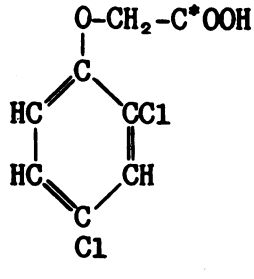
Although the morphological and physiological responses of plants to 2,4-dichlorophenoxyacetic acid (2,4-D) have been extensively studied, virtually nothing is known concerning the chemical reactions of this substance *in vivo* particularly as related to its biological effects. Evidence has been obtained by two different methods that a substantial proportion of the 2,4-D applied to a bean seedling can be recovered therefrom in apparently unchanged form (**3, 7**), but the possibility must be recognized that the recovered growth-regulator may represent only the excess beyond the amount that actually contributes to the reactions resulting in the observed developmental responses.

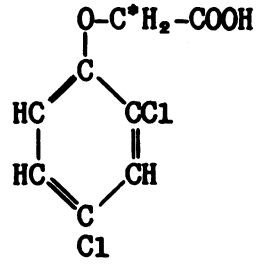
As the initial phase of a study of the transformations of C^{14}-labeled 2,4-D in higher plants, attention was directed to the production of radioactive carbon dioxide since such information would be required in the eventual formulation of a C^{14} balance sheet and might also be expected to contribute to understanding of the pathway of metabolism. The present paper describes the results of these experiments. Subsequent reports will deal with other metabolic products.

Experimental procedure

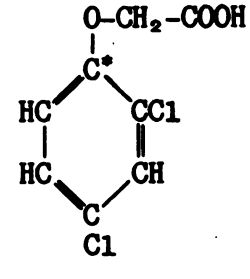
The compounds used were 2,4-dichlorophenoxyacetic acid-1-C^{14}, 2,4-dichlorophenoxyacetic acid-2-C^{14}, and 2,4-dichlorophenoxy-1-C^{14}-acetic acid (hereafter abbreviated as 2,4-D-1-C^{14}, 2,4-D-2-C^{14}, and 2,4-D-R-C^{14}) with specific activities of approximately 1, 0.5, and 0.02 mc./mmole, respectively.



2,4-D-1-C^{14}



2,4-D-2-C^{14}



2,4-D-R-C^{14}

The melting points, distribution on paper chromatograms, and physiological activities, as determined by bioassay (1), were identical with those of ordinary 2,4-D.

A modification of the gas counting system of JANNEY and MOYER (4) was used for measurement of activities. A stainless steel ionization chamber was found preferable to the brass chamber employed in the original instrument because of its apparent freedom from memory effects. Measurements were made at one atmosphere CO_2 pressure, 10 millimoles of gas being required to fill the chamber. With small samples, ordinary CO_2 was added to bring the total pressure to this value. Of a large number of experiments only those results in which the measured activities were at least four times the background values are reported here.

The general experimental procedure consisted of applying labeled 2,4-D to young bean plants (*Phaseolus vulgaris* var. Black Valentine) or parts thereof, collecting the evolved CO_2 in alkali, liberating CO_2 from an aliquot of the carbonate, and determining its radioactivity. The 2,4-D was applied by placing on the plant a 0.005 ml. drop of a solution of the desired concentration in 95% ethanol containing 1% Tween-20. During the period of collection of CO_2, the roots, or basal portions of the stems in the case of derooted plants, were immersed in a 0.01 M NaH_2PO_4 solution in order to minimize retention of CO_2.

Results

Since one of the objectives of these experiments was to obtain information which would be applicable to plants treated under greenhouse conditions, those conditions were simulated as closely as practicable. It was desired also to employ the smallest analytically feasible dosages of 2,4-D in view of the very great sensitivity of beans to this compound (1). The seedlings were cultured in the greenhouse to a stage just prior to unfolding of the first trifoliolate leaf from the bud. The roots were carefully washed free of soil and placed in NaH_2PO_4 solution. The 2,4-D was applied to the terminal buds and the plants were placed in a 28-liter glass jar which was sealed with a sheet of glass provided with inlet and exit tubes. The plants were illuminated from above by a bank of Daylight fluorescent lamps giving 900 fc at the primary leaves. Air from the jar was dispersed into fine bubbles by a fritted glass disk and passed first through an absorbing tower containing alkali and then through a barium hydroxide solution to verify completeness of absorption of CO_2. The entire apparatus was constructed in duplicate so that two sets of plants could be utilized concurrently.

The time course of $C^{14}O_2$ production from the side-chain is shown in figure 1. During the first 24-hour period a much greater fraction of the carboxyl carbon than of the methylene carbon is converted into CO_2. Subsequently, the rate of evolution from carboxyl diminishes while that from methylene increases. The total production of $C^{14}O_2$ from the methylene carbon during the first few days after treatment is considerably less than

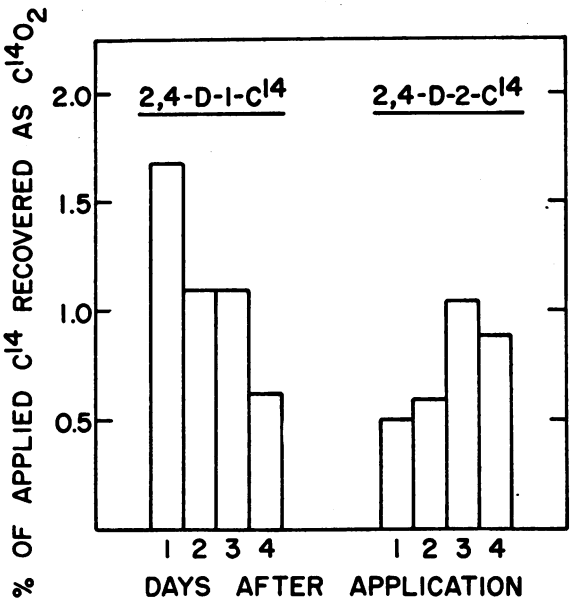


FIG. 1. Time course of $C^{14}O_2$ production by entire bean plants from side-chain-labeled 2,4-D. Dose, 1.5 μg. per plant, applied on terminal bud; light intensity, 900 fc; photoperiod, 14 hours; and ventilation rate, 14 liters per hour (Expt. 1098).

that from the carboxyl carbon. Data from seven additional experiments are presented in table I, from which it is seen that the fraction of the applied activity recoverable as CO_2 is not greatly influenced by conditions of illumination or by a sevenfold increase in dose. Experiment 818 was carried out in continuous darkness and experiment 979 in continuous light. Experiments 913, 930, and 857 were carried out under a photoperiod of 12 hours with normal air supplied during the light period and CO_2-free air during the dark period. Experiment 1097 was performed with normal air and a photoperiod of 14 hours. For the eight experiments the average per-

TABLE I

PRODUCTION OF $C^{14}O_2$ FROM LABELED 2,4-D APPLIED TO TERMINAL BUDS OF INTACT BEAN PLANTS.

Expt.	2,4-D applied per plant		Collection period	Per cent. of applied activity recovered as CO_2 from		mμmoles $C^{14}O_2$ evolved per plant from	
				2,4-D-1-C^{14}	2,4-D-2-C^{14}	2,4-D-1-C^{14}	2,4-D-2-C^{14}
	μg.	*mμmoles*	*days*				
818	1.5	6.8	3	5.1	0.7	0.34	0.048
913	1.5	6.8	4	6.8	1.4	.46	.095
930	1.5	6.8	4	5.3	1.0	.36	.068
979	1.5	6.8	4	6.2	1.1	.42	.075
857	1.5	6.8	5	7.2	2.2	.49	.15
1097	10.0	45.3	4	4.5	2.9	2.04	1.31

TABLE II

PRODUCTION OF $C^{14}O_2$ FROM LABELED 2,4-D APPLIED TO PRIMARY LEAVES OF INTACT BEAN PLANTS.

Expt.	2,4-D applied per plant		Per cent. of applied activity recovered as CO_2 from		mμmoles of $C^{14}O_2$ produced per plant from	
			2,4-D-1-C^{14}	2,4-D-2-C^{14}	2,4-D-1-C^{14}	2,4-D-2-C^{14}
	μg.	*mμmoles*				
1096	10	45.	4.4	2.8	2.0	1.3
1082	100	453.	2.9	1.2	13.0	5.5

centage of the applied activity recovered as CO_2 per day was 1.5 from 2,4-D-1-C^{14} and 0.4 from 2,4-D-2-C^{14}.

In order to ascertain whether a similar result would be obtained with still larger doses, recourse was had to leaf applications inasmuch as the sensitivity of the plant to leaf treatment is considerably lower than to bud application (1). A drop of 2,4-D solution was placed upon each of the primary leaves of the plant; otherwise the procedure was as already described. The data presented in table II were obtained with a collection period of three days at a ventilation rate of 14 liters of normal air per hour and a light intensity of 900 fc. Experiment 1096 was conducted with a photoperiod of 14 hours and 1082 with a photoperiod of 10 hours. The results of experiment 1096 (table II) and experiment 1097 (table I) indicate that, for a 10 μg. dose of 2,4-D, the same fraction is converted to CO_2 whether appli-

TABLE III

PRODUCTION OF $C^{14}O_2$ FROM LABELED 2,4-D APPLIED TO PRIMARY LEAVES OF BEAN PLANTS IN UNVENTILATED CONTAINERS.

Expt.	2,4-D applied per plant		Collection period	Per cent. of applied activity recovered as CO_2 from		mμmoles $C^{14}O_2$ evolved per plant per day from	
				2,4-D-1-C^{14}	2,4-D-2-C^{14}	2,4-D-1-C^{14}	2,4-D-2-C^{14}
	μg.	*mμmoles*	*days*				
519*	0.3	1.4	4	3.5	....	0.012	....
588†	0.5	2.3	2	2.1	....	.024	....
519*	1.0	4.5	4	3.0	....	.034	....
579**	1.0	4.5	3	1.5	....	.022	....
519*	3.0	13.6	4	2.4	0.8	.082	0.027
374*	5.0	22.6	6	4.1	....	.154	....
534*	5.0	22.6	2	....	0.2	....	.023
588†	5.0	22.6	2	1.1	0.4	.124	.045
498*	10.0	45.3	5	4.1	1.2	.369	.109
579**	10.0	45.3	3	1.2	0.5	.180	.076

*Continuous darkness.
**14-hr. photoperiod; intensity = 800 fc.
†14-hr. photoperiod; intensity = 800 fc, derooted plants.

cation is made to the leaf or to the bud; with a 100 μg. dose, a somewhat smaller fraction appears as CO_2.

In table III are listed results from several other trials conducted in unventilated desiccators or bell jars in which large surfaces of the absorbing alkali were exposed. Under these conditions of possible oxygen deficiency, the rate of conversion of 2,4-D to CO_2 appears to be appreciably smaller than in the ventilated cultures. During the collection periods of two to five

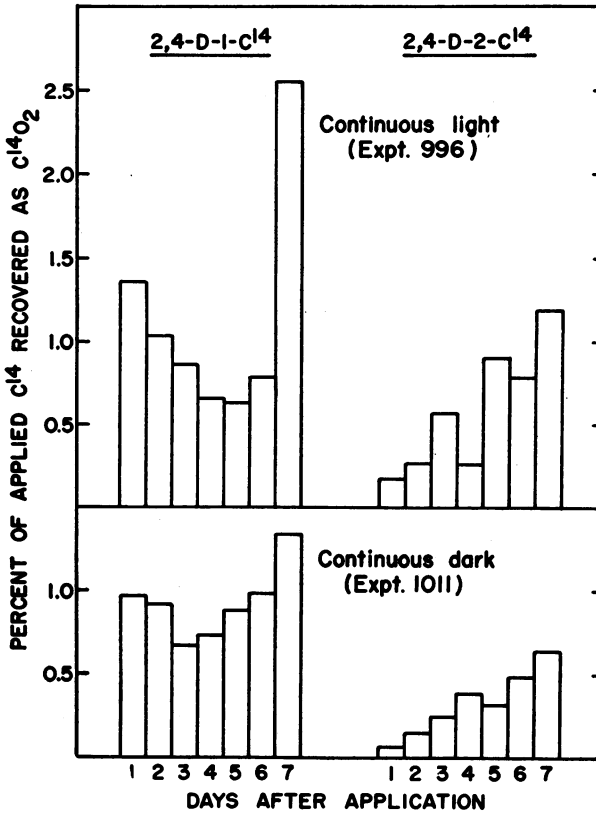


FIG. 2. Time course of $C^{14}O_2$ production by bean explants from side-chain-labeled 2,4-D. Dose, 1.5 μg. per plant, applied on terminal bud; light intensity, 900 fc; ventilation rate, 14 liters per hour.

days, the rate of conversion of the carboxyl was, in these tests also, about three times as great as that from the methylene carbon.

Owing to the large amount of $C^{12}O_2$ produced by intact bean plants relative to the amount of $C^{14}O_2$ derived from 2,4-D and to the consequent low specific activity of the total CO_2, the smallest doses which it was feasible to employ were larger than those required to elicit a marked morphological response. In order to study smaller doses, use was made of explants, consisting of 5 to 10 cm. of the stem bearing the terminal bud and cotyledons but lacking roots and primary leaves. The buds of such explants when treated

with 2,4-D developed leaves exhibiting the characteristic formative malformations (1); but much less CO_2 was evolved per explant than from intact plants, and many more could be accommodated in the respiration chambers.

The time course of $C^{14}O_2$ production in two experiments with such material is shown in figure 2. As with the entire plants, the rate of CO_2 production from the carboxyl carbon tends to diminish during the first few days while that from the methylene carbon increases. Long term experiments with explants have the difficulty that treatment with 2,4-D aggravates the

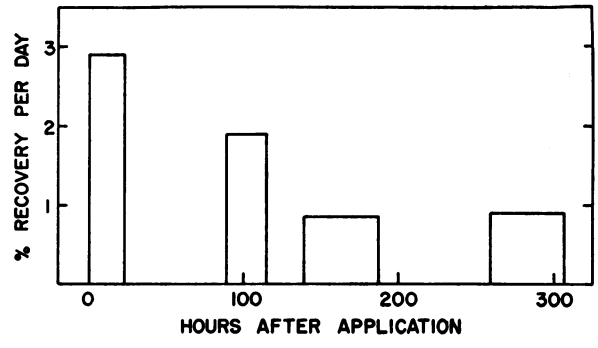


FIG. 3. Time course of $C^{14}O_2$ production by bean explants from 2,4-D-1-C^{14}. Dose, 0.04 μg. per plant, applied on terminal bud; light intensity, 900 fc; photoperiod, 14 hours (Expt. 652).

susceptibility to attack by microörganisms so that growth of molds on the tissue is frequently apparent after five or six days at high humidities. The rising rate of $C^{14}O_2$ evolution during the latter part of the experiment is probably related to this circumstance as is indicated by the experiment illustrated in figure 3. Treated explants were enclosed with alkali for a day, after which the containers were left open for a two day period, the alkali having been removed and the radioactivity measured. The explants were then enclosed with fresh alkali for a second period, and the alternation of opening and closing was repeated twice more, four fresh portions of absorb-

TABLE IV

RELATION BETWEEN DOSE OF 2,4-D-1-C^{14} AND AMOUNT OF $C^{14}O_2$ PRODUCED BY BEAN EXPLANTS.

2,4-D applied per explant		Number of experiments averaged	$C^{14}O_2$ production	
			mμmoles/explant/day	Per cent. of applied dose recovered/day
μg.	*mμmoles*			
0.04	0.18	2	0.0053	2.95
.10	.45	1	.0071	1.57
.15	.68	5	.0110	1.62
.20	.91	1	.0085	0.93
1.5	6.8	8	.0720	1.06
2.0	9.1	2	.0673	0.74
4.6	20.8	1	.125	0.60

ing solution being used in all. In this fashion it was possible to maintain the explants reasonably free of mold for 13 days and in large measure to prevent the increase in rate of $C^{14}O_2$ production. The total recovery of C^{14} following application of 0.15 μg. carboxyl-labeled 2,4-D per bud was 10.4% and, as the time of actual collection amounted to only 47% of the total duration of the experiment, it may be estimated that approximately 22% of the applied C^{14} escaped from the plants as CO_2.

With increasing doses of 2,4-D, the total amount of $C^{14}O_2$ evolved also increases but the fraction converted to CO_2 tends to diminish. Table IV summarizes the results of 20 experiments with explants using collection periods of two to four days. These data are plotted in figure 4, together with the dose-response curve for repression of leaf expansion (1).

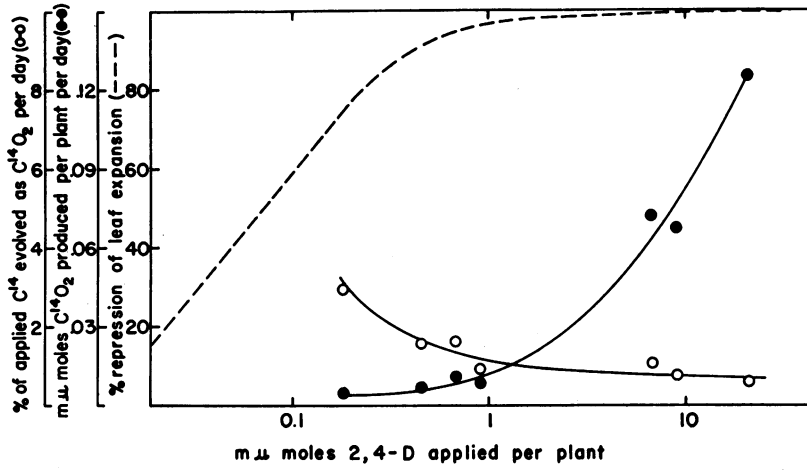


FIG. 4. Formative activity (leaf repression) and $C^{14}O_2$ production from 2,4-D-1-C^{14} as functions of applied dose of 2,4-D.

Owing to the relatively low specific activity of the available ring-labeled 2,4-D, it was necessary to employ much higher doses of this substance compared with the chain-labeled compounds. In a series of experiments with doses up to 200 μg. applied to leaves or buds of entire plants or to explants, no radioactivity was found in the evolved CO_2 during collection periods of two to six days, whence it may be concluded that less than 0.2% of ring carbon atom 1 was liberated as CO_2.

Discussion

During the course of these experiments, two reports of the formation of CO_2 from 2,4-D have appeared. The first, by HOLLEY *et al.* (3), states that radioactive CO_2 is produced by plants treated with 2,4-D-1-C^{14} but provides no data. The second, by FANG *et al.* (2), describes a single experiment in which bean plants treated with 100 μg. 2,4-D-2-C^{14} (50 μg. on each primary leaf) evolved in three days an amount of $C^{14}O_2$ corresponding to

17.5% of the applied C^{14}. So far as can be judged, this experiment was very closely similar to our Expt. 1082 (table II) in which only 1.2% of the applied C^{14} was liberated as CO_2. While this discrepancy may be due to some unrecognized diversity in plant material or environmental conditions, a difference in analytical methods also may be responsible. The technique employed by Fang *et al.* was not described in detail, and no information was given concerning the ratio of net counting rate to background so that it is difficult to assess their activity data which were obtained by extrapolation to an infinitely thin sample, a method involving considerable uncertainty (**5**).

Whether the production of CO_2 from 2,4-D is related to the physiological action of the growth regulator cannot be ascertained from the present results. It is suggestive, however, that the fraction converted to CO_2 is relatively independent of the amount of applied 2,4-D in the range of supramaximal doses but tends to rise rather sharply in the range of submaximal doses, figure 4. On the other hand, the data in tables II and III demonstrate a decomposition of 2,4-D applied to nearly mature leaves although these organs do not themselves manifest morphological responses and, in the experiments carried out in darkness, would be expected to export very little of the growth regulator to the meristematic cells (**6**).

Summary

Radioactive carbon dioxide is produced by plants which have been treated with 2,4-dichlorophenoxyacetic acids containing C^{14} in either the carboxyl or the methylene positions. The evolution of $C^{14}O_2$ continues at a relatively low rate during a period of several days. The initial rate of production from carboxyl-labeled 2,4-D is several times that from methylene-labeled 2,4-D. No carbon dioxide is evolved from the ring carbon at position 1.

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