STUDIES IN PLANT METABOLISM. III. ABSORPTION, TRANSLOCATION AND METABOLISM OF RADIOACTIVE 2,4-D IN CORN AND WHEAT PLANTS[1, 2, 3]

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The use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a selective herbicide has become quite extensive. In general, dicotyledonous plants are more sensitive to 2,4-D treatment than are most plants of the monocotyledonous types (7), although very little is known regarding the factors responsible for the difference in sensitivity. Recently, by using an isotopically labeled herbicide, Wood *et al.* (10) found that bean plants absorbed and translocated 2-iodo^{131}-3-nitrobenzoic acid (INBA) more readily than barley plants. Later, Mitchell *et al.* (8) extended their work to oat and corn plants. They suggested that the growth-inhibiting effect of INBA in the bean plant and its failure to produce significant inhibition in barley, oat and corn plants might be due to the differences in the manner in which INBA reacts with the plant constituents in each case.

Gallup and Gustafson (3) using 2,4-dichloro-5-iodo^{131}-phenoxyacetic acid were also able to demonstrate that the absorption of this growth regulator by the monocotyledonous plants is slower than by the dicotyledonous ones. The translocation to the apical portion of the plant is very rapid in broadleaved plants, but much less rapid in the grasses.

Holley, Boyle and Hand (4) using carboxyl-C^{14}-labeled 2,4-D demonstrated the presence of free 2,4-D in bean plant homogenate. Later Holley (5) using the same labeled preparation was able to show the presence of an ether-insoluble, water-soluble compound. Weintraub *et al.* (9), by using either carboxyl- or methylene-C^{14}-labeled 2,4-D, demonstrated that the C^{14} from the applied 2,4-D in bean plants is incorporated into other substances within a few days. The radioactive carbon becomes distributed among a variety of plant constituents, including acids, sugar, dextrins, starch, pectin, protein and cell wall substances.

In a publication from this laboratory (2) it was found that with bean plants the radioactive 2,4-D was readily absorbed and the radioactivity was found in various parts of the bean plant. In a continuation of this study (6) two radioactive compounds, in addition to unchanged 2,4-D, were found in an 80% alcohol extract of the stems of bean plants treated with either α-methylene- or carboxyl-C^{14}-labeled 2,4-D. It seemed worthwhile to extend this work to some 2,4-D resistant plants. The results here report the absorption, translocation and metabolism of radioactive 2,4-D by corn and wheat plants.

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EXPERIMENTAL METHODS AND MATERIALS

The methods used for the combustion of the plant samples and the measurement of radioactivity have been described previously (2). A sample of carboxyl-C^{14}-labeled 2,4-D, with a specific activity of 5.3×10^6 counts per minute per mg, was synthesized in our laboratory and used in these experiments.

The corn plants (*Zea mays saccharata* var. Golden Bantam) and wheat plants (*Triticum vulgare* var. Hard Winter White) used in these experiments were grown under greenhouse conditions in potted soil. A 95% ethanol solution containing 0.1% radioactive 2,4-D and 0.5% Tween-20 (w/v) was used for treatment. The treatment was made to plants which were grown to the third or fourth leaf stage. Unless otherwise indicated, the corn plants received 20 μg of 2,4-D on the tip of the first or second leaf, while 10 μg were used in treating the wheat plants. In the study of absorption and translocation of C^{14}, the plants were harvested and sectioned into different parts as indicated in the tables. The sections were pooled and dried immediately in a vacuum oven at 60°C for 24 hours. An aliquot of each ground sample was analyzed for C^{14} by the usual wet combustion procedure. The activity of $BaCO_3$ was counted and corrected to zero thickness. Duplicate determinations were made from all $BaCO_3$ samples. In every case an accuracy of 5% or better was realized.

For the identification and determination of 2,4-D and other radioactive compounds, the fresh plant material was homogenized in a Waring blendor with 80% ethanol. The materials were quantitatively transferred to a volumetric flask by rinsing several times with 80% alcohol and made to 250 ml with the same solvent. The radioactivity of the extracts was determined by direct plating in a stainless steel cup and then converted into radioactivity expressed as $BaCO_3$ by using a conversion factor (6). The radioactivity of the alcohol-insoluble residues was determined by the usual wet combustion procedure. The total activity of the plant was obtained from the summation of activity of the alcohol-soluble and alcohol-insoluble residues. The amount of 2,4-D present in the plant tissue was determined by the technique of one-dimensional paper partition chromatography. The extract, containing enough radioactivity for an accurate measurement, was applied to a spot about 7 cm from the end of strips of Whatman #1 filter paper (1×22 in) by means of a fine eye dropper. Usually duplicate strips were run. One was developed with a phenol-

TABLE I

ACCUMULATION OF RADIOACTIVITY IN CORN SEEDLINGS HARVESTED AT DIFFERENT TIMES AFTER TREATMENT WITH 20 μG CARBOXYL-C^{14}-LABELED 2,4-D

PLANT PART	SPECIFIC ACTIVITY OF PLANT MATERIAL COUNTS/MIN/MG *				AVERAGE DRY WEIGHT PER PLANT PART MG				TRANSLOCATION AND ACCUMULATION OF C^{14} %			
	1 DAY	3 DAYS	5 DAYS	8 DAYS	1 DAY	3 DAYS	5 DAYS	8 DAYS	1 DAY	3 DAYS	5 DAYS	8 DAYS
1st leaf †	2.9	7.1	...	...	10.3	10.8	...	...	0.03	0.07	...	...
2nd leaf	...	...	3.8	1.9	...	...	30.8	26.9	...	...	0.12	0.03
3rd leaf	0.9	0.0	4.3	9.5	22.7	56.8	46.8	52.0	0.02	0.00	0.21	0.52
4th leaf	3.7	3.7	28.0	21.0	5.7	48.8	23.5	60.2	0.02	0.17	0.68	1.33
5th leaf	...	14.1	...	14.3	...	9.7	...	16.9	...	0.13	...	0.25
Stem & sheath	90.7	33.1	121.3	86.0	35.7	74.1	50.5	43.2	3.06	2.33	6.26	3.90
Root	9.2	4.1	10.4	15.0	126.5	148.5	160.5	131.6	1.10	0.58	1.73	1.83
Total	...	...	...	...	...	...	...	...	4.23	3.28	9.00	7.86

The plants harvested on the first and third days were treated on the second leaves and the plants harvested on the fifth and eighth days were treated on the first leaves. The treated leaves were discarded. Each group consisted of 10 plants which were grown to the fourth-leaf stage. (1 μg 2,4-D = 5370 counts per minute.)

* Counts/min/1 mg dry sample.

† Lowermost leaf.

H_2O and the other with an ammonium hydroxide-*n*-butanol (1 : 4) system. After drying, the chromatogram was cut into 1-cm sections starting from the original spot and the radioactivity of each section was determined directly. A Tracerlab windowless gas flow counter was used when the radioactivity of the chromatogram was low. The following expressions were used for the determination of relative radioactivity of compounds containing C^{14} in 80% alcohol extract and in calculation of the amount of 2,4-D present in fresh plant tissue:

$$\frac{\text{Radioactivity of the spot}}{\Sigma \text{ radioactivity of each section}} \times 100 = \text{Relative radioactivity in per cent}$$

$$\frac{\text{Total alcohol-soluble activity} \times \text{relative activity}}{\text{Specific activity of 2,4-D} \times \text{weight of fresh sample in gm}} = \text{Amount of 2,4-D per gm fresh sample}$$

RESULTS AND DISCUSSION

In all experiments with corn and wheat seedlings, the accumulation of C^{14} activity in the nontreated parts of the plants was much lower than was found in bean plants (2) as indicated in tables I and II. Corn plants translocated and accumulated slightly more C^{14} than did the wheat seedlings. When 2,4-D was applied on the tips of the lowermost leaves of corn plants, the absorption and translocation of C^{14} was found to be somewhat higher than when 2,4-D treatment was made on the second lowermost leaves. Most of the activity accumulated in the root, stem and sheath of the treated corn plants. The growing leaves (4th or 5th) usually showed a higher specific activity than was found in the other leaves, which indicated that the transport of C^{14} to the apical portion of the plant is more rapid. In a separate experiment, which is not included in table I, the results indicated that when 2,4-D was applied to the first leaf the sheath of the first leaf had a specific activity of 447 cpm per mg dry sample while the sheath of the second leaf contained 8.7 cpm per mg, and of the third leaf 9.4 cpm per mg. The great difference in C^{14} activity between the sheaths may be due to impedients in them or in the stems which interfere with the transport of 2,4-D

TABLE II

ACCUMULATION OF RADIOACTIVITY IN PARTS OF WHEAT SEEDLINGS WHICH WERE HARVESTED AT DIFFERENT TIMES AFTER TREATMENT WITH 10 μG CARBOXYL-C^{14}-LABELED 2,4-DICHLOROPHENOXYACETIC ACID TO THE 1ST LEAF

PLANT PARTS	SPECIFIC ACTIVITY OF PLANT MATERIAL COUNTS/MIN/MG *		AVERAGE DRY WEIGHT PER PLANT PART MG		TRANSLOCATION AND ACCUMULATION OF C^{14} %	
	4 DAYS	7 DAYS	4 DAYS	7 DAYS	4 DAYS	7 DAYS
2nd & 3rd leaves †	31.3	58.1	20.1	5.7	1.31	0.69
Stem & sheath	23.6	11.2	12.6	9.9	0.62	0.23
Root	5.8	3.2	9.1	13.5	0.11	0.09
Total	...	...	...	...	2.04	1.01

Each group consisted of 20 plants. Plants were grown to third-leaf stage. (1 μg 2,4-D = 5370 counts per minute.)

* Counts/min/1 mg dry plant sample.

† 2nd & 3rd lowermost leaves.

or radioactive compounds from the treated leaf to the nontreated one.

In the experiment with wheat seedlings, only between 1 to 2% of applied C^{14} activity was found in plant parts from which the treated leaf was excluded. Most of the activity was present in the growing leaves, less in the stem and sheath, and least in the root. This pattern differed slightly from that found in the experiment with corn plants.

A fairly high accumulation of radioactivity in the untreated part of the treated leaf in the corn plant, as indicated in table III, revealed that 2,4-D was also readily absorbed by the resistant plant. This free 2,4-D may be found in a relatively large concentration in the treated leaf of the plant harvested after one day of treatment. It was comparable to the concentration which was found in bean stems (6) yet no physiological effect was observed in the corn plant. The uneven distribution of 2,4-D and also the difference in relative concentration found in the alcohol extract of plant parts in both one-day and three-day samples suggested first, that the transport of 2,4-D toward apical parts (4th leaf) is quicker than its movement into the root; and second, 2,4-D may be incorporated into other compounds more rapidly in the root than in the stem. The 2,4-D concentration dropped considerably in all plant parts of samples harvested three days after treatment, while at the same time, the C^{14} activity in other alcohol-soluble compounds and the alcohol-insoluble residue was increased.

The results of the determination of free 2,4-D and other 80% alcohol-soluble compounds containing C^{14} in the 2,4-D treated leaf (the treated section was excluded) of both corn and wheat plants are shown in figures 1 and 2. The corn plants were harvested in groups of four at 1, 3, 7, 9 and 14 days and the wheat plants were harvested in groups of ten at 1, 4 and 7 days after treatment. Only the treated leaves were used in this study. Pure radioactive 2,4-D was found to have an R_f value between 0.72–0.76 in the phenol-H_2O system and 0.62–0.64 when butanol-ammonium hydroxide (4:1) was used. A radioactive spot with an identical R_f value developed with either solvent was found in the alcohol extract of the corn and wheat plants. The radioactive spot was eluted with alcohol and was subsequently co-chromatographed with a known amount of 2,4-D in both solvents. In each chromatogram, only one spot corresponding to the 2,4-D was found. Further evidence of the identity of the 2,4-D was given when a known amount of 2,4-D was added to the plant extract. The subsequent determination of the radioactivity of the paper chromatogram gave an increase in the activity of the 2,4-D spot which corresponded to the amount of 2,4-D added.

It is evident that the 2,4-D becomes a component of several compounds. The compound in the corn and wheat leaves which is quantitatively most important is one which we have designated as unknown 3, with an R_f value of 0.83 in phenol-H_2O and 0.85 in butanol-ammonium hydroxide. The amount of this compound increased with time for nine days to 58% and then remained relatively constant in the corn plants. Concomitantly the level of 2,4-D decreased. A significant but much smaller amount of the compound designated as unknown 1 was present. This compound has an R_f value 0.57 in phenol-H_2O and 0.04 in butanol-ammonium hydroxide and was the major radioactive alcohol-soluble compound formed in bean plants (6). In the time course study with wheat seedlings, a maxi-

TABLE III

DISTRIBUTION OF RADIOACTIVITY IN CORN PLANTS AFTER TREATMENT WITH 20 μG CARBOXYL-C^{14}-LABELED 2,4-D TO EACH PLANT. 2,4-D WAS APPLIED ON THE TIPS OF THE 2ND LEAVES

	TOTAL RADIO-ACTIVITY IN PLANT PARTS	80% ALCOHOL SOLUBLE		80% ALCOHOL INSOLUBLE		C^{14} FROM 2,4-D IN ALCOHOL EXTRACT OF PLANT PARTS †	2,4-D PER 1 GM FRESH SAMPLE
		TOTAL RADIO-ACTIVITY	SPECIFIC ACTIVITY *	TOTAL RADIO-ACTIVITY	SPECIFIC ACTIVITY *		
	$cpm \times 10^3$	$cpm \times 10^3$	$cpm \times 10^3/gm$	$cpm \times 10^3$	$cpm \times 10^3/gm$	%	μg
Expt. 1. Harvested after one day of treatment, 20 plants in this group							
Untreated part of treated leaf, 4.2 gm	240.2	236.0	56.0	4.2	1.00	69	7.21
2nd leaf sheath, 3.8 gm	81.7	80.5	21.2	1.2	0.32	57	2.25
4th leaf, 6.3 gm	17.3	16.5	2.6	0.75	0.12	90	0.43
Stem, 2.1 gm	20.4	19.9	9.6	0.54	0.26	82	1.46
Root, 27.8 gm	40.1	36.1	1.3	4.0	0.14	56	0.14
Expt. 2. Harvested after three days of treatment, 18 plants in this group							
Untreated part of treated leaf, 4.6 gm	111.0	104.0	22.6	7.0	1.50	13	0.55
Stem & sheaths, 13.4 gm	44.2	27.7	2.1	16.5	1.10	56	0.22
Root, 26.8 gm	11.0	7.0	0.26	4.0	0.15	10	0.005

(1 μg 2,4-D = 5370 counts per minute.)
* Counts per minute per 1 gm fresh sample.
† Data obtained from paper chromatograms.

mum formation of unknown 3 was observed after 24 hours of treatment. Both unknown 1 and unknown 2 (6) were also present.

In an earlier report from this laboratory (6) it was found that the unknown 1 formed in bean plants is a 2,4-D complex since treatment with dilute acid or emulsin released free 2,4-D. Unknown 3, when treated in a like manner, also gave free 2,4-D. Therefore, it is tentatively suggested that this complex may be similar in nature to unknown 1, but that it may involve conjugation of 2,4-D with different compounds. The formation of the complex as was suggested earlier might be a detoxification mechanism in bean plants. The formation of unknown 3 might be a similar mechanism used by the corn and wheat seedlings. At any rate, there is a marked difference in the type of compounds formed after treating the bean plant (susceptible) on one hand and the corn and wheat plants (resistant) on the other.

At the present date, no information is available regarding the identity of all of these unknown compounds. However, in preliminary work, a small but isotopically pure sample of residue-C^{14}-labeled unknown 1 and unknown 3 had been isolated and hydrolyzed. The unknown 1 was hydrolyzed by boiling with 2 *N* HCl for 2 hours while with unknown 3, 2 *N* $Ba(OH)_2$ was used. After the solutions were neutralized, the paper chromatograms of these hydrolysates were developed with phenol-H_2O or butanol-ammonium hydroxide. Many radioactive spots found on these chromatograms revealed the complexity of both unknown 1 and unknown 3 molecules. (Radioactive spots found in chromatograms from residue-C^{14}-labeled unknown 1 developed in phenol-H_2O were at R_f 0.00, 0.21, 0.28, 0.35, 0.42, 0.55 (unk. 1), 0.68, 0.74 and 0.84; radioactive spots found in chromatograms from residue-C^{14}-labeled unknown 3 developed in butanol-ammonium hydroxide were at R_f 0.03, 0.11, 0.34, 0.48, 0.62, 0.70, 0.85 (unk. 3) and 0.99.) The residue-C^{14}-labeled unknown 3 was prepared from corn seedlings which were exposed to $C^{14}O_2$ for 3 days under greenhouse conditions and then treated with non-radioactive 2,4-D. The treated leaves were harvested after 24 hours and homogenized with 80% alcohol. Paper chromatograms of this alcohol extract were prepared and the radioactive spot corresponding to unknown 3 was cut off and eluted with alcohol. The eluate was redeveloped with a different solvent system. Three different solvent systems were used, namely, phenol saturated with water, ammonium hydroxide and *n*-butanol (1 : 4) and *n*-butanol-propionic acid-H_2O (12 : 5.6 : 8). This process was repeated until only one radioactive spot appeared. The residue-C^{14}-labeled unknown 1 was prepared from bean plants in the same manner.

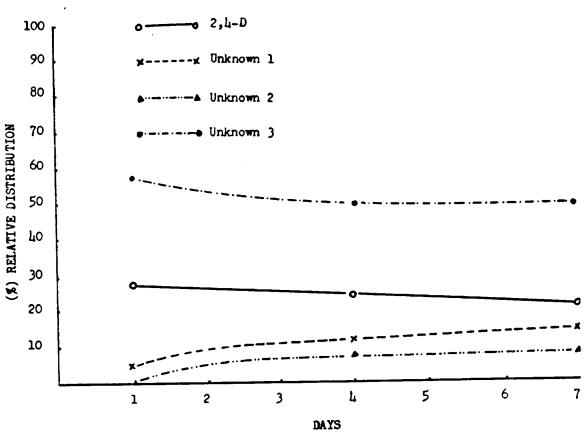

FIG. 1. Per cent distribution of 80%-alcohol-soluble radioactive compounds present in the leaf of corn seedlings treated with 20 μg of carboxyl-C^{14}-labeled 2,4-D. Plants harvested after varying intervals of time.

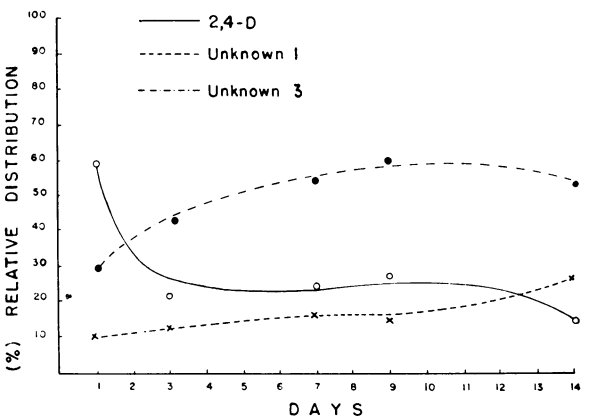

FIG. 2. Per cent distribution of 80%-alcohol-soluble radioactive compounds present in the leaf of wheat seedlings treated with 10 μg of carboxyl-C^{14}-labeled 2,4-D Plants harvested after varying intervals of time.

Further work regarding the identity of unknown 1 and unknown 3 is in progress and will be reported in a separate paper.

SUMMARY

A study has been made of the absorption and translocation of carboxyl-C^{14}-labeled 2,4-D by the corn and wheat plants. It was found that this growth regulator is absorbed by the monocots but at a slower rate in comparison with bean plants. The translocation of C^{14} to the apical portion of the corn and wheat plants was very slow. There seems to be a block in the intercalary meristem of the monocotyledonous stems and leaves of young plants.

At least a part of the applied 2,4-D is incorporated in a few days into other compounds. Two major compounds which we designated as unknown 1 and unknown 3 were found in the 80% alcohol extracts of leaves from both 2,4-D treated corn and wheat plants. The latter unknown, which was found in a greater concentration, indicated a difference in metabolism of 2,4-D from that found in the bean plants.

The identity of the major unknown radioactive compounds has not been established. However, the complexity of the molecule of these two 2,4-D conjugates has been demonstrated.

The question of how the monocotyledonous and

dicotyledonous plants react differently on 2,4-D treatment has not yet been answered but the slower absorption and translocation within the monocots and the rate of detoxification (formation of 2,4-D complex) may be the contributing factors.

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INHIBITION OF GROWTH OF CHLORELLA PYRENOIDOSA BY BETA-EMITTING RADIOISOTOPES[1, 2]

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Many studies have been made during the past 50 years of the effects of ionizing radiations on plants, animals and microorganisms, but to our knowledge no extensive studies have been made on the effect of these radiations on Chlorella. Neither have attempts been made to grow this organism in the presence of radioactive elements, except in tracer experiments. Mutations of Chlorella have been produced, however, by Granick (3) with X-ray.

This paper reports (1) the methods used in growing algae in the presence of beta-emitting radioactive elements, and (2) the effect of several concentrations of tritium oxide, phosphorus-32, sulphur-35 and the equilibrium mixture Sr^{90}-Y^{90} on the growth of Chlorella. The results presented, which are largely descriptive, are preliminary to other extensive studies now being made on the effect of tritium oxide on cellular processes and biosynthetic reactions of Chlorella.

Materials and Methods

The culture of Chlorella, #7516 of the American Type Culture Collection, was grown on agar slants and transfers were made at monthly intervals. Algae to serve as inocula in experiments were transferred from slants at approximately 6-week intervals to one liter of sterile inorganic nutrient solution (Knop, modified). A modified, low-form, 3-liter culture flask mounted on an electrically driven rocker platform served as the container. The algae were grown under white fluorescent light of 100 to 300 fc, and aerated with a stream of 8 to 9% CO_2 in air. After 4 to 6 days' growth 900 ml of solution were drawn off 2 to 3 times a week and replaced with sterile nutrient solution. The algae withdrawn served as a source of inoculum for the experiments with the beta-emitting isotopes.

Algae were grown in the presence of each of the four isotopes in 20×150 mm tubes. The nutrient solution was that of Myers (4). Nutrient solutions containing tritium oxide were prepared as follows. Fifteen ml of nutrient solution, adjusted to pH 5.5, and distilled water were added to each tube. After autoclaving the solutions were cooled and tritium oxide was added. Cells to serve as inocula were removed from the continuous culture, centrifuged and then suspended in sterile nutrient solution. The suspension was adjusted to an optical density of 0.60 (660 mμ, Beckman Model DU Spectrophotometer) and then 1.0 ml of the suspension was added to each tube. The final volume in each tube was 18.0 ml.

Nutrient solutions containing the other beta-emitting isotopes were prepared somewhat differently. Six ml of nutrient solution were added to each tube. After sterilization 0.01 to 0.02 ml of 0.1% methyl red in alcohol was added. The radioactive isotope was added,

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