AN ABSTRACT OF THE THESIS OF

John Butts Bourke (Name) for the Ph. D. (Degree) in Biochemistry (Major)

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Title Studies on the Metabolism of n-Propyl N,N-Di-n-Propyl Thiolcarbamate in Legumes

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Abstract approved (Major professor)

The absorption, translocation and metabolism of a new herbicide, n-propyl-C^{14} N,N-di-n-propyl-thiolcarbamate (PDPC) were studied in both peanut and soybean plants. These studies were carried out on plants of different ages and treated at two rates of application. The residue content of the plants was also determined from germination to maturity.

Radioautograms demonstrated a general distribution of radioactivity throughout the plant although the above ground portions contained a somewhat higher content. Increasing the rate of application resulted in a greater uptake of PDPC but this was not proportional. The uptake increased somewhat faster than the rate of application.

The residue content of both soybean and peanut seedlings was extremely low at harvest. The maximum concentration, which was reached two to three weeks after treatment, was quickly reduced. At the end of the 26 week growing period all the plant parts had negligible residue levels. Gas chromatography was used to establish the validity of the residue determining procedures.

Experiments carried out on the effect of PDPC on germinating seedlings and its subsequent breakdown demonstrated the inhibitory effect of this compound on its own metabolism. Seedlings allowed to imbibe PDPC demonstrated a reduced ability to break it into ethanol soluble components. The ability to convert the herbicide into respiratory carbon dioxide was also reduced as was the incorporation into cellular tissue. The absorption of PDPC, however, was not affected.

Age appears to affect the ability to break down PDPC. Germinating seeds were inhibited in their metabolism of the herbicide to a much larger extent than seedlings which were allowed to germinate in water before being treated. This applied not only to the breakdown of PDPC into metabolites but also the metabolism of the ethanol soluble intermediates. The seedlings germinated in the presence of PDPC, however, rapidly recover and within eight to ten days inhibition is small.

Generally it may be stated that while uptake and translocation of n-propyl-C^{14} N,N-di-n-propylthiolcarbamate are not materially affected by PDPC at the concentrations studied, there is a inhibition to the metabolism of this compound when applied as a pre-emergence herbicide. The seedlings rapidly recover and the residue content at harvest is low.

STUDIES ON THE METABOLISM OF n-PROPYL N,N-Di-n-PROPYL THIOLCARBAMATE IN LEGUMES

by

John Butts Bourke

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APPROVED:

Redacted for Privacy

Associate Professor of Agricultural Chemistry

In Charge of Major

Redacted for Privacy

Chairman of Department of Chemistry

Redacted for Privacy

Chairman of the School Graduate Committee

Redacted for Privacy

Dean of Graduate School

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Introduction

Herbicides had their beginning in 1934 when Koyl isolated a compound from human urine which was markedly active in promoting cell elongation in plants. This compound, later isolated from yeast, was found upon purification to be indole-3-acetic acid.

Koyl's discovery was followed in 1935 by Zimmerman's discovery of α-naphthaleneacetic acid and in 1938 by Irvine's 2-napthoxy-acetic acid, all of which exhibited growth regulating properties. Finally in 1942 the age of herbicides was opened by Zimmerman who first reported the remarkable growth regulating properties of 2,4-dichlorophenoxyacetic acid (2,4-D) (7, p. 44-52).

Today there are many chemicals in commercial use as weed killers which are applied as dusts, sprays, and emulsions to the foliage as well as to the soil. It is esimated by the United States Department of Agriculture (U. S. D. A.) that these are applied to about 90 million acres or one tillable acre out of 20 in the 48 contiguous states.

One group of soil applied herbicides used primarily as pre-emergence herbicides is the carbamates. First described in 1945 by Templeman these herbicides now find wide usage (31, p. 10). This group includes among others, isopropyl N-phenyl-carbamate (IPC) and its phenyl chlorinated derivative (CIPC), 2-chloroally-di-ethyl-di-thiocarbamate (CDEC) or "Vegadex", and ethyl N,N-di-n-propyl thiolcarbamate (EPTC). All are particularly effective in controlling

various grasses in many broad leaf crops. Of the carbamates the group to be considered here is the thiolcarbamates, and in particular, n-propyl-N,N-di-n-propyl thiolcarbamate, (PDPC). Due to the length of the chemical name of this herbicide, it will hereafter be designated PDPC. This abbreviation has not to the author's knowledge been used before. This compound belongs to the family of analogs of EPTC which also includes the new herbicide, n-propyl-N,N, -ethyl-n-butyl thiolcarbamate, (Tillam).

EPTC, the first of this family, was originally described by Antognini of the Stauffer Chemical Company in 1957. At that time it was found to be effective against all grasses and many broad leaf weeds when applied at rates of three to ten pounds per acre before the crop emerged. The action of the chemical was found effective over a wide range of soil temperatures, soil types and rainfall. It has a low acute mammalian toxicity, the oral LD 50 to male albino rats being reported at 1.63g/Kg and the dermal LD 50 is 2.64g/Kg in rabbits (2, p. 3-1).

EPTC has since been found effective on weed control in many crops. Satisfactory control of weeds in potatoes (1, p. 146-148) was reported while no residue was found in the harvested crop (24, p. 216-217). Crops of navy and kidney beans were also satisfactorily protected from weed competition with EPTC (21, p. 318-324). Weeds were controlled in tomatoes but some injury resulted (1, p. 146-148) while wild oats were controlled in sugar beets. A wide range of application rates resulted in a better than 85% kill (40,

p. 268-278). EPTC has been used in the control of weeds in ornamental crops such as gladioli (8, p. 53-55) but some damage to the bulb was found (13, p. 5-7) at rates most effective for weed control. Protection in alfalfa and birdsfoot trefoil does not appear satisfactory; however, the crop is not harmed nor is yield decreased (38, p. 291-299) (16, p. 859).

Of particular concern in this thesis is the absorption, translocation, detoxification and metabolism of thiolcarbamates in peanuts. Various herbicides of the triazine and urea type have been tried with some success in this crop (33, p. 175-184). MCPA and MCPB were most adequate. EPTC was found to give 80% control with little or no harm to the crop (25, p. 139-144). Mixtures of herbicides allowed the use of lower rates of the component herbicides and increased the spectrum of weeds controlled.

Several analogs of EPTC have been used in the control of weeds. In general the symmetrically substituted thiolcarbamates were ineffective while the unsymmetrically substituted compounds were more effective. Tertiary butyl dipropylthiolcarbamate gave good control of several annual and perennial grass weeds with no injury in a wide variety of vegetables. The legumes; peas, lima beans and soybeans, were damaged by this herbicide (35, p. 8985).

n-Propyl N,N-ethyl-n-butyl thiocarbamate was as effective as EPTC in controlling several weed species in potatoes; however, the efficiency was somewhat less than that of simazine (34, p. 269-276).

PDPC was found to be only slightly less effective than EPTC in the control of watergrass in corn. When applied at two, four, and

eight pounds per acre as a pre-emergent herbicide, PDPC was found to damage up to 9% of the corn, although the per acre yield of the treated was still higher than in the untreated controls (22, p. 12-14). Both EPTC and PDPC were found to control all weeds except morning glory in tobacco when applied at three pounds per acre in three post-planting applications. No effect on tobacco yield or flavor could be detected (14, p. 115-118) (30, p. 63-68).

Any study considering the effectiveness of the thiolcarbamates must include analysis of the factors affecting the availability of the compound to the plant. It has been shown that the persistence of EPTC in the soil is influenced by the solvent carrier system, the soil temperature and the addition of surfactants to the carrier system (12, p. 463-476). Preliminary studies have shown that placement of EPTC in intimate contact with the soil particle by incorporation beneath the surface increased its retention and thus prolonged the action on the plants. Shallow incorporation has been shown to be much more effective than deep incorporation (39, p. 105-111). For the greatest effectiveness it has been demonstrated (30, p. 63-68) that EPTC must be uniformly and completely incorporated immediately following application.

The influence of soil moisture on EPTC activity is quite pronounced. Much more effective control was obtained using dry or moist soil rather than wet soil (26, p. 52-56). Additional effectiveness was obtained where weed control was increased with cultivation and irrigation. Of the thiolcarbamates Tillam is less

affected by temperature change; PDPC somewhat more, and EPTC the most (20, p. 3). Leaching occurs in the same order.

The loss of volatile EPTC is apparently reduced by its absorption on soil particles. A part of the volatile EPTC is bound so strongly as to be unavailable to the plant. Air dried soil adsorbs more herbicide than soil with a moisture content near field capacity (3, p. 88-90) (19, p. 564-574). It has also been shown that the amount of herbicide required for effective weed control must be increased as the organic content of the soil increases (44, p. 9-14).

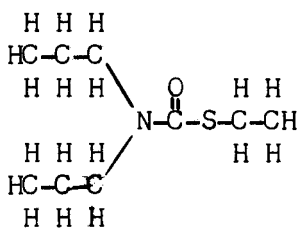
Very little has been reported concerning the effect of the thiolcarbamates on soil microorganisms. EPTC, however, has been shown to reduce the respiration of soil microorganisms, the inhibition differing in various soil types (9, p. 589-598). Methyl dithiocarbamate has been shown to inhibit the action of soil urease (29, p. 9590) but no mechanisms for these observations have been proposed.

In addition to their value as herbicides the thiocarbamates have found some application as nematocides. Ethyl dimethyl-thiocarbamate was successful in the control of the root-knot nematode (5, p. 4818). Chlorobenzyl-thiocarbamate used as an insecticide was effective in the control of spidermites (35, p. 8985) while several dithiocarbamates were very effective in the control of the fungus Septoria in oats (10, p. 620-627). The phenoxypropylthiocarbamates are showing promise as tranquilizers (15, p. 919-924)

while ethyl di-ethylaminoethylisopropyldithiocarbamate is proving as effective as quinine in the treatment of malaria (37, p. 2758).

Since very little information was found in regard to absorption, translocation and metabolism of these herbicides this study was undertaken and only three of these compounds will be considered here.

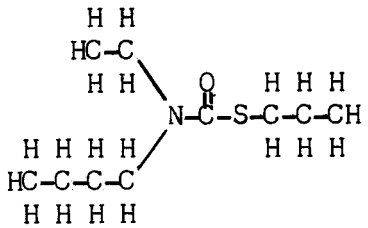
Ethyl N, N-di-n-propylthiolcarbamate (EPTC). EPTC has a boiling point of 127°C at 20 mm of Hg, a d^{30} of 0.9543 g/ml and an $n^{30}D$ of 1.4755 (36, p. 54). It is a clear liquid having a vapor pressure of 1.97×10^{-2} mm of Hg at 24°C and a ΔH vaporization of 14.5 Kcal/mole. The solubility in water at 25°C is 375 ppm and the ΔH solution is -3930 cal/mole. The structure of EPTC is:



EPTC may be prepared by stabilizing at 5 to 200 μ a dispersion of 16.9 g of sodium in 50 ml of xylene by oleic acid and 50 g of ethyl mercaptan. Xylene is slowly added under argon at 25 to 36°C. The sodium mercaptide is refluxed while 120 g of di-n-propylcarbamoyl chloride is added over a period of 17 minutes. The mixture is then boiled for three hours. Removal of the toluene gives 90% EPTC (41, p. 300).

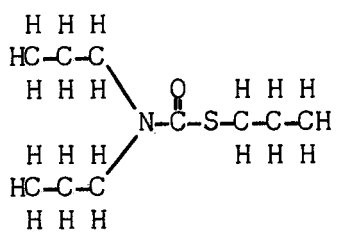
n-Propyl N, N-ethyl, n-butyl thiolcarbamate (Tillam). Tillam has a vapor pressure of 4.8×10^{-3} mm of Hg at 24°C and a ΔH

vaporization of 12.0 Kcal/mole. Its solubility in water is 90 ppm at 25°C with a ΔH solution of -2740 cal/mole (20, p. 3) (43, p. 9-14). The structure of Tillam is:



Tillam can be prepared in the same manner as EPTC by the substitution of propylthiol for ethylthiol and ethyl butyl carbamoyl chloride for di-n-propylcarbamoyl chloride (41, p. 300).

n-Propyl N, N-di-n-propyl thiolcarbamate PDPC. PDPC has a vapor pressure of 5.41×10^{-3} at 24°C and a ΔH vaporization of 13.5 Kcal/mole. Its solubility in water is 109 ppm at 24°C and the ΔH of solution is -3240 cal/mole (20, p. 3). The structure of PDPC is:



PDPC is also prepared in the same manner as EPTC with the substitution of propylthiol for ethylthiol. This gives as 85% yield of PDPC having a boiling point of 149 to 150°C at 30 mm of Hg (41, p. 300).

The analysis for the thiolcarbamates has been designed for EPTC but works equally well on the other analogs.

Batchelder and Patchett (4, p. 214-216) developed a coloremetric method for the determination of EPTC residues in crops which depended on the hydrolysis of EPTC by concentrated sulfuric acid to dipropylamine. This in turn was reacted in a hexane-water system with CS_2 in the presence of ammonia and copper to form the cupric dithiocarbamate complex. This complex was then determined in the hexane solution at 440 mμ . The determination of 0.02 ppm of residue was found possible. In this determination hexane was used as the extraction media.

Gutenmann and Lish (24, p. 216-217) improved the extraction method by using a hexane-isopropanol-water extraction system. This resulted in a system which was less prone to the formation of troublesome emulsions.

One of the first methods for the direct extraction of EPTC-S^{35} residues from crops was developed by Fang (17, p. 770-771) and utilized steam distillation techniques. The sample was steam distilled and the distillate continuously extracted with iso-octane using a volatile oil still. The radioactivity in the iso-octane was then determined and the quantity of EPTC computed from the specific activity.

Hughes and Freed (28, p. 381-382) developed a quantitative method for measuring EPTC utilizing gas liquid chromatography. The sample to be analyzed was extracted by the method of Fang (17, p. 770-771) and the residue determined with a Beckman Model GC-2 gas chromatograph containing a four foot Apiezon-L column packed with

celite. The sensitivity of their detector was found to limit the sensitivity of the determination to 10 μg. A similar method was developed by Hindin and Dunston (27, p. 8773) using a six foot column of Chromosorb W coated with Dow 11 silicone grease.

A limited amount of material is available on the absorption, translocation, and distribution of EPTC in plants; however, nothing is available concerning the other thiolcarbamates.

Crafts (11, p. 14-17) using labeled EPTC showed that the herbicide was rapidly absorbed through the leaves and translocated to the roots. Fang (18, p. 295-298) applying S^{35} labeled EPTC to the soil before germination of the seeds showed an uptake of the label and translocation through the plant. The above-ground portion of the plants contained more S^{35} than the roots. Increased application of the herbicide resulted in increased absorption but the increase was not proportional.

Yamaguchi (45, p. 374-380) applied EPTC-S^{35} to the roots and leaves of a number of plants. The pattern of absorption, translocation, and distribution was found to be similar in both tolerant and susceptible species. Leaf application resulted in a movement primarily to the growing areas while root application resulted in distribution throughout the plant.

The effect of the thiolcarbamates on metabolism has only occasionally been studied. Stevens et al. (42, p. 215-222) found that there was no significant effect on acetate absorption or metabolism following treatment of pea seedlings with EPTC. Bourke

(7, p. 44-52) found no significant decrease in glucose absorption following treatment with EPTC; however, glycolysis was somewhat inhibited. No effect was detected in the anabolic processes.

In general the research work on the thiolcarbamates has revolved around their field testing with very little attention to their effect on the biochemical processes.

In order for a herbicide to be effective not only must it be selective, (i.e., destroying the unwanted weeds and not harming the crop to be protected), but it must also be metabolized by the plant in order not to leave any undesirable residue. This thesis is concerned with the residues found throughout the life cycle of the plant.

The amount of residue found depends on a number of factors such as absorption from the soil, translocation, distribution, breakdown into metabolites and final conversion to carbon dioxide. All of these functions will be examined with relation to rate of herbicide application and age at which treatment occurred. In addition the effect of pre-exposure to PDPC will be studied to determine if a preadaptation or inhibition is induced.

Analysis of such datum as biological half life will aid in determining the length of time necessary after treatment for reduction of residue content to a safe level. Comparison of the results obtained from PDPC experiments with those from the other thiolcarbamates will give a broader picture of the overall effect of these compounds on the physiological and biochemical processes occurring in plants.

Materials and Methods

All the chemicals used in this thesis work were of the highest purity available. The compound under investigation, n-propyl-1-C^{14} n,n-di-n-propyl thiolcarbamate (PDPC-C^{14}) was supplied by Research Specialties Co. in two millicurie units having a specific activity of 3.11 millicuries per millimole. Stauffer Chemical Company supplied the carrier PDPC which had a purity of 98.8%.

Residue Determination. Newberg silty loam was sifted and placed in 25 pound freezer tins 14 inches high and 9.75 inches in diameter. The area of each was calculated to be 30.635 square inches or 4.883×10^{-6} acres. Fourteen peanut seeds, variety North Carolina 4X, or 25 soybean seeds, variety Lee, were planted in each pot. A total of eight pots were used for each crop, of which two were controls, three treated at the rate of one pound per acre and three at four pounds per acre. Each of the test pots was treated with 33.2 μc of radioactive PDPC plus enough carrier to bring the concentration to the desired level. The herbicide was dissolved in 100 ml distilled water and applied by means of a plastic squirt bottle. Table I shows the treatment scheme.

The plants were watered daily and given occasional applications of nutrient solution (6, p. 55) which contained the following compounds per liter of water: $Ca(NO_3)_2.4H_2O$ - 1.18g; KNO_3 - 0.51g; KH_2PO_4 - 0.14g; $MgSO_4.7H_2O$ - 0.46g; $FeSO_4.7H_2O$ - 0.005g. One ml of a minor element solution was added to each liter of the above nutrient

Table I

Planting and Treatment Scheme

Crop	Pot	Number Seeds	Concentration	μc PDPC-C^{14}	mg PDPC-C^{14}	mg Carrier PDPC
Peanut	1	19	0	0	0	0
"	2	19	1lb/A	33.2	2.214	0
"	3	19	4lb/A	33.2	2.214	6.642
"	4	19	1lb/A	33.2	2.214	0
"	5	19	4lb/A	33.2	2.214	6.642
"	6	19	1lb/A	33.2	2.214	0
"	7	19	4lb/A	33.2	2.214	6.642
"	8	19	0	0	0	0
Soybean	1	28	0	0	0	0
"	2	28	1lb/A	33.2	2.214	0
"	3	28	4lb/A	33.2	2.214	6.642
"	4	28	1lb/A	33.2	2.214	0
"	5	28	4lb/A	33.2	2.214	6.642
"	6	28	1lb/A	33.2	2.214	0
"	7	28	4lb/A	33.2	2.214	6.642
"	8	28	0	0	0	0

solution. The minor element solution contained the following compounds per liter of water: H_3BO_3 - 0.6g; $MnCl_2.4H_2O$ - 0.4g; $ZnSO_4$ - 0.05g; $CuSO_4.5H_2O$ - 0.05G; $H_2MoO_4.4H_2O$ - 0.02g.

Pests were controlled using TEPP (tetraethyl pyrophosphate) smoke generators and tetradane sprayed on the foliage. The peanut seeds were treated with Arasan 75 fungicide before planting, while the soybean seeds were untreated.

The greenhouse temperature was maintained at 80° F (both night and day) during the entire experiment. The light was supplemented with Sylvania VHF Gro-Lux flourescent lights and maintained at a 14 hour day. Plants were harvested according to the schedule shown in Table II by digging up the entire plant as long as this was possible. When the plants became too large to remove intact they were clipped off at ground level. The final harvest was made by digging up the entire plant in order to obtain both the roots and nuts.

The harvested plants were dissected into foliage, stems, roots, cotyledons, seed and seed pod. The plants were then weighed and dried in a vacuum oven at 20 to 30 mm Hg at 60° C. The dried plants were reweighed and ground in a Wiley Micromill in order to enable them to pass through a 20 mesh sieve.

Table II

Plant Harvest Schedule

Peanut Harvest

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
9/27	2	control	dig	4		foliage	6.80	0.89
						stem	10.3	1.21
						root	5.35	0.41
	2	1lb/A	dig	5		foliage	6.30	0.82
						stem	11.2	1.48
						root	7.2	0.63
	2	4lb/A	dig	5		foliage	5.8	0.78
						root	12.4	1.77
						stem	8.5	0.84
10/3	3	control	dig	4		foliage	9.00	1.21
						stem	11.78	1.53
						root	3.56	0.41
	3	1lb/A	dig	7		foliage	15.05	1.99
						stem	20.15	2.46
						root	9.83	1.13
	3	4lb/A	dig	5		foliage	8.04	1.20
						stem	12.75	1.64
						root	8.25	0.85
10/17	5	control	dig	4		foliage	12.87	2.05
						stem	16.18	2.38
						root	3.27	0.62

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
	5	1lb/A	dig	5		foliage	16.10	2.65
						stem	19.52	2.85
						root	6.29	0.87
	5	4lb/A	dig	5		foliage	14.49	2.42
						stem	17.69	2.59
						root	5.03	0.81
10/31	7	control	dig	4		foliage	14.30	2.17
						stem	17.05	2.33
						root	5.51	0.88
	7	1lb/A	dig	6		foliage	32.95	5.28
						stem	32.82	6.69
						root	6.92	1.12
	7	4lb/A	dig	6		foliage	38.60	5.67
						stem	37.52	5.43
						root	10.41	1.30
11/21	10	control	dig	4		foliage	34.44	5.58
						stem	31.37	5.06
						root	4.37	0.98
	10	1lb/A	dig	6		foliage	52.44	9.29
						stem	47.35	8.37
						root	7.37	1.65
	10	4lb/A	dig	6		foliage	44.00	7.64

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
						stem	41.41	6.51
						root	7.49	1.58
12/19	14	control	clip	4		foliage	43.19	7.81
						stem	42.84	8.18
						nut, stem	7.14	1.24
	14	1lb/A	clip	6		foliage	46.42	9.17
						stem	46.73	10.85
						nut, stem	4.21	0.82
	14	4lb/A	clip	6		foliage	53.56	10.93
						stem	55.00	11.68
						nut, stem	2.11	0.53
3/13	26	control	dig	4		foliage		2.18
						stem		4.29
						root		0.21
						nut (6)		2.105
						nut, coat & stem		.495
	26	1lb/A	dig	6		foliage		8.04
						stem		8.40
						root		1.105
						nut (14)		9.00
						nut, coat & stem		3.16
	26	4lb/A	dig	6		foliage		7.59

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
						stem		8.51
						root		1.03
						nut (9)		4.875
						nut, coat & stem		1.54
				Soybean Harvest				
11/23	2	control	dig	10		foliage	2.98	0.34
						stem	6.13	0.435
						root	2.05	0.14
						cotyledon	3.50	0.30
	2	1lb/A	dig	14		foliage	4.41	0.64
						stem	7.66	0.65
						root	2.33	0.18
						cotyledon	4.31	0.40
	2	4lb/A	dig	14		foliage	4.26	0.63
						stem	7.19	0.64
						root	2.90	0.24
						cotyledon	4.68	0.40
11/30	3	control	dig	8		foliage	4.10	0.70
						stem	6.81	0.69
						root	1.47	0.15

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
						cotyledon	1.54	0.18
	3	1lb/A	dig	11		foliage	5.38	1.10
						stem	8.08	1.06
						root	0.78	0.20
						cotyledon	1.89	0.23
	3	4lb/A	dig	11		foliage	6.23	1.22
						stem	9.17	1.20
						root	0.99	0.19
						cotyledon	1.77	0.21
12/7	4	control	dig	8		foliage	6.97	1.30
						stem	9.10	1.26
						root	2.04	0.15
	4	1lb/A	dig	12		foliage	10.63	2.16
						stem	13.51	2.30
						root	3.19	0.33
	4	4lb/A	dig	12		foliage	9.14	1.84
						stem	12.33	2.09
						root	2.95	0.30
12/14	5	control	dig	6		foliage	5.65	1.31
						stem	7.41	1.32
						root	1.16	0.13
	5	1lb/A	dig	9		foliage	10.44	2.40
						stem	12.36	2.50

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
						root	2.49	0.34
	5	41b/A	dig	9		foliage	10.32	2.24
						stem	13.84	2.71
						root	2.26	0.31
1/5	8	control	cut	6		foliage	13.52	2.94
						stem	12.98	3.90
	8	11b/A	cut	9		foliage	19.76	5.21
						stem	19.71	5.29
	8	41b/A	cut	7		foliage	15.77	3.70
						stem	14.32	3.40
2/1	12	control	cut	5		foliage	8.41	2.06
						stem	8.31	2.32
	12	11b/A	cut	5		foliage	13.64	3.65
						stem	13.97	4.11
	12	41b/A	cut	6		foliage	14.92	4.21
						stem	15.38	4.66
3/29	20	control	cut	4		foliage	5.43	1.43
						stem	6.51	1.72
						pod	5.25	1.48
						seed		
	20	11b/A	cut	6		foliage	7.90	2.15
						stem	9.23	2.60
						pod	6.54	1.69

Table II
(Continued)

Plant Harvest Schedule

Date	Age Weeks	Rate of Conc. Treatment	Method of Harvest	Number Plants	Code	Tissue	Fresh Weight	Dry Weight
						seed		
	20	41b/A	cut	5		foliage	8.25	2.51
						stem	10.35	3.00
						pod	11.81	3.44
						seed		
5/14	26	control	cut	8		foliage	15.90	4.80
						stem	21.80	7.80
						pod	5.10	4.10
						seed	4.95	4.50
	26	11b/A	cut	13		foliage	18.30	5.64
						stem	34.40	10.95
						pod	6.90	5.32
						seed	6.65	6.00
	26	41b/A	cut	11		foliage	17.00	5.25
						stem	29.60	10.25
						pod	12.70	7.40
						seed	12.20	8.90

A portion of the ground tissue was steam distilled and the distillate was continuously extracted with two ml of iso-octane. One-half ml of the iso-octane extract was then counted in the liquid scintillation counter. Corrections were made for counting efficiency and the μg quantities of PDPC calculated.

From the remaining ground tissue 100 mg was placed in a one inch stainless steel planchet and counted with a thin window gas flow geiger counter. Corrections were made for counting efficiency and the μg equivalents of PDPC were calculated.

Metabolism of PDPC by Seedlings. The metabolism of soybean seedlings was determined on non-imbibed seedlings and seedlings that had imbibed water for 48 or 72 hours. These experiments were carried out in the following manner.

To 50 ml beakers containing ten g of soil, six uniform soybean seeds were added. Fifty μg of PDPC in five ml of water was then added and the beakers placed in a seed germinator maintained at 25° C. Samples were collected daily for seven days and analyzed.

Soybean seeds to be imbibed were placed between moist paper towels for either 48 or 72 hours, then removed and selected for uniformity. Six of these uniform 48 hour imbibed seeds were placed in a beaker containing ten grams of soil. Four and one-quarter ml of water containing 50 μg of PDPC was added to each beaker and the beakers placed in the germinator.

Seedlings imbibed for 72 hours were placed in a 100 ml beaker containing 20 grams of soil and then nine ml of water containing 50 μg of PDPC-C^{14} was added to each beaker.

The different amounts of water added to the various age seedlings maintained a constant soil moisture in the beakers. If excess water was added, the breakdown of PDPC-C^{14} was decreased and the seedlings finally died.

The imbibition of water by soybean seedlings planted in soil was studied in order to determine the maximum time necessary to complete this phenomena. Graph I plots the percent moisture on a dry weight basis of seedlings grown for 25 hours. Complete imbibition was reached after 24 hours, thus allowing the seedlings 24 more hours to develop before being treated with PDPC.

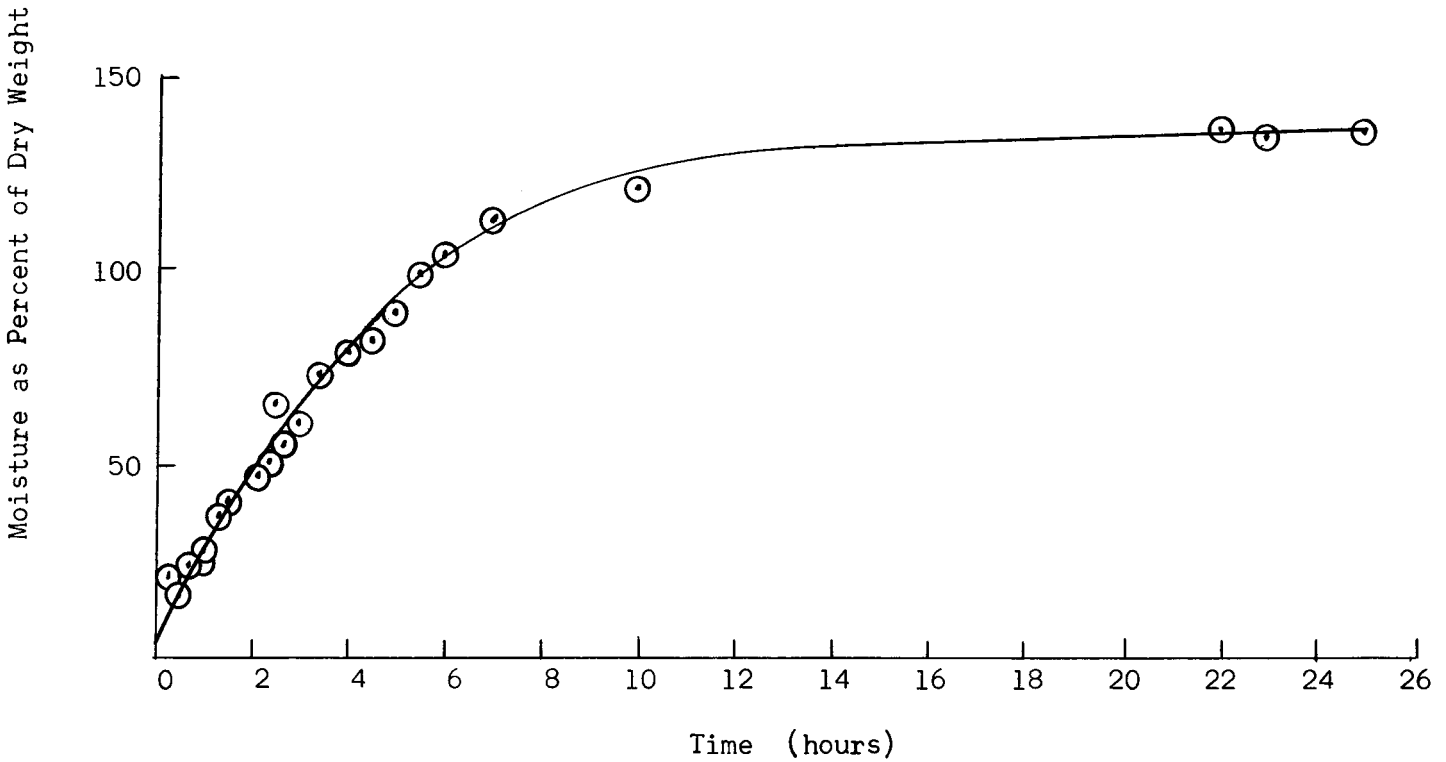
At the appropriate time one beaker of each was removed from the germinator, the seedlings removed, washed to remove all soil particles, and ground in 95% ethanol. The ethanol extract was made up to ten ml and 0.5 ml counted in a manner to be described later.

One ml of the ethanol solution was added to the previously described steam distillation stills and the PDPC distilled into iso-octane. This was counted as described in a following section.

Several runs were performed in which some of the seedlings were allowed to imbibe in a five ppm PDPC carrier solution for 48 hours and some in tap water. These were then removed and samples run in the above manner. The reason for this type of experiment was to study the effect of pre-exposure of PDPC to the seedlings.

GRAPH I

Imbibition of Water by Soybean Seedlings in Soil



For the same reason respiratory carbon dioxide experiments were run using both pre-exposed and non-pre-exposed seedlings. These were carried out in a respirometer as shown in Figure I. Five ml of a 2 x 10^{-2} M phosphate buffer, six uniform 48 hour old seedlings and five ml of water containing 50 μg of PDPC-C^{14} were added to each flask. One half the flasks containing seedlings germinated in water saturated paper towels and one half containing seeds germinated in paper towels saturated with a five ppm PDPC carrier solution.

The carbon dioxide was trapped in 0.5 N sodium hydroxide solution which was changed at various intervals. The sodium carbonate was precipitated as the barium salt with 10% barium chloride, filtered on one inch diameter fiber glass filter paper, dried with ethanol and ether, and counted as described in the section on counting procedure.

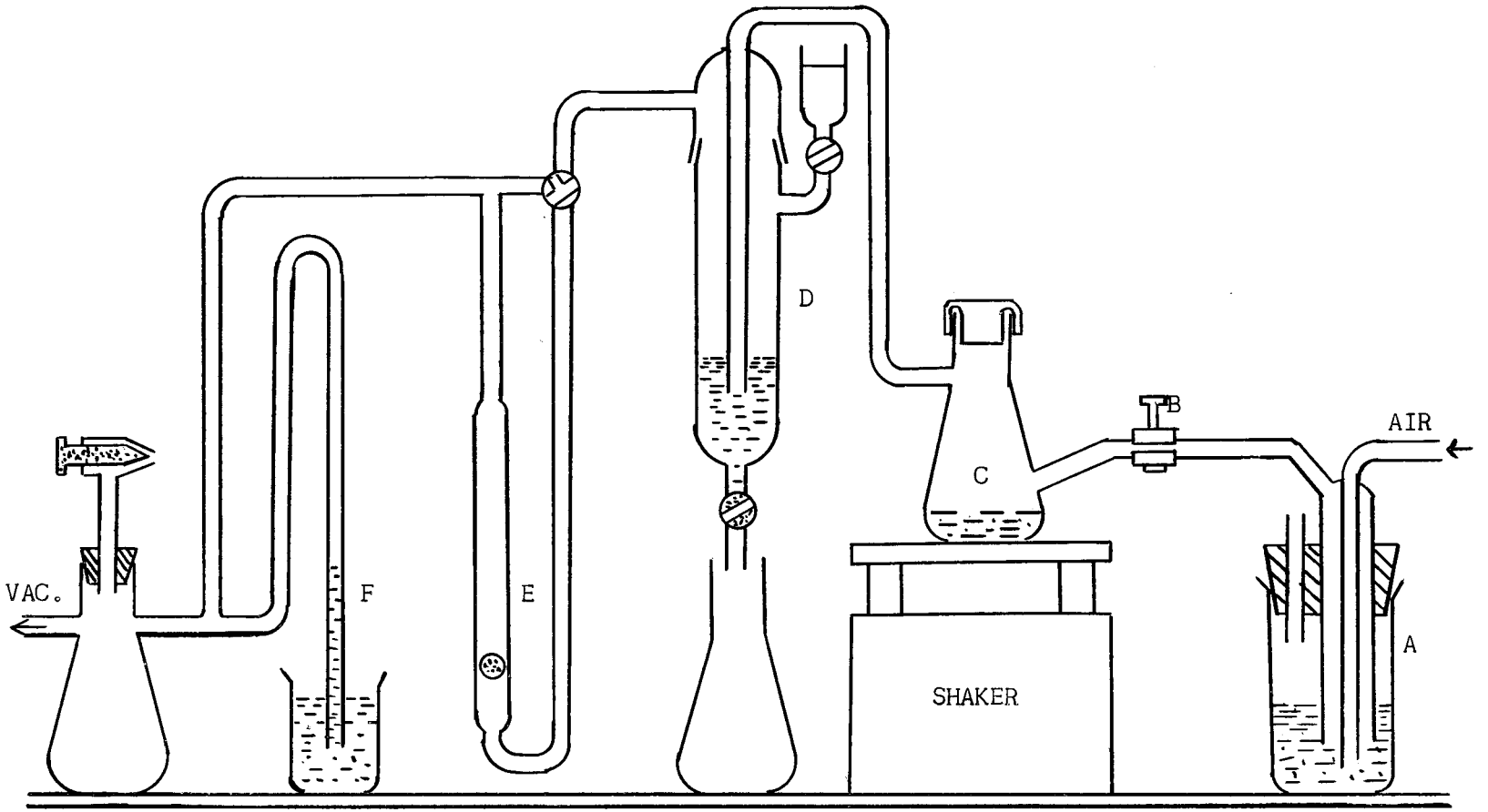
The tissue was ground in 95% ethanol, made to a final volume of ten ml, and 0.5 ml counted as described in the counting procedure. One ml of ethanol was steam distilled in the manner described and the iso-octane extract also counted.

The dried tissue was counted as previously described for the residue determinations. All counting values were converted to μg of PDPC or total equivalents of PDPC.

Iso-octane Extract - Counting Procedure. One-half ml of the iso-octane extract was added to two ml of redistilled toluene and

FIGURE I

RESPIRATOR



A. CO_2 Scrubber
B. Pinch Clamp
C. Respiration Flask
D. CO_2 Absorber
E. Flowmeter
F. Manometer

2.5 ml of a scintillation mixture containing 0.4% PPO and 0.005% POPOP in toluene. This system was found to have a counting efficiency of 53.8% (Graph II) and was used in counting all samples following steam distillation into iso-octane. One μg of PDPC-C^{14} was found to give 16,920 cpm in this system.

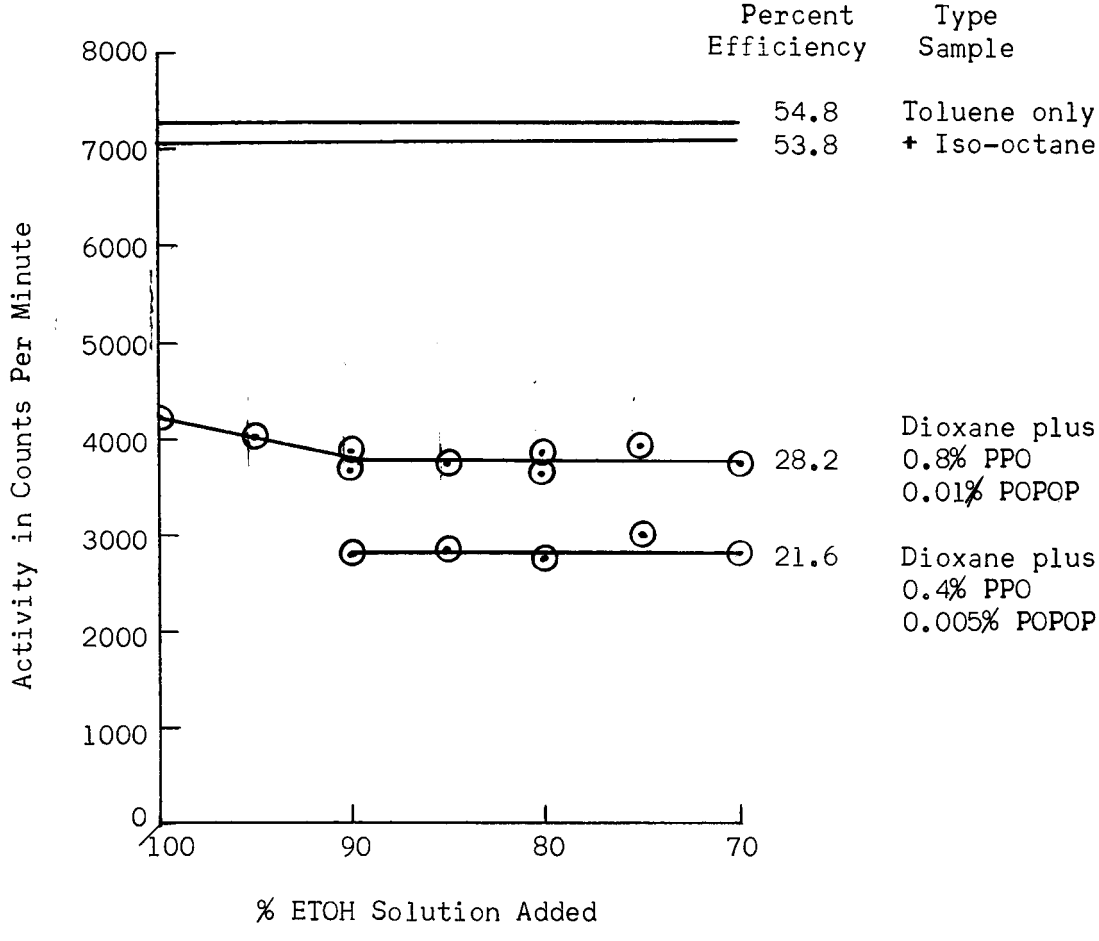
Ethanol Extract - Counting Procedure. It was deemed desirable to count the ethanol extract in a liquid scintillation system because of the increased sensitivity available. Such a system containing alcohol, however, introduces new problems of quenching, which can have a large effect on efficiency, and the solubility of the alcohol. The latter problem can be easily solved by using a toluene-dioxane system in place of the conventional pure toluene system.

It was found that the use of two ml of toluene and 2.5 ml of a dioxane solution containing 0.4% PPO and 0.005% POPOP gave a system into which 0.5 ml of ethanol extract could be easily dissolved. By varying the water content of the ethanol extract it was found that a constant efficiency of 21.6% could be obtained over an ethanol concentration of 70 to 90% (Graph II).

In order to increase the efficiency, the concentration of scintillator was doubled to give 0.8% PPO and 0.01% POPOP in dioxane. When 2.5 ml of this double strength dioxane mixture was added to two ml of toluene and 0.5 ml of ethanol solution, the curve shown in Graph II was obtained. The water content of the ethanol solution can be varied from 10 to 30% while maintaining a

GRAPH II

Counting Rate of Samples as Affected by Ethanol



constant counting efficiency of 28.2%. This system, which was found to give 8341 counts per minute per μg equivalent of PDPC-C^{14}, was used for counting all ethanol extracts.

Dried Tissue - Counting Procedure. Dried tissue was counted by weighing out 100 mg into a one inch stainless steel planchet and counting in the thin window gas flow geiger counter attached to a Tracer Lab super scaler. The efficiency was determined by combustion experiments using both the dried tissue and C^{14} labeled benzoic acid of known specific activity (Table III). The efficiency for foliage and stem was found to be 1.40% and for root tissue 1.58%.

Method of Residue Extraction. The sample to be distilled was added to 100 ml of distilled water in a 250 ml boiling flask and the flask attached to an oil extraction still containing two ml iso-octane in the sidearm. This method was tested using PDPC-C^{14} in the concentrations shown in Table IV. After distilling for one hour approximately 96% of the PDPC was recovered, after two hours 99% and after three hours recovery was at 100%. A three hour extraction period was used in these experiments and found in frequent checks of various types of samples to be adequate, resulting in complete recovery of the compound.

Gas Chromatography. Gas chromatographs were run on steam distillate samples collected from many runs. These were evaporated to a final concentration of about 0.4 μg/μl and analyzed on a Beckman GC-2A gas chromatograph equipped with a hydrogen flame

Table III

Determination of Dry Tissue Counting Efficiency

A

Benzoic Acid-C^{14} Combustion

Sample Weight mg	Yield mg	% Recovery	DPM	CPM Gas-Flow of $BaCO_3$	CPM Scintillation of $BaCO_3$	% Efficiency Gas-Flow	% Efficiency Scintillation
4.8	53.50	97.9	26,400	2145	5636	8.14	21.3
4.0	45.00	102	21.950	1860	4692	8.48	21.3
Average		99.9				8.31	21.3

B

Determination of Counting Efficiency of Dry Plant Tissues using a Gas-Flow G. M. Detector

Sample Type	mg Combusted	Yield $BaCO_3$	Dry Tissue cpm/.1g Gas-Flow	Carbonate cpm/.1g Scintillation	DPM/.1g Dry Tissue	Average DPM/.1g	% Efficiency Average
foliage	4.1	31.95	22.2*	316	1480	1580	1.40
foliage	4.0	31.00		358	1680		
stem	4.0	31.35	65.1*	1000	4690	4520	1.40
stem	4.0	30.85		925	4350		
root	5.0	33.25	78.7*	1095	5140	4965	1.58
root	4.6	30.60		1020	4790		

* Average of Several Values

Table IV

Determination of Recovery of PDPC-C^{14}
by a Continuous Steam Distillation

Extraction Number	Sample Number	Added R1607-C^{14} µg	Extraction Period	Total CPM Extracted	% Total Recovery From Time Zero
A	A-1	1	0-1	17092	98.8
A	A-2		1-2	130	99.6
A	A-3		2-3	36	100
B	B-1	2	0-1	29808	94.8
B	B-2		1-2	1568	99.8
B	B-3		2-3	136	100
C	C-1	3	0-1	48528	97.0
C	C-2		1-2	908	99.5
C	C-3		2-3	104	100
D	D-1	4	0-1	64116	98.9
D	D-2		1-2	728	99.8
D	D-3		2-3	84	100

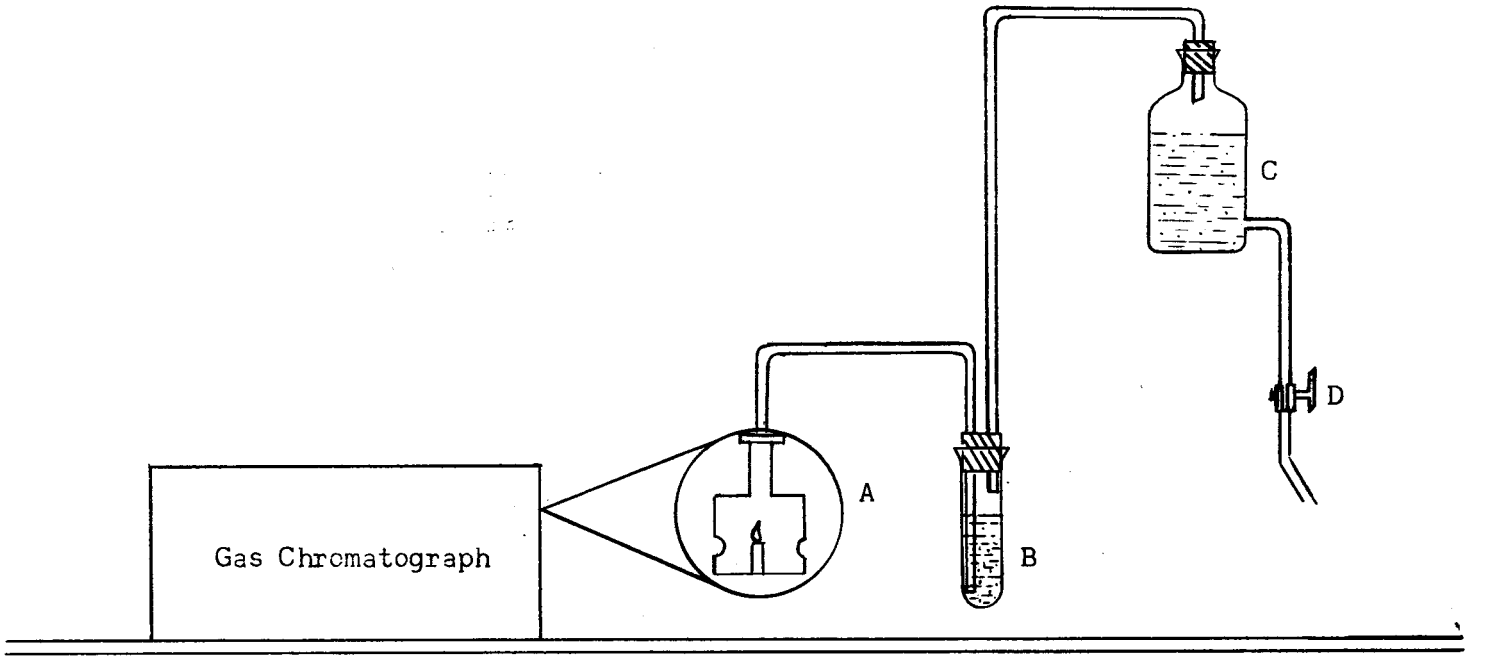
detector. The carrier gas was helium run at 25 psi pressure. Hydrogen and air for the flame detector were run at 14 and seven psi respectively. All chromatographs were run at 160° C through a 1/8 inch by 26 foot column filled with 5% polyestersuccinate on 40/60 chromosorb. The exhaust stack from the hydrogen flame was connected to a carbon dioxide trap containing 2N NaOH solution. The bubble rate was regulated by a Mariotte bottle. The entire system is pictured in Figure II.

Samples corresponding to the peaks were collected, Na_2CO_3carrier added, and the sample precipitated with 10% $BaCl_2$. The $BaCO_3$was then filtered on one inch fiber glass filter paper. The filters were rinsed with 95% ethanol and dried with ethyl ether. The dried sample was placed upside down in a glass scintillation counting vial, two ml of toluene scintillation mixture added and counted in the Tricarb liquid scintillation counter at an efficiency of 21.3%. This was determined as described in the Dried Tissue Counting Procedures.

Preparation of Radioautograms. Radioautograms of the sample plants were made following harvest of the intact plant. Harvested plants were washed well with water, blotted dry and pressed between two pieces of filter paper suspended in a warm air stream. The pressed plants were then placed on sheets of no screen medical X-ray film and placed in the dark for between two to three months. At the end of the exposure period the radioautograms were developed, washed and dried.

FIGURE II

Carbon Dioxide Sample Collector for Gas Chromatograph



A. Hydrogen Flame Detector B. CO_2 Absorber C. Mariotte Bottle D. Pinch Clamp

One-dimensional paper chromatograms were run on the ethanol extracts of seedlings in the following way: enough 95% ethyl alcohol extract of the seedlings to give at least 100 counts after application was removed and applied as a line across the paper strip. The chromatogram was then developed in a butanol-acetic acid-water (4:1:1) solvent system for 16 hours. The developed chromatograms were then scanned for radioactivity on a Forro gas flow chromatogram scanner connected to a Tracer Lab SC-34A rate meter, and an Angus-Esterline recorder. Radioautograms were then made from the chromatograms in the same manner as described above for dried plants.

Results and Discussion

Evaluation of Radioautograms. The radioautograms made from two, three and five week old peanut seedlings show a distribution of activity through the entire seedling (Figure III). Table V, Graph III and Graph IV give the average concentration of PDPC and total equivalents of PDPC found in the various parts of the seedling. These values are proportional to the density of exposure on the radioautograms.

Table V

Total Microgram Equivalents of PDPC and Total Residue found in an Average Peanut Seedling

Amount in µg found as	Age in Weeks	1 lb per acre			4 lbs per acre		
		foliage	stem	root	foliage	stem	root
Equivalents	2	0.083	0.350	0.288	0.496	3.02	2.49
"	3	0.146	0.460	0.342	0.948	3.60	2.00
"	5	0.314	0.476	0.238	1.30	1.04	1.62
Residue	2	0.003	0.056	0.091	0.024	0.807	0.364
"	3	0.005	0.105	0.098	0.040	0.636	0.468
"	5	0.010	0.049	0.051	0.037	0.233	0.177

As would be expected the plant parts closest to the source have the highest concentration of radioactive material while those farthest from the source have the lowest. At the earlier stages of growth the cotyledons have the greatest concentration, probably due to the passive absorption of PDPC into the imbibing seedlings. Likewise, the roots have a high unit concentration. The rapid

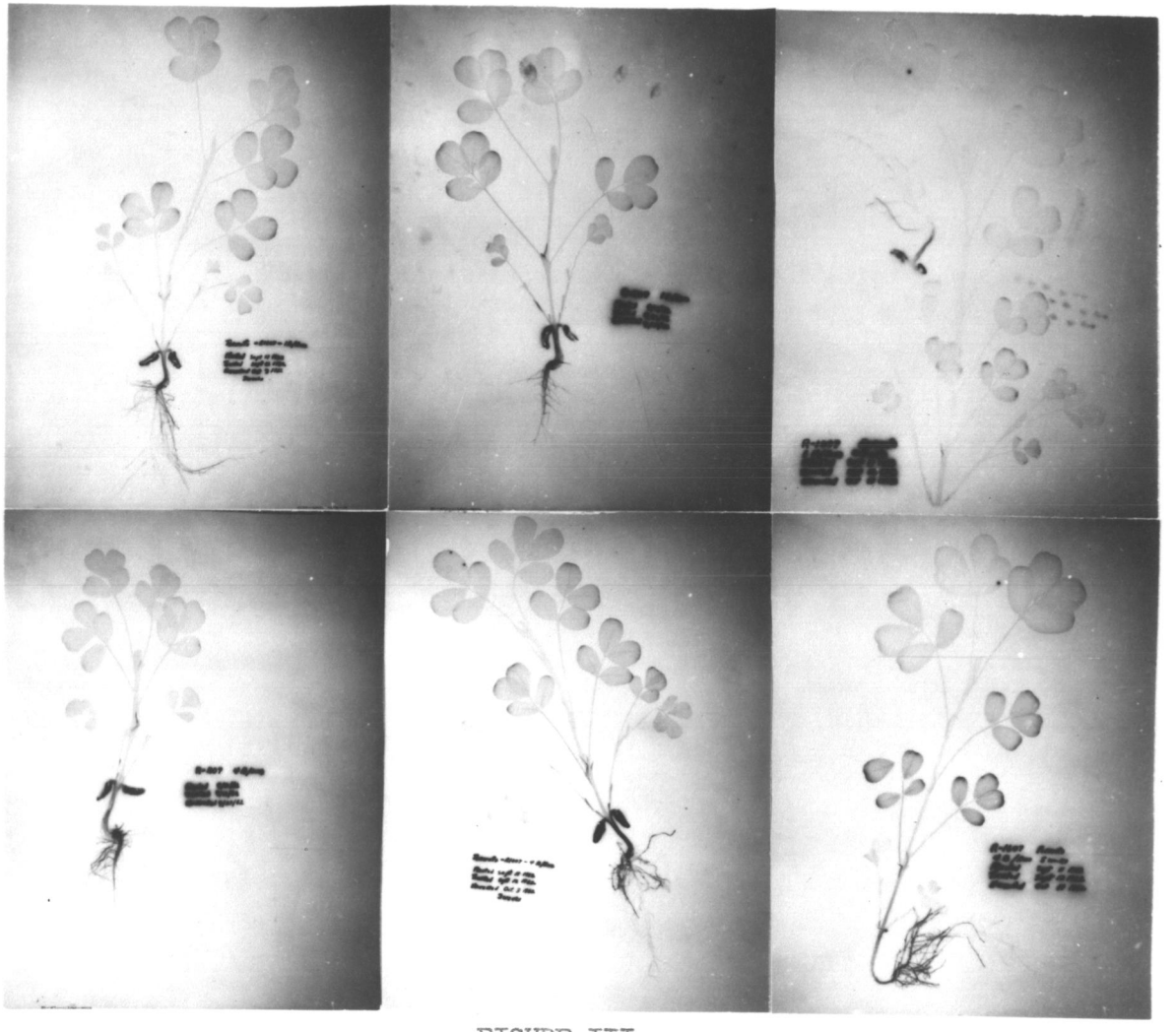
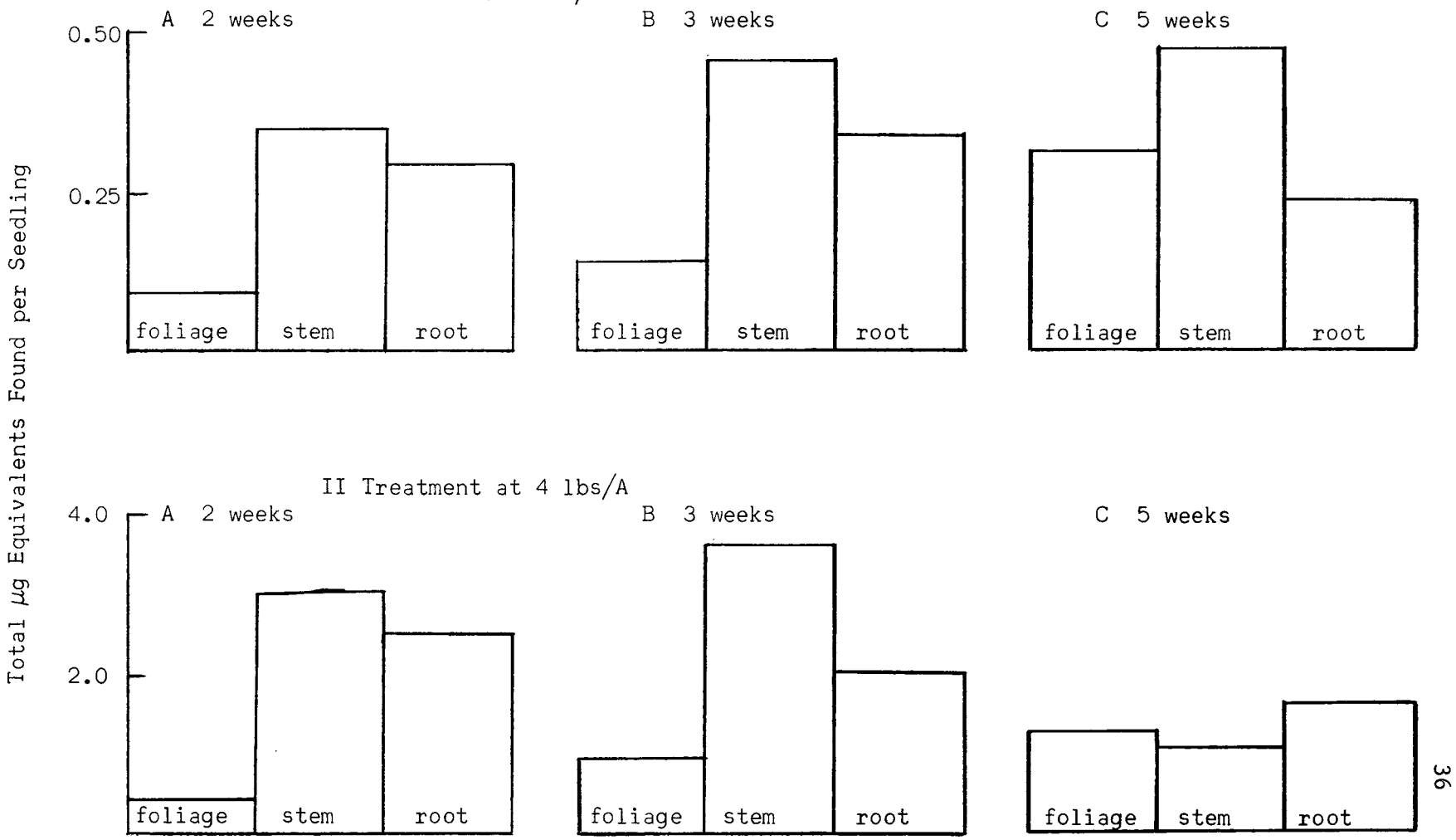


FIGURE III

Radioautograms of Peanuts: Left to Right, 2,3,5 Weeks Old; Top to Bottom 1 and 4 Pounds per Acre Application.

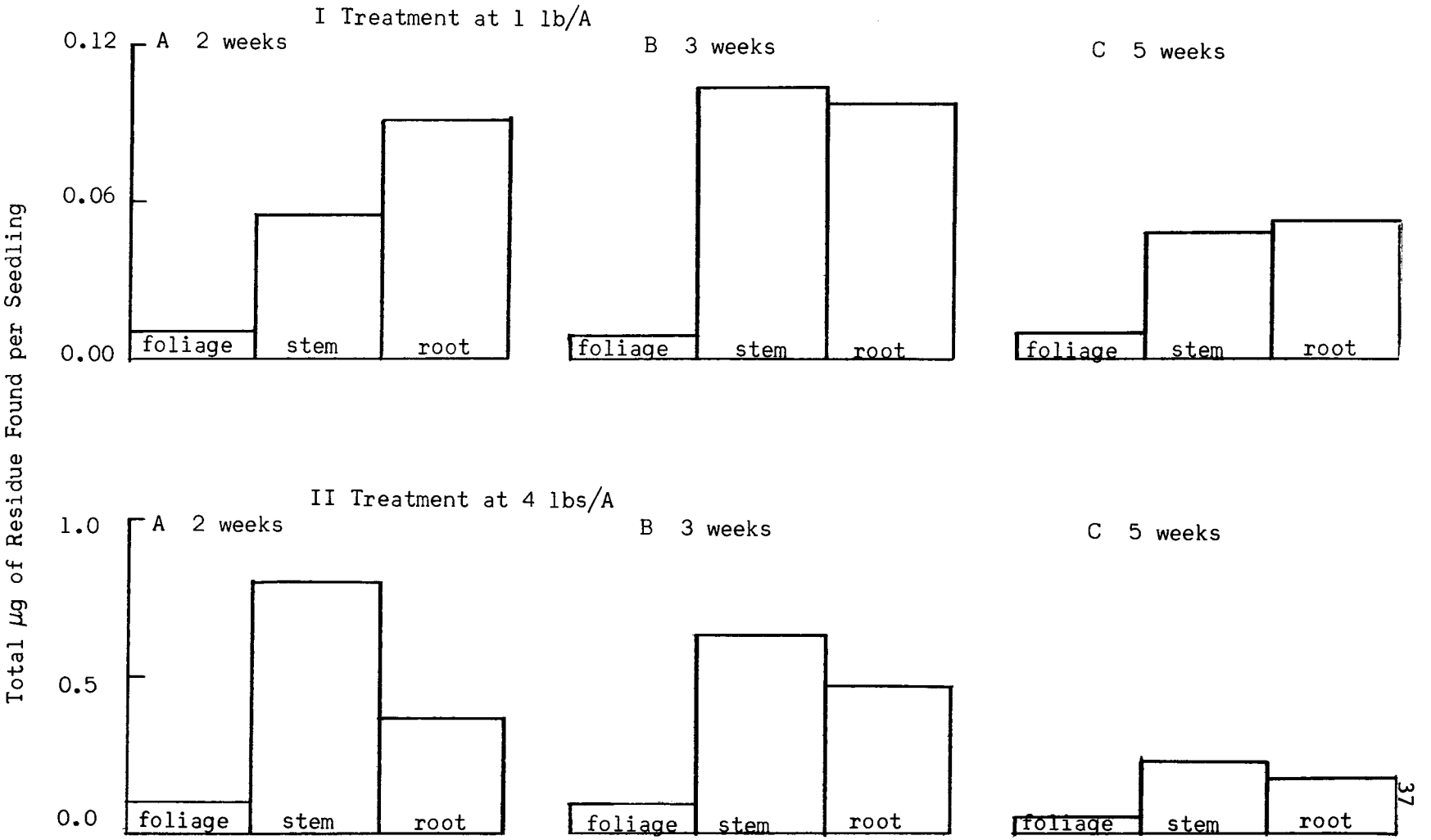
GRAPH III

Total Equivalents of PDPC Found in Average Peanut Seedling



GRAPH IV

Total Amount of Residue Found in Average Peanut Seedling



movement of PDPC from the roots is evident by the amount found in the stems and foliage. The entire stem contains more material than the roots although the concentration per gram is lower. Translocation upward is continued to the foliage and distribution throughout the leaf is quite uniform. Translocation from one leaf to another appears to be restricted to some degree since the younger leaves contain a lower density of material. This new foliage probably depends on the root absorption for its supply of radiochemical.

The continued uptake of herbicide from soil containing one pound per acre by the roots is evident by the increased amount in the stem and foliage without loss from the roots. Treatment with four pounds per acre, however, may have some effect on absorption of new herbicide from the soil since increasing leaf content results in a decreased stem and root content. This is demonstrated in Graph III which shows the equivalents of PDPC found in the various plant parts at different ages.

It is interesting to note that the percentage of activity present as residue is lowest in the foliage and highest in the stem. Whether this is due to a decreased ability to translocate the residue or to an increased metabolism in the foliage is not know. Translocation of residue from root to stem appears to be quite active.

In general, the radioautograms demonstrate the ability of the plant to translocate the activity, either as residue or metabolite,

through the plant and that this translocation is active at both concentrations studied.

Radioautograms made from two and three week old soybean plants also show a distribution of radiocarbon throughout the seedling (Figure IV). Treatment at one pound per acre results in the greatest concentration of radioactive material in the roots and cotyledons. Translocation, however, is rapid toward the stem and foliage until at three weeks the total amount is almost evenly divided among the four parts. The residue (Table VI) remains fairly constant indicating a reduced ability to be translocated or an increased metabolism in the stem and foliage. The four week radioautogram of a seedling treated at one pound per acre indicates that more radioactive material is in the older foliage than in the new foliage. This would suggest that translocation from the mature tissue is negligible and once incorporated in the leaf is only slightly remobilized. The higher concentration in the meristematic tissue than in the previous leaves can be explained by the generally increased cell sap concentration of all metabolites prior to expansion of the leaf cells.

Treatment at four pounds per acre results in an increased uptake and more even distribution of total activity throughout the plant. The leaves show the same decrease in concentration of radioactive material with decreasing age of tissue. As in the seedlings treated with one pound per acre the roots and cotyledons contain the

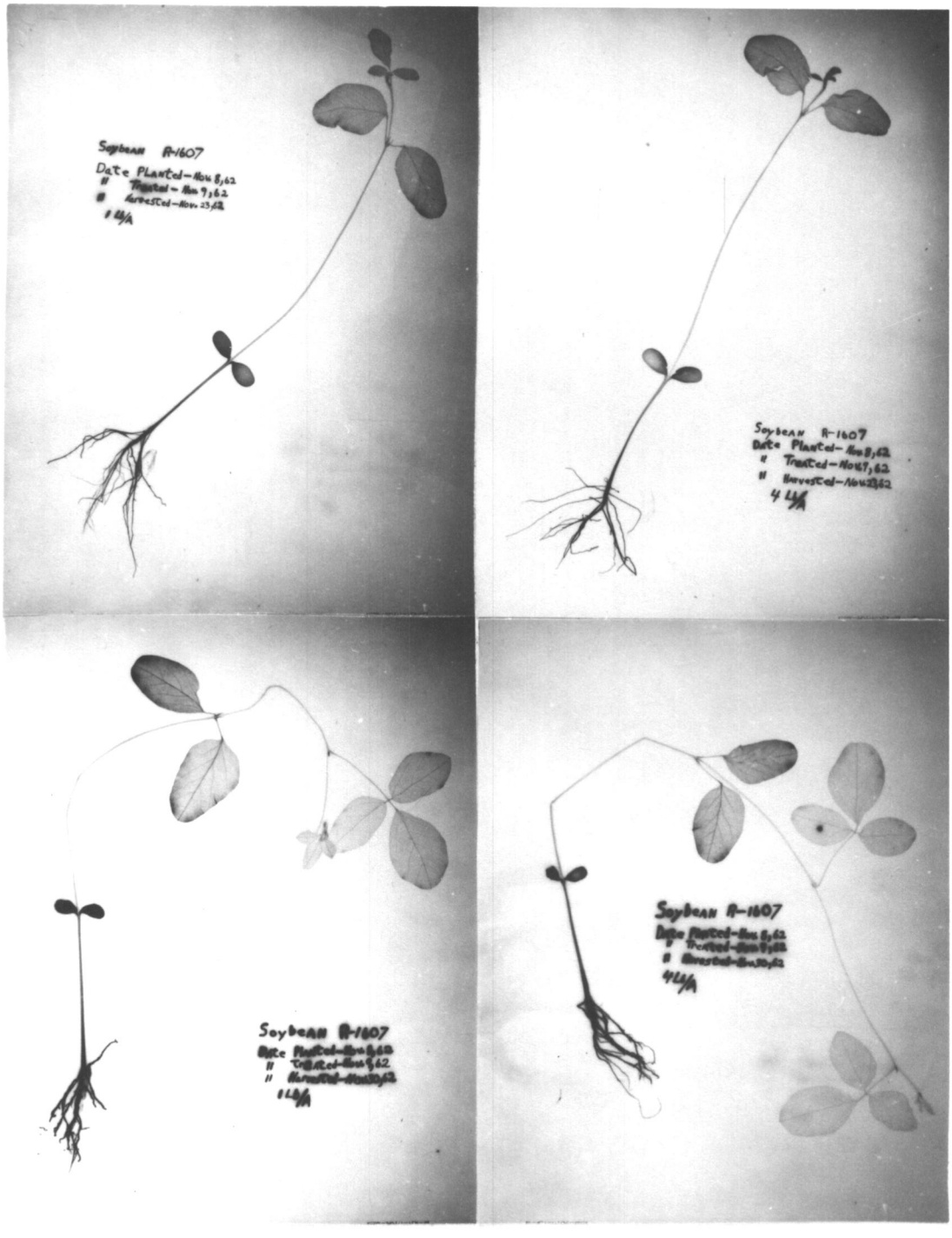


FIGURE IV

Radioautograms of Soybeans: Left to Right, 1 and 4 Pounds per Acre Application; Top to Bottom, 2,3 Weeks Old.

Table VI

Total Micrograms of Equivalents of PDPC and Residue
found in an Average Soybean Seedling

Amount in µg	Age Weeks	1 lb per acre				4 lbs per acre			
		foliage	stem	cotyledon	root	foliage	stem	cotyledon	root
Equivalents	2	0.015	0.017	0.296	0.270	1.00	1.16	1.16	1.65
"	3	0.187	0.208	0.195	0.247	1.17	1.35	1.12	1.42
Residue	2	0.0006	0.004	0.003	0.007	0.011	0.046	0.013	0.077
"	3	0.0007	0.005	0.003	0.007	0.007	0.027	0.018	0.083

greatest concentration per unit area; however, the total amount of material does not differ greatly from that of the stem.

The total amount of residue found in the various tissues remains small in comparison to the total amount of radioactive material. As in the case of the seedlings treated at the lower rate, the residue found in the various parts remains fairly constant over the time period studied. Again, it may be stated that while the translocation of radioactive material is quite rapid and general, the concentration of residue remains small (Graph V and VI). The final result is a leveling off of the total distribution of both radioactive material and residue in all plant parts.

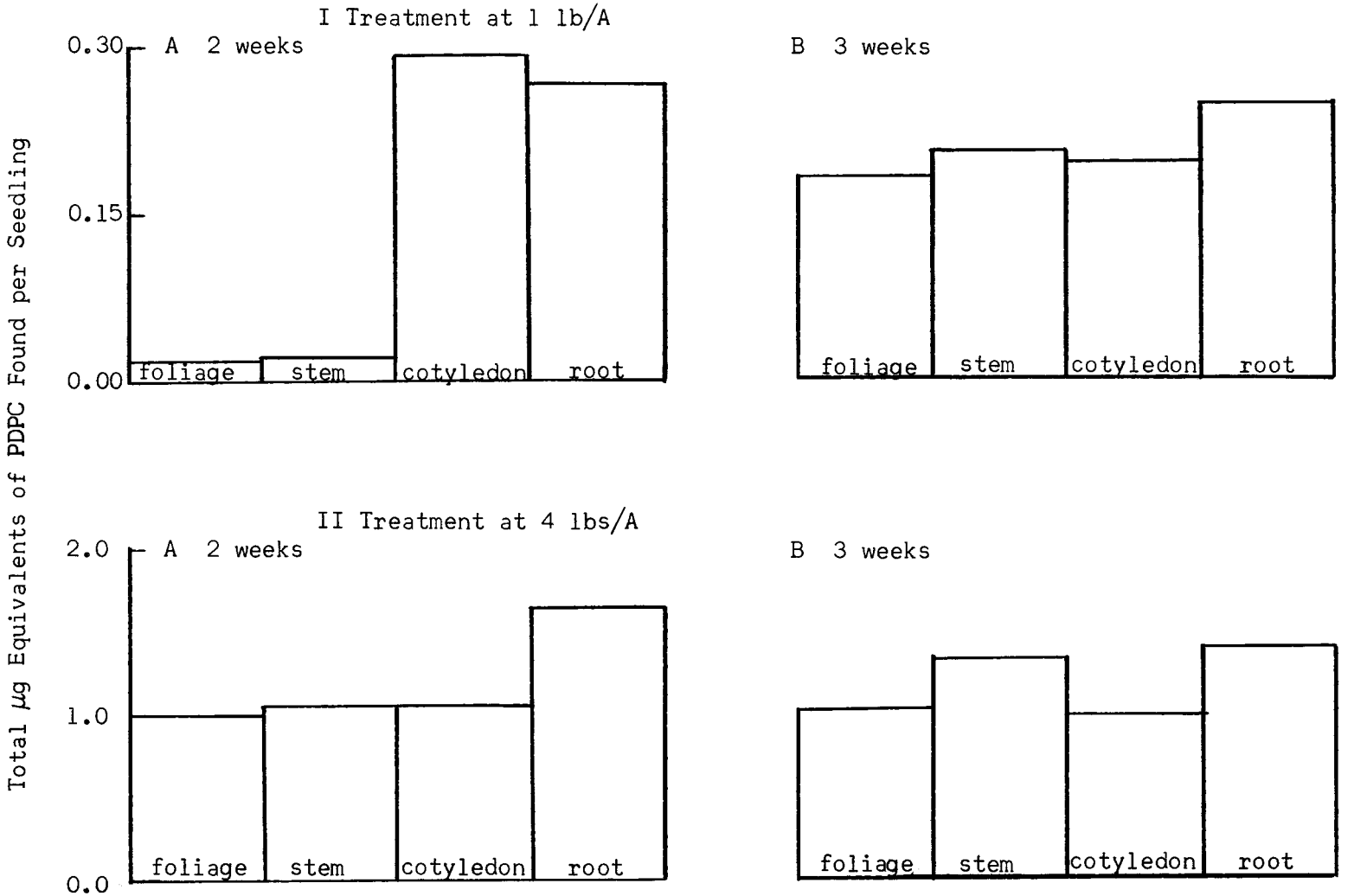
The radioautograms of soybean and peanut seedlings treated with PDPC-C^{14} generally resemble those obtained by Yamaguchi (45, p. 374-280) and Fang (18, p. 295-298) using EPTC-S^{35}. As was the case with EPTC-S^{35}, absorption and translocation of PDPC-C^{14} was rapid with more radioactivity being found in the aerial portions of the plants. This was true for both the amount of residue and the total activity.

Increasing the application rate of PDPC increased the amount of uptake from the soil, but as was the case following treatment with EPTC-S^{35}, this was not proportional.

Evaluation of PDPC Breakdown and Residues in Peanuts. Analysis for the activity of both unchanged and metabolized PDPC showed (Table VII) as would be expected, the highest concentration in the roots. A maximum was reached between two and three weeks after treatment and rapidly decreased in the following weeks. When

GRAPH V

Total Amount of PDPC Equivalents Found in Average Soybean Seedling



GRAPH VI

Total Amount of PDPC Residue Found in Average Soybean Seedling

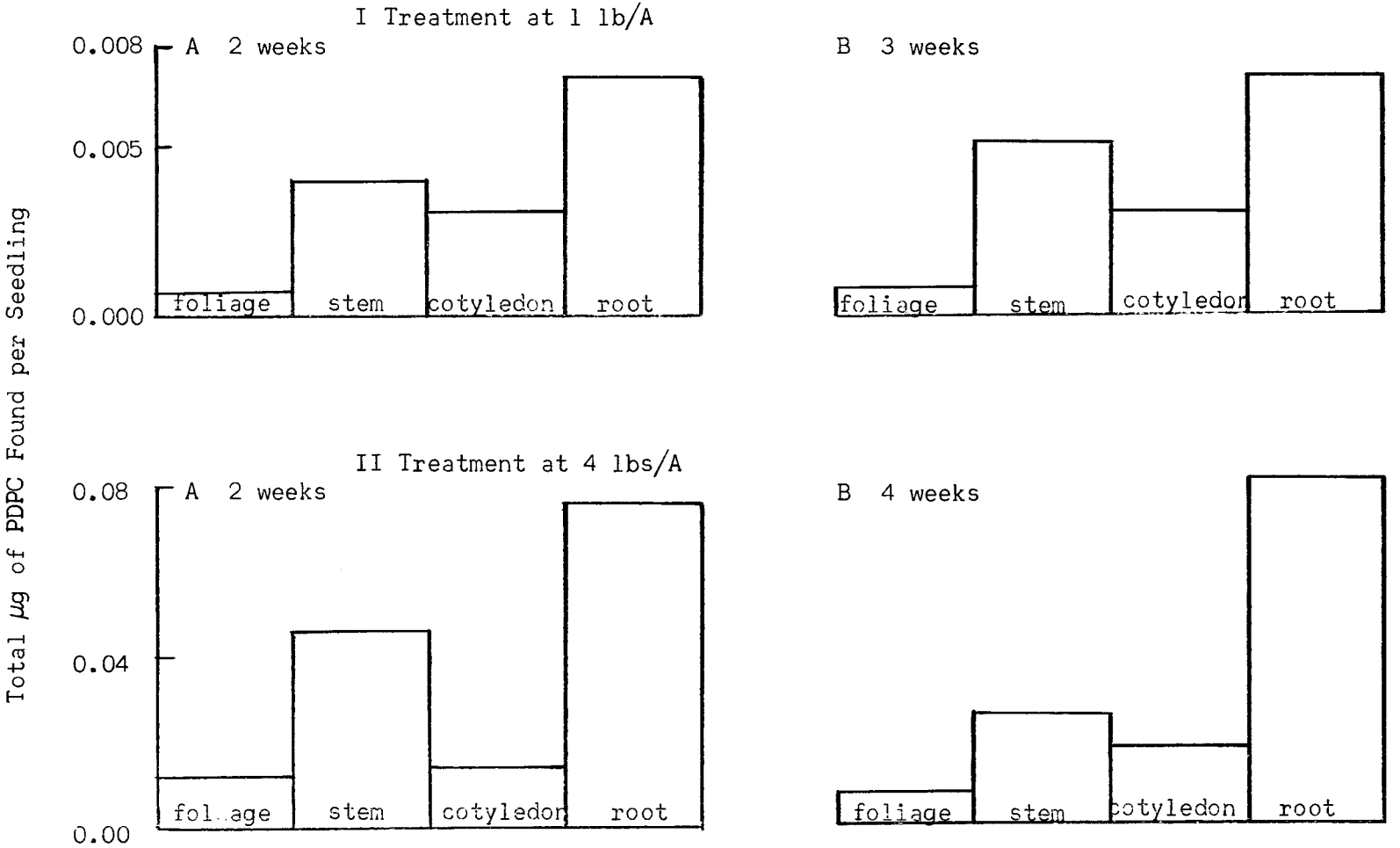


Table VII

Analysis of Residues in Dry Peanut Tissue at Various Ages

Tissue	Age Weeks	Treatment lbs/acre	µg/g Dry Tissue PDPC	µg/g Dry Tissue Equivalents	PDPC Equivalents	% Moisture
foliage	2	1	0.070	0.504	0.040	87.0
stem	2	1	0.188	1.180	0.116	86.8
root	2	1	0.723	2.280	0.317	91.2
foliage	2	4	0.152	3.180	0.047	86.5
stem	2	4	2.280	8.480	0.249	85.8
root	2	4	2.170	14.84	0.146	90.3
foliage	3	1	0.019	0.515	0.037	86.8
stem	3	1	0.299	1.310	0.228	87.9
root	3	1	0.605	2.110	0.286	88.5
foliage	3	4	0.168	3.732	0.045	85.1
stem	3	4	1.940	10.96	0.177	87.0
root	3	4	2.761	11.76	0.235	89.7
foliage	5	1	0.0181	0.594	0.031	83.5
stem	5	1	0.0854	0.836	0.102	85.4
root	5	1	0.291	1.370	0.213	86.3
foliage	5	4	0.076	2.692	0.284	83.4
stem	5	4	0.448	2.000	0.224	85.4
root	5	4	1.091	10.00	0.109	84.0
foliage	6	1	0.008	0.320	0.024	84.0
stem	6	1	0.005	1.060	0.005	79.6
root	6	1	0.087	0.736	0.118	83.9
foliage	6	4	0.032	1.428	0.022	85.4
stem	6	4	0.065	1.548	0.042	85.5
root	6	4	0.406	5.120	0.793	87.4
foliage	10	1	0.043	0.400	0.106	82.3
stem	10	1	0.011	0.281	0.041	82.3
root	10	1	0.033	0.440	0.075	77.6
foliage	10	4	0.147	1.480	0.099	82.6
stem	10	4	0.387	1.296	0.299	84.3
root	10	4	0.286	2.196	0.131	78.9

Table VII
(Continued)

Analysis of Residues in Dry Peanut Tissue at Various Ages

Tissue	Age Weeks	Treatment lbs/acre	µg/g Dry Tissue PDPC	µg/g Dry Tissue Equivalents	PDPC Equivalents	% Moisture
foliage	14	1	0.019	0.296	0.064	81.4
stem	14	1	0.010	0.155	0.063	76.6
fruit	14	1	0.092	0.104	0.089	79.2
foliage	14	4	0.090	0.472	0.207	78.5
stem	14	4	0.036	0.672	0.055	78.5
fruit	14	4	0.014	0.344	0.041	75.0
foliage	26	1	0.013	not de-	not de-	not de-
stem	26	1	0.031	termined	termined	termined
root	26	1	0.013	counts		
seed, husk				too low		
& stem	26	1	0.005			
seed	26	1	0.001			
foliage	26	4	0.042			
stem	26	4	0.028			
root	26	4	0.132			
seed, husk						
& stem	26	4	0.038			
seed	26	4	0.007			

herbicide was applied at the rate of one pound per acre, a maximum of 2.28 μg per gram of dry tissue was found. Treatment at the rate of four pounds per acre resulted in a maximum of 14.84 μg per gram of dry tissue. At the end of ten weeks when the determination of total activity in the roots was terminated, the concentrations had dropped to 0.44 μg and 2.19 μg per gram of dry tissue for application rates of one and four pounds per acre, respectively.

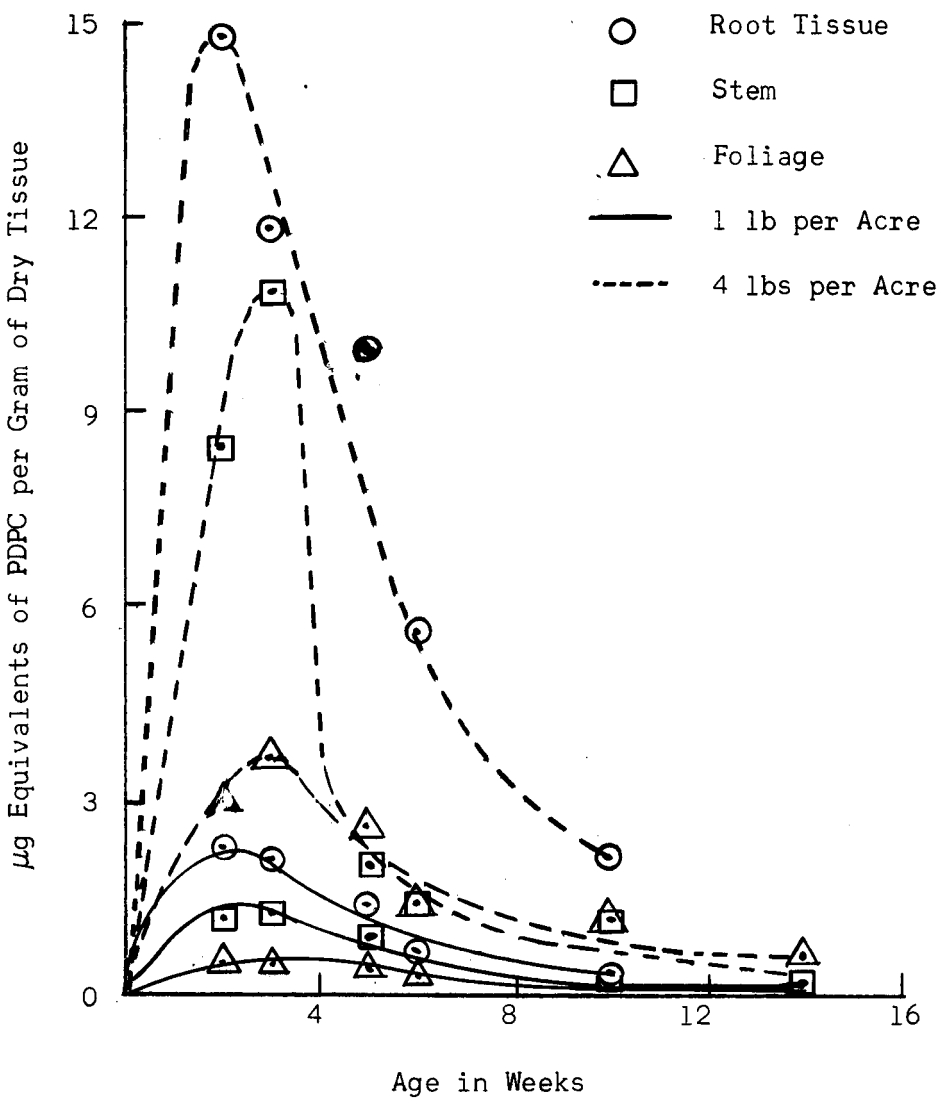
In a similar manner, a maximum of 1.180 and 8.48 μg/g dry stem tissue was found following one and four pound per acre applications. This was followed by a rapid decrease to 0.16 and 0.67 μg per gram of dry tissue after 14 weeks. The changes in the foliage are much less pronounced and the maximum was reached after only three weeks. Application of herbicide at one pound per acre resulted in a maximum concentration of 0.59 μg while application at four pounds per acre gave a maximum of 2.69 μg per gram of dry tissue. At the end of the 14 week period these values had dropped to 0.30 and 0.47 μg per gram of dry tissue respectively for one and four pound per acre application rates.

Graph VII shows the time course plot of equivalents of PDPC found during the 14 week period. The values found for each harvest are shown in Table VII.

Graph VIII which is similar to the above graph for the total content of PDPC equivalents is given for the PDPC residue. Although the maximum is found at a slightly earlier age, the roots as expected

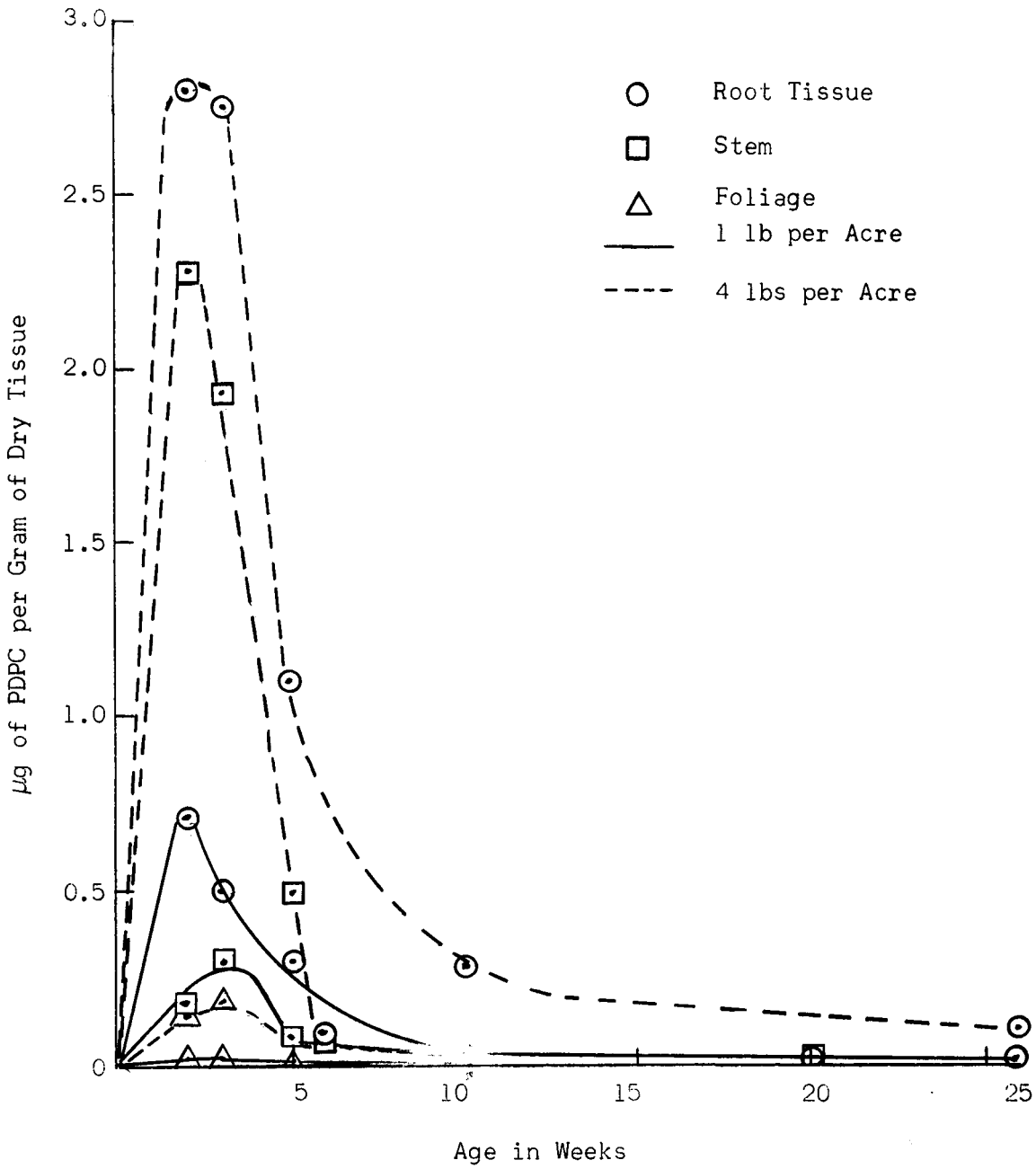
GRAPH VII

μg Equivalents of PDPC Found per Gram of Peanut Tissue



GRAPH VIII

μg of PDPC Found per Gram of Peanut Tissue



have the highest concentration. Application at the rate of one pound per acre resulted as a maximum of 0.72 μg of PDPC residue after two weeks. At the four pound rate a maximum concentration of 2.76 μg was found after three weeks treatment. The concentration of PDPC dropped rapidly to 0.03 and 0.27 μg per gram of dry tissue after ten weeks and 0.01 and 0.13 μg after 26 weeks for one and four pound per acre application rates, respectively.

The concentration in the stem also reached a maximum at between two and three weeks which rapidly decreased to about 0.01 μg per gram of dry tissue for both rates of application at the 26 week harvest time. The concentration of PDPC in the foliage never exceeded 0.04 μg per gram of dry tissue following treatment at one pound per acre; however, treatment at four pound per acre gave a maximum of 0.168 μg per gram of dry tissue after three weeks. The final 26 week concentrations, however, were found to be 0.01 and 0.04 μg per gram of dry tissue, respectively, for application rates of one and four pound per acre.

The seed pod and pedicels were analyzed individually only at the final harvest, otherwise they were included with the nut for analytical purposes. These showed a residue level generally five times that of the nut itself, being somewhat comparable to the foliage. The nut contained only a trace of residue at harvest and the uncertainty of this figure is largely due to the extremely low counting rate. For example, the iso-octane extract of the peanut

treated at four pounds per acre gave only three counts per minute per gram dry tissue when analyzed at the final harvest.

The ratio of unchanged residue to total ethanol soluble equivalents of PDPC was found to be fairly constant. In the foliage it remained between 0.05 and 0.1, in the stem between 0.05 and 0.25, and in the root between 0.1 and 0.3. There is a gradual lowering of the values with age, as would be expected, due to the decreased herbicide concentration in the soil. There is also a decrease in the ratio from root to foliage due to a larger portion of herbicide being metabolized in the tissues closer to the source.

Generally it may be concluded that while the uptake of herbicide from the soil may be quite rapid, the ability of the peanut plants to metabolize PDPC is also quite rapid. Not only is the original herbicide quickly broken down into metabolites but the metabolites are soon lost, the amount remaining at harvest being quite small. The nut, the only portion directly consumed by humans, has a residue content of only one to seven parts per billion, far below all tolerance levels.

The moisture content of the plants is not affected by treatment as may be seen in Table VII. It is generally found to range between 77 and 90%.

Evaluation of PDPC Breakdown and Residues in Soybean Plants. The general pattern of degradation and accumulation of PDPC residues in soybean plants is quite similar to that in peanuts. The roots possess the highest concentration of both residue and total

equivalents of PDPC (Table VIII). The maximum is again reached at two weeks and falls off rapidly (Graph IX and X). The maxima for residue and equivalent of PDPC found in the roots were respectively 0.52 and 21.00 μg per gram dry tissue at the rate of one pound per acre and 5.28 and 112.0 at four pounds per acre. At the end of five weeks these values have decreased to 0.07 and 6.86 at one pound per acre and 0.25 and 45.20 at four pounds per acre.

Again the same pattern was found in the stem with the maxima following treatment at one pound per acre being 0.086 μg of residue and 3.67 μg of equivalents per gram of dry tissue. At the end of five weeks these values had been cut to about one fourth in the plants treated at the one pound rate and one tenth in the plants treated at the four pound rate. At harvest the residue levels had dropped to two and 17 parts per billion for the one and four pound rates, respectively.

In the foliage the equivalents of PDPC paralleled those found in the stem but at a slightly lower value. The concentration of residue in the foliage, however, was found to be at a considerably lower level than in the stem. Treatment at one pound per acre resulted in residue concentrations less than twenty parts per billion through the life span. At harvest this value had dropped to four parts per billion. Treatment at four pounds per acre resulted in a two week maximum of 0.34 μg per gram of dry tissue; however, this had dropped to five parts per billion at harvest time.

Table VIII

Analysis of Residue in Dry Soybean Tissue at Various Ages

Tissue	Age Weeks	Treatment	µg/g Dry Tissue PDPC	µg/g Dry Tissue Equivalents	PDPC Equivalents	% Moisture
foliage	2	1	0.013	3.37	0.004	86
stem	2	1	0.086	3.67	0.023	91
root	2	1	0.524	21.00	0.025	92
cotyledon	2	1	0.102	10.35	0.010	91
foliage	2	4	0.337	22.24	0.015	85
stem	2	4	0.996	25.52	0.039	91
root	2	4	5.280	112.0	0.047	93
cotyledon	2	4	0.442	40.40	0.011	91
foliage	3	1	0.007	1.840	0.004	80
stem	3	1	0.052	2.120	0.024	87
root	3	1	0.363	13.60	0.267	74
cotyledon	3	1	0.128	9.350	0.137	88
foliage	3	4	0.060	10.540	0.006	80
stem	3	4	0.2448	12.360	0.020	87
root	3	4	4.820	82.48	0.059	81
cotyledon	3	4	0.984	60.00	0.016	88
foliage	4	1	0.016	1.208	0.013	80
stem	4	1	0.031	1.008	0.031	83
root	4	1	0.057	9.050	0.006	90
foliage	4	4	0.132	7.960	0.171	80
stem	4	4	0.876	6.160	0.142	83
root	4	4	0.102	61.60	0.002	90
foliage	5	1	0.162	1.250	0.136	77
stem	5	1	0.022	1.240	0.018	80
root	5	1	0.067	6.860	0.010	86
foliage	5	4	0.034	3.640	0.009	78
stem	5	4	0.071	2.556	0.028	80
root	5	4	0.252	45.20	0.006	86
foliage	8	1	0.003	0.391	0.006	74
stem	8	1	0.004	0.361	0.011	73
foliage	8	4	0.014	2.488	0.006	76
stem	8	4	0.022	1.840	0.012	76

Table VIII
(Continued)

Analysis of Residue in Dry Soybean Tissue at Various Ages

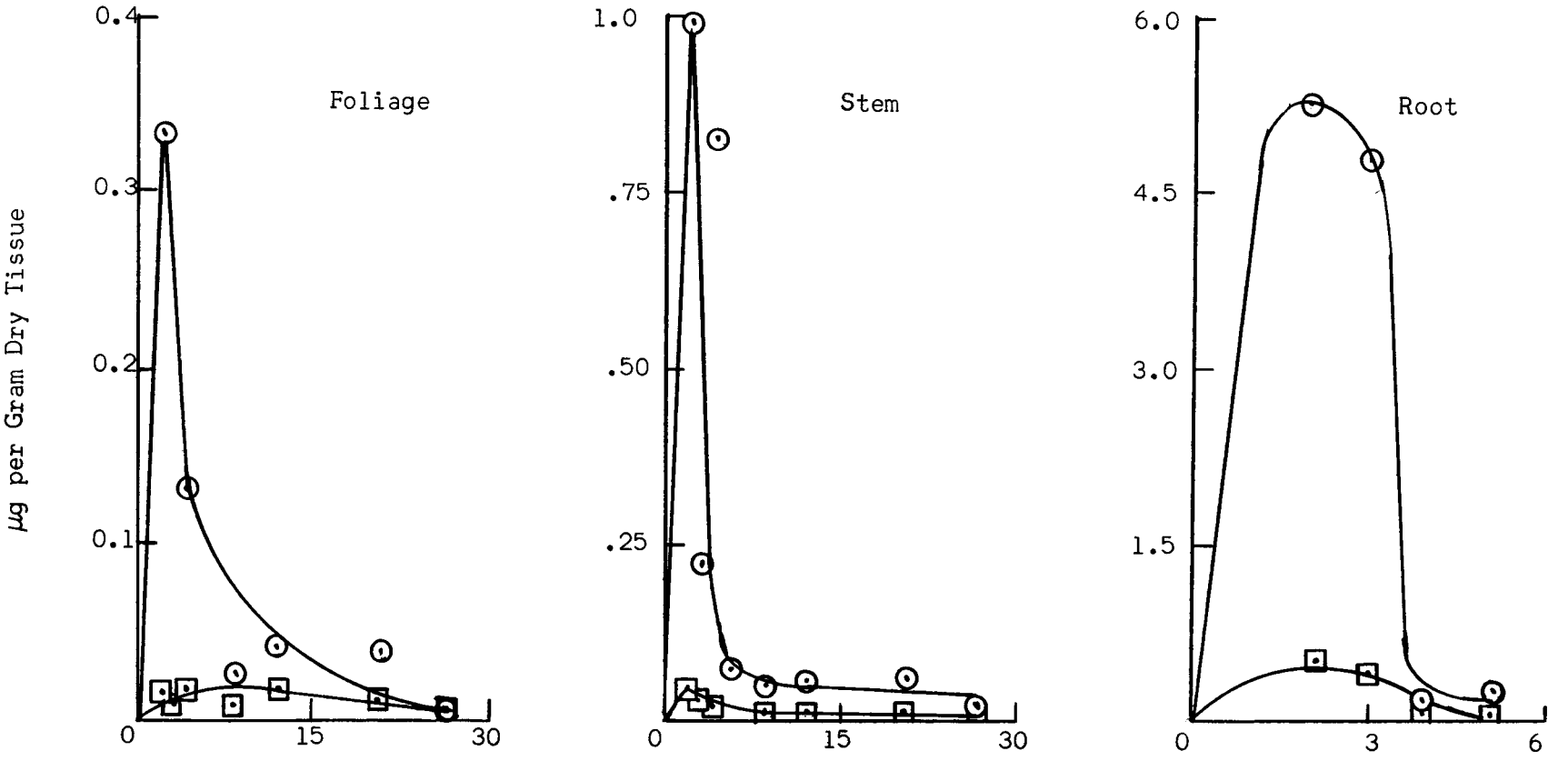
Tissue	Age Weeks	Treatment	µg/g Dry Tissue PDPC	µg/g Dry Tissue Equivalents	PDPC Equivalents	% Moisture
foliage	12	1	0.014	0.346	0.039	73
stem	12	1	0.010	0.352	0.029	71
foliage	12	4	0.040	0.816	0.049	72
stem	12	4	0.049	1.046	0.047	70
foliage	20	1	0.006	not de-	not de-	71
stem	20	1	0.014	termined	termined	73
pod	20	1	0.001	because		72
seed	20	1	0.006	of low counting		71
foliage	20	4	0.038	rate		73
stem	20	4	0.066			70
pod	20	4	0.012			71
seed	20	4	0.025			70
foliage	26	1	0.004			69
stem	26	1	0.002			68
pod	26	1	0.004			23
seed	26	1	0.001			9.8
foliage	26	4	0.005			69
stem	26	4	0.017			65
pod	26	4	0.015			42
seed	26	4	0.017			27

GRAPH IX

Ethanol Soluble Equivalents of PDPC Found in Soybean Seedlings at Various Ages

GRAPH X

Amount of PDPC Found in Soybean Seedlings at Various Ages



The cotyledons were found to continue to accumulate both residue and metabolites until they abscinded, at which time they still contained considerable quantities of both forms (Table VIII). During the first two weeks, while still affixed to the seedling, they contained both herbicide residue and total equivalents in ratios proportional to the original rate of application, i.e., one to four. At three weeks, however, the cotyledons receiving four pound per acre had doubled the concentration of both residue and equivalent while those treated at the rate of one pound contained the same concentrations as at two weeks.

Analysis of the seed and seed pod at both 20 and 26 weeks revealed only very small concentrations of residue. Application of one pound per acre resulted in less than six parts per billion of residue in either the seed or pod. At four pounds per acre the residue at harvest was less than 20 parts per billion. As in the case of the peanuts, the moisture level in the plants was not affected by the treatment as Table VIII shows.

The same general conclusions may be reached concerning the ratio of PDPC residue to total PDPC equivalents in the soybeans as was found in the peanuts. The ratios are somewhat lower, the foliage falling between .004 and .04, the stem falling between .01 and .05, and the root falling between .002 and .05. The few exceptions were probably due to poor recoveries of residue during steam distillation.

Both soybeans and peanuts are crops tolerant of PDPC, thus making this herbicide useful for the control of grassy weeds. Since

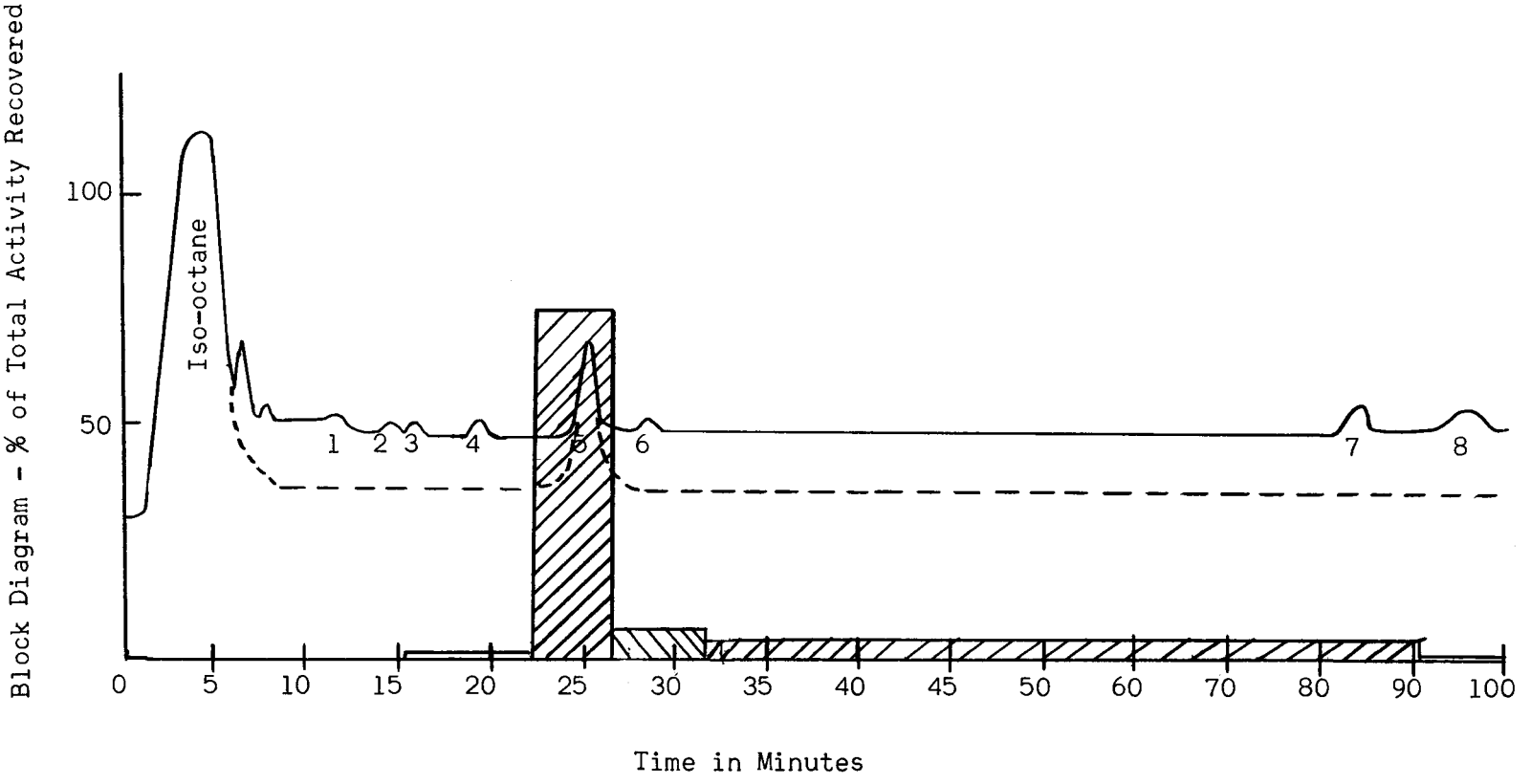
the residue in the seeds, after pre-emergent treatment, is below pharmacological limits they may be used directly as food or as a source of oil. The stem, foliage, seed pods and seed pulp are all low enough in residue so as to allow their use as stock feed without cause for concern. Higher concentrations of residue can be expected if post-emergent application is utilized but the final concentration would have to be determined.

Gas Chromatography of Iso-octane Extract. A number of iso-octane extracts were combined and evaporated to a small volume in a stream of compressed air. Gas chromatographs were then made of this extract in order to verify the assumption that all recovered activity present was unchanged PDPC. Graph XI shows a scan from the gas chromatograph of the iso-octane extract and a sample of known PDPC. Peak five represents the PDPC while the rest of the peaks are from other plant volatile constituents. The bar graph at the bottom shows the percent of radioactivity recovered in each region. The majority of radioactivity (about 80%) was recovered under the PDPC peak. The remaining amount coming off in progressively smaller quantities is probably due to trailing of material because of the type of collection system used.

The collection system was open to the atmosphere at the hydrogen flame and excess air drawn through by means of a vacuum. An aluminum tube connecting the burner to the CO_2 trap would often act as a condenser becoming partially plugged with water. This

GRAPH XI

Analysis of Radioactivity in Gas Chromatographic Peaks of PDPC



would cause a partial retention of CO_2 which would not be absorbed in the CO_2 trap immediately after formation.

It is apparent, however; that most, if not all, the radioactivity did originate from the PDPC-C^{14} peak. The original assumption that the activity was in the form of PDPC is thus verified.

Effect of PDPC on Germination and Herbicide Metabolism. The general pattern of PDPC metabolism as discussed thus far suggests that the early stages of germination and growth may be responsible in determining the manner of breakdown of this compound. The ratio of unchanged compound to total activity remains fairly constant once it has been established and the rate of breakdown is quite rapid at the onset of the first series of experiments reported.

In a series of experiments to be described here the effect of age on the metabolism of PDPC was studied. The experiments were carried out as described in the section of Methods and Materials.

From Table IX it is evident that seedlings germinated in the presence of PDPC contained considerably more total ethanol soluble activity than those allowed to imbibe water before being treated. Likewise, the total concentration of free PDPC is also greater in the former. The seedlings germinated in PDPC were not able to metabolize this compound as rapidly as those germinated in water. Graph XII shows the amount of total ethanol soluble activity as a function of seedling age. This activity is due primarily to unchanged PDPC as may be seen in Table IX or in Graph XIII. The

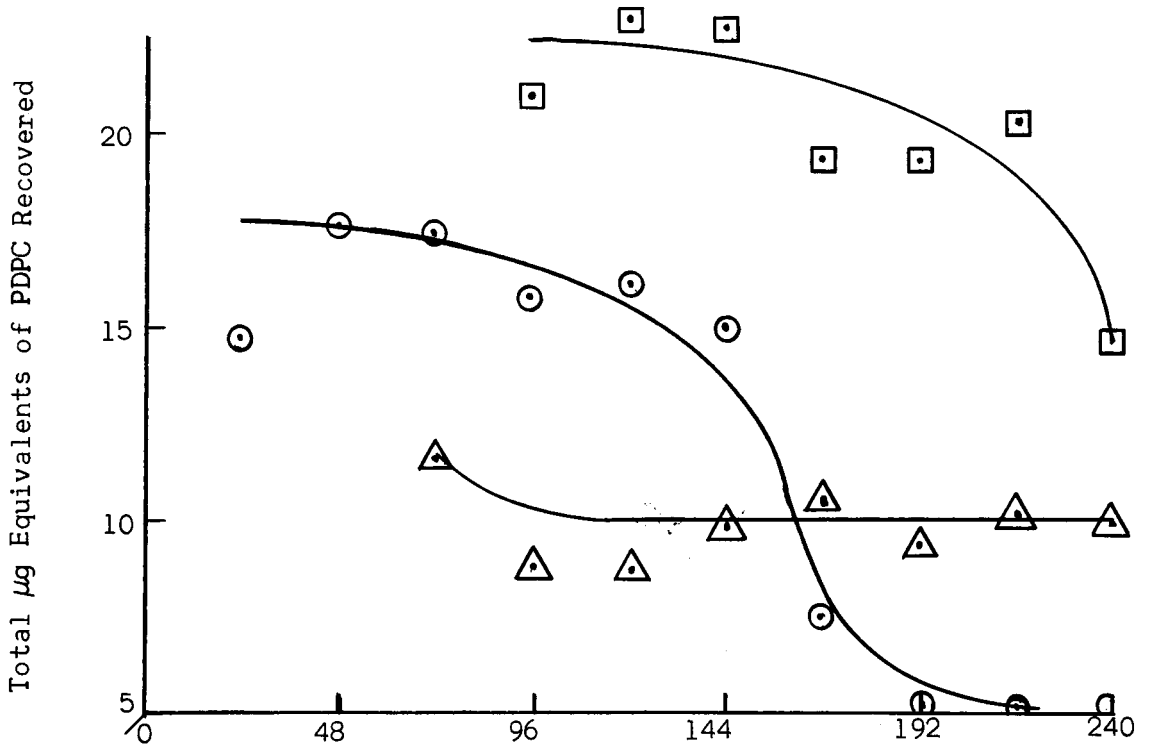
Table IX

Breakdown of PDPC in Young Soybean Seedlings

Time Pretreated in H_2O	Time in PDPC	Age of Seedlings	Total μg Equiv.	Number Determined	μg PDPC	PDPC Equiv.
0	24	24	14.3±2.5	3	15.6±2.9	1.091
0	48	48	17.9±1.1	5	18.8±1.7	1.055
0	72	72	17.7±3.4	4	17.1±5.0	0.962
0	96	96	15.9±3.5	4	18.8±.04	1.185
0	120	120	16.6±0.7	5	18.1±5.8	1.106
0	144	144	15.5±1.5	3	9.7±.02	0.626
0	168	168	7.5±1.9	3	3.9±2.8	0.532
0	192	192		1	3.1±	
0	216	216	4.6±0.3	2	2.1	0.460
0	240	240	5.1	1	1.6	0.319
48	24	72	11.8±2.8	3	10.8±3.1	0.917
48	48	96	8.8±0.1	3	2.9±1.1	0.329
48	72	120	8.6±0.8	5	1.8±0.8	0.206
48	96	144	9.9±0.5	5	1.3±0.3	0.128
48	120	168	10.7±0.9	5	1.4±0.2	0.131
48	144	192	9.5±1.8	4	0.9±0.3	0.094
48	168	216	10.4±1.7	4	1.1±0.5	0.104
48	192	240	9.9±2.7	2	1.6±0.5	0.162
48	216	264	10.5±3.5	2	3.0±0.6	0.289
72	24	96	21.4±0.3	2	22.6±0.2	1.060
72	48	120	23.1±0.9	2	20.5±0.4	0.887
72	72	144	16.4±2.6	4	4.7±1.2	0.287
72	96	160	19.9±3.2	2	6.1±2.4	0.305
72	120	192	19.3±0.9	3	4.6±2.1	0.240
72	144	216	20.3±.06	3	3.1±1.5	0.154
72	168	240	14.7+2.5	2	1.7+0.2	0.119

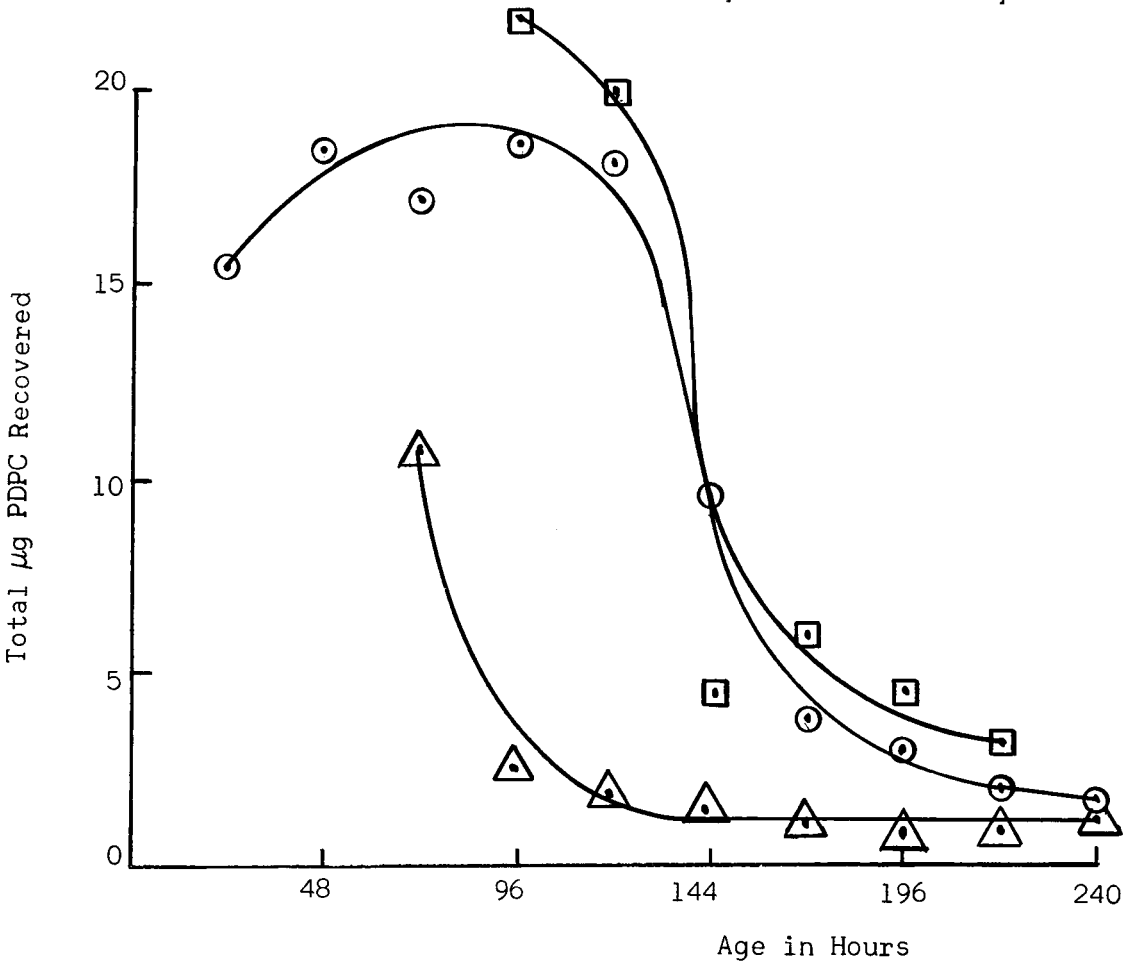
GRAPH XII

μg Equivalents of PDPC Extracted by Ethanol from Soybean Seedlings



GRAPH XII

μg of PDPC Extracted by Ethanol from Soybean Seedlings



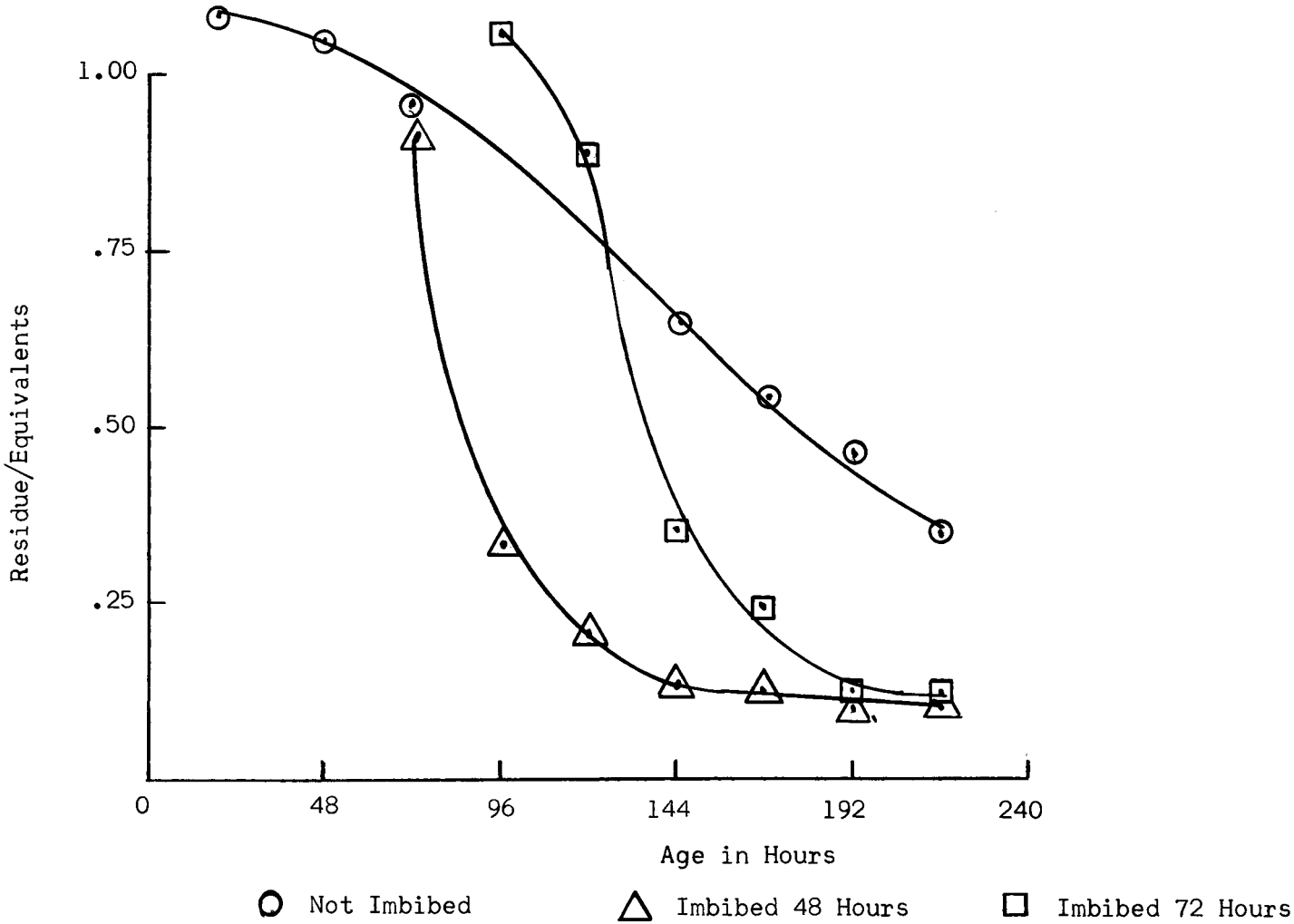
PDPC appears to inhibit the formation of a certain enzyme system for its own metabolism since the seedlings containing less herbicide were able to degrade it more quickly and convert it to CO_2. The seedlings germinated in water are not subjected to the action of a large concentration of herbicide and are able to form during germination the necessary enzyme system for its complete metabolism. For this reason the concentration of both total ethanol soluble material and unchanged residue remain fairly constant. Thus, seedlings germinated in the presence of PDPC require a longer time to form the necessary enzyme systems; however, they appear to recover during the later stages of growth.

Seedlings allowed to germinate for 72 hours before treatment with PDPC gave results not readily correlated with the previous experiments. It is the opinion of the author that light may play an inhibitory role by decreasing the ability of the seedling to metabolize the ethanol soluble metabolites of PDPC to CO_2. Another alternative explanation is that the respiratory CO_2 is re-utilized in photosynthesis and thus not liberated. The conversion of residue PDPC to metabolite is quite rapid once established and the concentration falls to that of the other type treatments after 240 hours.

This effect if further demonstrated by data in Graph XIV which shows the ratio of residue to total ethanol soluble material as a function of age. The seedlings germinated in water show a rapid conversion of PDPC to ethanol soluble material while the seedlings

GRAPH XIV

Metabolic Ratio as a Function of Age and Treatment



germinated in the presence of PDPC were not capable of such a rapid conversion. Seedlings germinated for 48 hours before treatment had the smallest ratio of residue to total ethanol soluble material, demonstrating a rapid destruction of residue. Seedlings germinated for 72 hours in water had this ability reduced, greatly at first but to a lesser degree as treatment time was extended. Those seedlings germinated in PDPC had the ability to metabolize the residue reduced the most and recovery slowed considerably.

Paper chromatograms of the ethanol soluble fraction revealed the presence of four radioactive compounds. Two of these contained most of the radiation with only a trace or very small percentage of activity found in the others. Table X lists the percent of activity found in the different compounds for the various types of experiments run.

In each type of treatment the youngest tissue showed the greatest activity in the third position. The older the tissue at the time of treatment the less activity found in position three and the more found in position four. As the seedlings within any treatment group ages there was a similar conversion of activity from positions three to four. After 240 hours treatment the seedlings showed slightly more activity in position four than three. The compounds found in position one and two contained only a trace of activity and this never reached a very high percentage. Seedlings grown in the dark resulted in similar chromatographic patterns with only slightly more activity found in position one and two.

Table X

Distribution of Activity found in Chromatograms made from the Ethanol Extract of Soybean Seedlings Grown in Dark

	% Activity found in				Age	Time	Time	Light
Position No.	1	2	3	4	Seedling	Pretreated	Treated	Condition
Rf Value	.239	.341	.549	.735	(hrs)	(hrs)	(hrs)	
	0	0	100	0	48	0	48	light
	T	T	99	T	96	0	96	light
	T	T	82	17	144	0	144	light
	9	7	48	36	192	0	192	light
	T	5	43	52	240	0	240	light
	T	T	79	21	96	48	48	light
	T	7	65	28	144	48	96	light
	T	11	48	41	192	48	144	light
	T	7	47	46	240	48	192	light
	6	11	42	41	288	48	240	light
	0	0	63	38	120	72	48	light
	11	9	40	40	168	72	96	light
	8	18	38	36	216	72	144	light
	T	13	46	42	264	72	192	light
	T	T	32	68	312	72	240	light
	T	T	99	T	48	0	48	dark
	T	T	99	T	96	0	96	dark
	14	6	55	25	144	0	144	dark
	13	6	66	15	192	0	192	dark

Table X
(Continued)

Distribution of Activity found in Chromatograms made from the Ethanol Extract of Soybean Seedlings Grown in Dark

	% Activity found in				Age	Time	Time	Light
Position No.	1	2	3	4	Seedling	Pretreated	Treated	Condition
Rf Value	.239	.341	.549	.735	(hrs)	(hrs)	(hrs)	
	9	6	71	14	96	48	48	dark
	14	5	64	17	144	48	96	dark
	11	T	76	12	192	48	144	dark
	15	5	57	24	240	48	192	dark
	T	T	T	T	120	72	48	dark
	8	2	63	27	168	72	196	dark
	11	5	55	29	216	72	144	dark
	--	4	48	48	264	72	192	dark

From the foregoing it would appear the PDPC applied directly to the seeds reduces the immediate breakdown of the herbicide, and probably the conversion of metabolite three to metabolite four. Germination for 72 hours prior to treatment also results in some inhibition to breakdown but does not affect the conversion of the compound in position three to that in four. Germination for 48 hours least affects the breakdown, but the compound in position three is not converted to that in position four as readily as in the older seedlings.

In order to determine if this phenomena is an effect of age or of the herbicide, several experiments were undertaken using seedlings of the same age, one half germinated 48 hours in water and the rest germinated 48 hours in a five ppm solution of nonradioactive PDPC. These experiments will be discussed in the following section.

Pre-exposure of Soybean Seedlings to PDPC. These experiments were carried out as described in the section on Methods. Table XI gives the data for soybean seedlings germinated for 48 hours either in water or a five ppm PDPC solution and grown in soil to an age of 216 hours. Graph XV shows the micrograms of either residue or total ethanol soluble material as a function of age for the two types of experiments. As can be seen germination in nonradioactive PDPC results in a decreased ability to degrade the PDPC-C^{14} at the early stages. The seedlings recover to normal at the end of the growing period. In a similar fashion the concentration of ethanol soluble

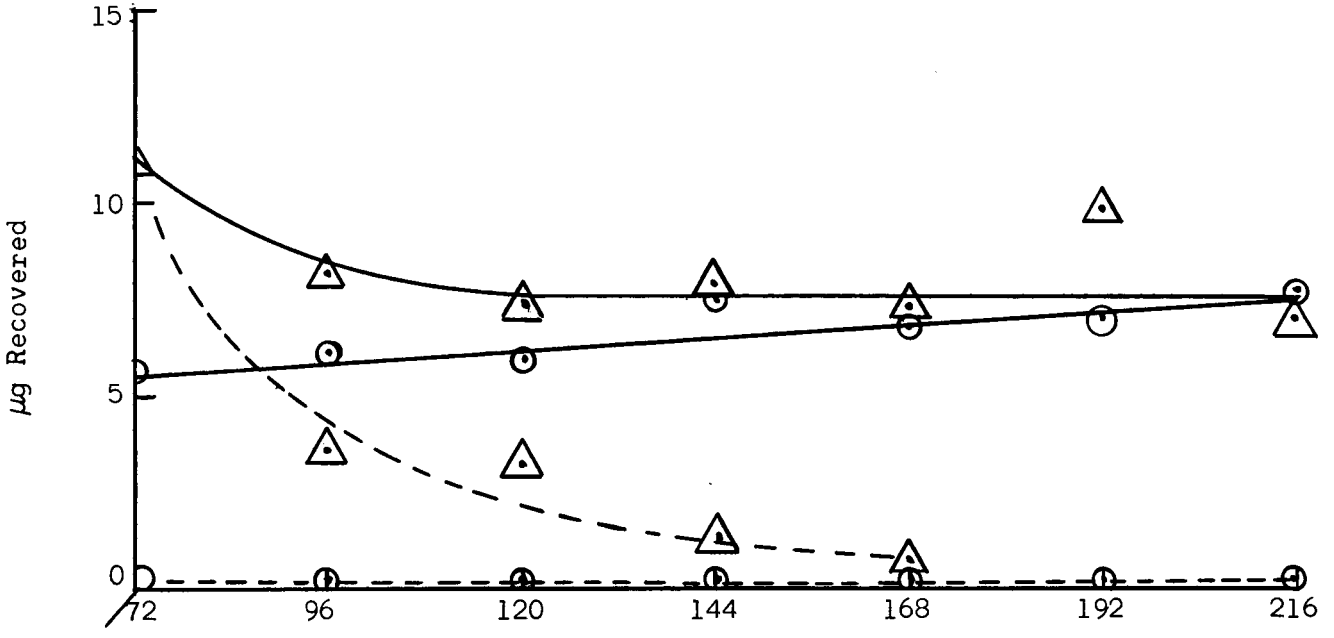
Table XI

Pre-exposure of Soil Grown Soybean Seedlings to PDPC

Pretreatment	Age (hrs)	No. Runs	µg Equivalents of PDPC	µg PDPC	PDPC Metabolize
H_2O	72	2	5.73±0.22	0.047±0.004	5.68
H_2O	96	2	6.12±0.46	0.064±0.009	6.06
H_2O	120	2	5.96±0.08	0.084±0.001	5.88
H_2O	144	3	7.52±0.45	0.088±0.003	7.43
H_2O	168	2	6.67±1.00	0.098±0.000	6.57
H_2O	192	1	7.00	0.095	6.90
H_2O	216	2	7.71±0.08	0.069±0.014	7.64
PDPC	72	3	11.05±0.57	11.19±0.39	0.00
PDPC	96	2	8.20±0.30	3.51±0.22	4.69
PDPC	120	2	7.47±0.14	3.37±0.82	4.10
PDPC	144	3	7.86±0.72	1.14±0.39	6.72
PDPC	168	2	7.15±0.12	0.98±0.13	6.17
PDPC	192	1	9.93		
PDPC	216	2	6.83±1.00		

GRAPH XV

Effect of Pre-exposure of Soil Grown Soybean Seedlings to PDPC



○ Imbibed in Water, △ Imbibed in PDPC, —— Total Equivalents, _ _ _ Residue

metabolites is initially high following germination in PDPC but it also returns to normal at an age of about 192 hours.

The amount of PDPC metabolized following germination in water remains fairly constant throughout the experimental period. The rate of breakdown is high which results in a very low level of residue. Pre-exposure to herbicide, however, results in an almost complete inhibition to degradation during the first 24 hour period. The rate gradually revives to approximately that of the control by 168 hours. These results indicate that either pre-exposure to or added accumulation of the herbicide causes an inhibition to its degradation into metabolites. This inhibition is due to pre-exposure and not to age.

Finally, in order to study the complete metabolism of the herbicide to carbon dioxide, experiments were run in a respirometer as described in the section on Methods. Table XII lists the carbon dioxide recovered per hour as a percent of absorption for seedlings grown to an age of 107 hours. Graph XVI plots this data as a function of age for both the seedlings germinated in H_2O and those germinated in five ppm solution of carrier PDPC. A total of eight runs were completed, three of which utilized seedlings germinated in PDPC.

As can be seen the recovery of radioactive carbon dioxide as a percent of total absorption is greatly reduced by pre-exposure to PDPC. The overall absorptions, however, are similar for the two types, 20.98 ± 0.06 µg for water germinated and 19.47 ± 0.56 µg for

Table XII

Hourly Recovery of Respiratory $C^{14}O_2$ as Percent of Absorption

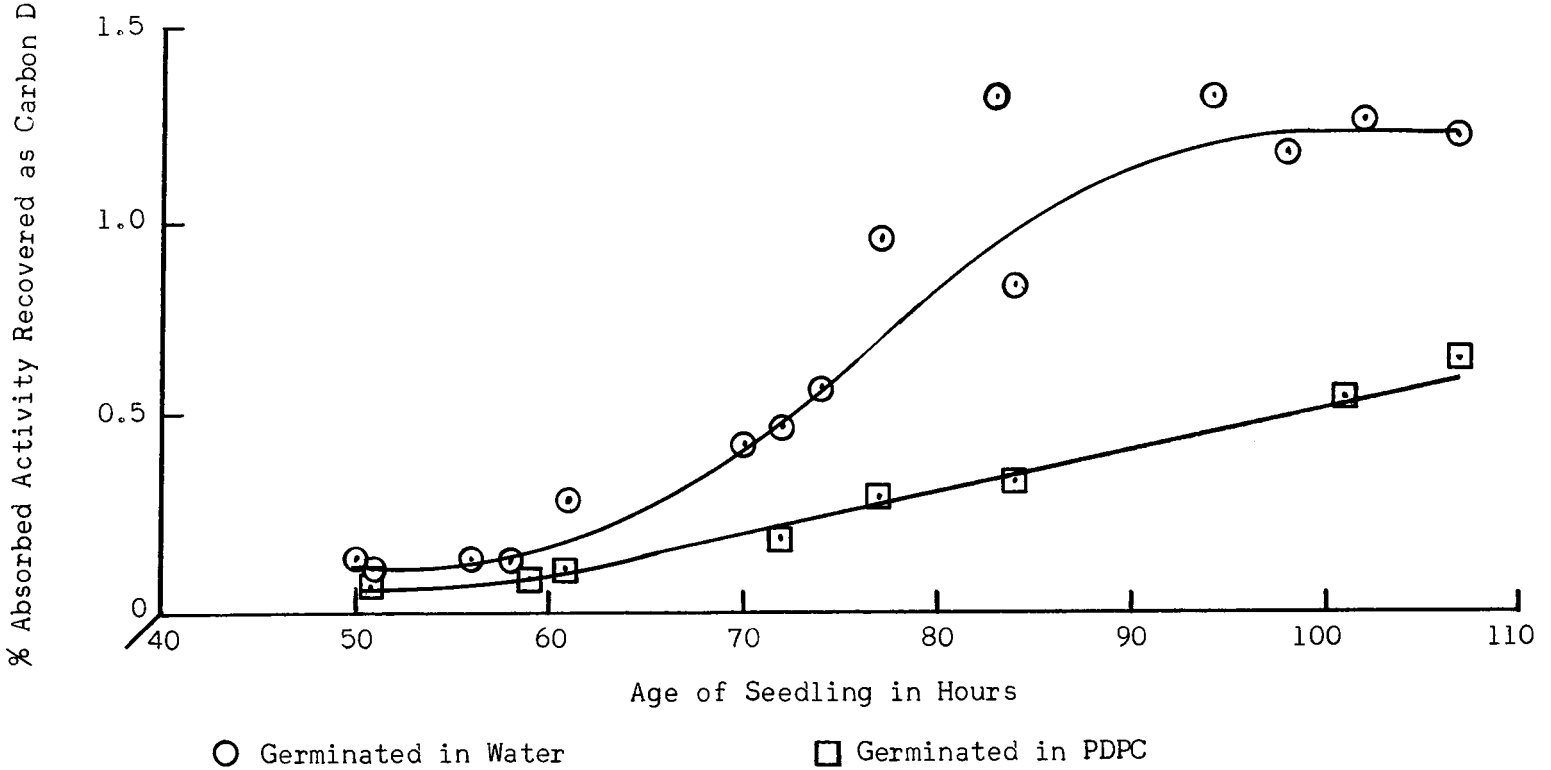
Germinated in Water			Germinated in PDPC Solution		
Age (hrs)	CO_2 Recovered per Hour as Percent of Absorption	Number Determinations	Age (hrs)	CO_2 Recovered per Hour as Percent of Absorption	Number Determinations
50	0.14±0.10	3	51	0.07±0.01	2
51	0.08±0.01	2	58	0.08±0.00	2
56	0.14±0.04	3	61	0.12±0.04	2
58	0.13±0.01	2	72	0.19±0.03	2
61	0.29±0.02	2	77	0.30±0.03	2
70	0.44±0.12	3	84	0.34±0.06	2
72	0.46±0.01	2	101	0.55±0.01	2
74	1.07±0.07	3	107	0.65±0.05	2
77	0.96±0.30	4			
83	1.34±0.10	3			
84	0.83±0.04	2			
94	1.34±0.15	3			
98	1.19±0.17	3			
101	0.92±0.18	2			
102	1.28±0.15	3			
107	1.23±0.12	3			

carrier germinated seedlings. Seedlings germinated in carrier produced 3.25 ± 0.15 µg equivalents of radioactive carbon dioxide while water germinated seedlings produced a total of 7.48 ± 0.44 µg equivalents. The difference in carbon dioxide production is reflected in the total ethanol soluble material which increased by 50%. The amount of PDPC converted into ethanol soluble material was increased from 9.90 ± 0.58 µg in water germinated seedlings to 14.74 ± 0.76 µg in carrier germinated seedlings.

These values compare favorably with those obtained from the previous set of experiments. In both, the pre-exposed seedlings

GRAPH XVI

Percent Total Absorption Recovered as Radioactive Carbon Dioxide per Hour



contained much more alcohol soluble activity than did those germinated in water.

Comparison of the values for residue PDPC is also good. Seedlings grown in water and pre-exposed to PDPC had a residue content of 15.00 μg while those germinated in water contained 5.21 μg. This increase is similar to that obtained from the previous experiments carried out using soil. This indicates that while the experiments carried out in water resulted in higher values at an earlier age both followed the same pattern of decreased PDPC metabolism.

The incorporation of C-14 into tissue was greatly decreased by pre-exposure. Seedlings germinated in water incorporated 3.59 ± 0.07 μg of PDPC equivalents into tissue while pre-exposure to carrier decreased this value to 1.47 ± 0.05 μg.

Thus the result of treatment of soybean seedlings during the early stages of germination results in a decreased conversion of PDPC-C^{14} to metabolites, a decrease in the conversion of metabolites to CO_2 and a decreased incorporation of metabolites into cellular tissues. The absorption of PDPC-C^{14}, however, is not affected, remaining very close to that found in the controls.

Summary

The experiments carried out for this thesis were designed to study the absorption, translocation, metabolism and residue content of n-propyl-C^{14} N,N-di-n-propylthiolcarbamate (PDPC) in soybean and peanut plants. The experiments described study these processes in germinating seeds, young seedlings and mature plants. The following are the most pertinent points discussed:

1. A method is given for the isolation of both unchanged residue and total ethanol soluble components of PDPC utilizing steam distillation techniques.

2. A method for the determination of residue and ethanol soluble components in a liquid scintillation counting system is discussed. Description of the counting systems and efficiencies are given.

3. A method for the collection of radioactive carbon dioxide from a gas chromatograph and comparison of activity with chromatographic peaks is given.

4. The general distribution of radioactivity in plants treated with PDPC-C^{14} is demonstrated with radioautograms. The above ground portions were found to contain a somewhat higher amount of radioactivity. Increasing the role of application resulted in an increased uptake but this was not proportional, a greater increase in uptake resulting. Remobilization of activity from the leaves was small.

5. The residue content of both peanuts and soybeans was extremely low at harvest. A maximum concentration, which was reached

two to three weeks after application, was quickly reduced. At the end of the 26 week growing period all the plant parts had negligible residue levels.

6. Either age or cellular concentration of herbicide appeared to affect the breakdown of herbicide. Seedlings which were allowed to imbibe water for 48 hours demonstrated a greater ability to detoxify the herbicide than seeds which were treated with PDPC-C^{14} throughout germination. These pre-exposed seedlings, however, were able to recover in time and function as normal plants.

7. Experiments carried out in which seeds were allowed to imbibe either carrier PDPC or water before being treated with PDPC-C^{14} demonstrated the inhibiting effect of this herbicide on its own metabolism. Seeds allowed to imbibe the herbicide showed a reduced ability to break it into ethanol soluble components.

8. The ability to convert PDPC-C^{14} into respiratory carbon dioxide was also reduced by the above treatment as was their incorporation into cellular tissue. The absorption of herbicide, however, was not affected.

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