A STUDY ON THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE METABOLISM OF ASPARTIC ACID AND GLUTAMIC ACID IN THE BEAN PLANT, PHASEOLUS VULGARIS, VARIETY BLACK VALENTINE

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INTRODUCTION

In the last decade 2,4-dichlorophenoxyacetic acid (2,4-D) was found to be an effective herbicide. What was once only a laboratory chemical now has great value in the field of weed control. An estimated 30 million pounds of 2,4-D are now being manufactured and used annually.

It was found in early research that very small amounts of 2,4-D produced an effect on plants which was very similar to that of indoleacetic acid, a naturally occurring plant hormone. Later studies disclosed that large amounts of 2,4-D had a herbicidal action. Thus 2,4-D became known as a herbicide of the hormone or growth-regulator type.

The action of hormone type herbicides is entirely different from that of contact type herbicides. Rather than killing only the tissue with which it comes into contact, the hormone type may affect the entire plant. In general, dicotyledonous plants are more susceptible to 2,4-D than are monocotyledonous plants. This selectivity allows effective suppression of most broad-leafed weeds occurring in grass and grain crops.

When 2,4-D is applied to bean plants, the first observable symptoms are the bending and twisting of the leaf petioles and stems and curling of the leaves. Then the stems become enlarged, and later, if a lethal dose had been applied, chlorosis occurs. This is followed by the death of the plant.

A recent review by Crafts (5, pp.253-282) reports that much work has been done revealing the apparent effects of 2,4-D on various plant systems. Little is known, however, about the actual mechanism of action of this compound.

Many methods of investigation have been used in attempting to solve this problem. One method has been to study the changes in protein and amino acid metabolism. Sell et al. (15, pp.295-299) working with red kidney bean plants have shown that the stems of 2,4-D treated plants may contain almost twice as much crude protein as do stems of control plants. Although the total amounts of the amino acids in the crude protein were greater in the stems of the treated plants, the percentages of the amino acids in the crude protein were different from those of the control plant. This data suggested to the authors that a change had occurred in the character of the protein.

The same authors (19, pp.289-293) later found that 2,4-D caused a slight decrease in crude leaf protein while the crude root protein remained approximately the same. Most of the amino acids determined were found to occur in slightly lower concentrations in the leaves and roots of the treated plants than was the case in control plants. The authors interpreted these facts as indications of translocation of the amino acids from the leaves and roots to the stem. Lesser amounts of aspartic acid were found in the leaves and roots of the treated plants while in the stem the percentage of aspartic acid in the crude protein was found to be 43.7% lower.

This work has been criticized by Payne and coworkers (12, pp.142-150). They reasoned that changes in amino acid content could be due either to free amino acids or to the amino acids from the protein. To clarify this point Payne and coworkers investigated the effect of 2,4-D on the free amino acids in the potato tuber. The free amino acids in the tubers were separated by paper chromatography. Their relative densities were determined with a Welch Densichron No. 2150 after being sprayed with 0.1% ninhydrin solution. By this method Payne and coworkers found that 2,4-D caused an

increase in free glutamic acid and a decrease in eleven other amino acids including aspartic acid. The decrease was explained as an increased catabolism of the free amino acids. The increase of glutamic acid was explained as an inhibition of the reaction of glutamic acid to α-ketoglutaric acid. Thus glutamic acid, due to its key position in transamination with other acids in protein synthesis, was not subjected to increased amino acid catabolism.

Payne et al. (13, pp.46-49) have subsequently shown that the crude protein increased in the tubers of the treated potato plants. It has been reported elsewhere (4, pp.393-405; 20, pp.50-58; 10, pp.322-333; and 17, pp.58-65) that the amount of protein or protein nitrogen decreases in the leaves and increases in the stems and roots of 2,4-D treated plants. Rebstock et al. (14, pp.639-643) have shown that proteolytic activity was markedly decreased in the leaf and slightly decreased in the root of the 2,4-D treated plant. This activity was significantly increased in the stem. Freiburg (9, pp.674-675) obtained similar results when dealing with proteinase and polypeptidase activities. There was an exception, however, in that he found an increase in activity in the roots of treated plants.

Using microbiological assay methods, Fang (6) has shown that the distribution of the amino acids derived directly from the Kreb's cycle remained unchanged in the leaves of bean plants treated with 2,4-D. Aspartic and glutamic acids decreased in both the stems and roots of the treated plants with a greater change in the stems. Analyses were made for the total amino acids in the plant material. Two possibilities were presented to explain the decreased amounts of amino acids; (a) the 2,4-D treatment might inhibit the synthesis of such amino acids, or (b) this treatment might speed up the oxidation rate of the amino acids.

The present work was carried out in order to clarify this point. The principal approach to this problem has been that of determining the rates of incorporation of carbon 14 into glutamic and aspartic acids in 2,4-D treated and control plants. With this objective in mind, the investigations were carried on employing ion exchange and paper chromatography. These techniques were used for the isolation, quantitative determination, and measurement of the specific radioactivities of these amino acids.

TREATMENT OF BEAN PLANTS

Twelve bean plants (Phaseolus vulgaris, variety Black Valentine) were grown in potted soil, four plants to a pot, under greenhouse conditions. The plants were allowed to grow until they were six to eight inches tall with the primary leaves fully unfolded and the second internode well defined. At this time two plants of each pot were treated with 50 micrograms of 2,4-D on the mid-rib of one primary leaf. The solution used was 0.1% of 2,4-D in 95% ethanol containing 0.5% Tween-20. The other two plants in each pot were left untreated as control plants.

A closed system was prepared as shown in Figure 1. This system consisted of a bell jar large enough to contain one set of four plants, a column of calcium chloride to absorb the excess moisture, a generator to produce radioactive carbon dioxide from $BaC^{14}O_3$ after the system was sealed, a Geiger-Müller tube to detect the radioactivity, and a pump to circulate the atmosphere.

One day after treatment, the first set of bean plants was put in this closed system and exposed to an atmosphere containing $C^{14}O_2$ for 24 hours under simulated greenhouse conditions. The radioactivity readings of

the atmosphere were periodically taken in order to check the amount of radioactivity absorbed by the plants. The second and third sets of bean plants were exposed to $C^{14}O_2$ in a similar manner, three days and seven days, respectively, after the treatment with 2,4-D. The amounts of $BaC^{14}O_3$ used for these three experiments were 4.3 milligrams, 6.3 milligrams, and 5.1 milligrams respectively (20.6 microcuries per milligram). The results of the radioactivity readings are shown graphically in Figure 2.

After the exposure to radioactive carbon dioxide the plants were harvested and divided into roots, stems, and leaves. The stems included the hypocotyl, petioles, and first and second internodes. After drying overnight at 55° C. in a vacuum oven, the samples were weighed, ground, and stored in a vacuum desiccator.

ISOLATION AND ANALYSES

Weighed amounts of each sample (100 milligrams) were autoclaved with ten milliliters of 2 N hydrochloric acid in sealed tubes for ten hours at fifteen pounds pressure. The excess hydrochloric acid was removed from the hydrolysate by repeated addition of

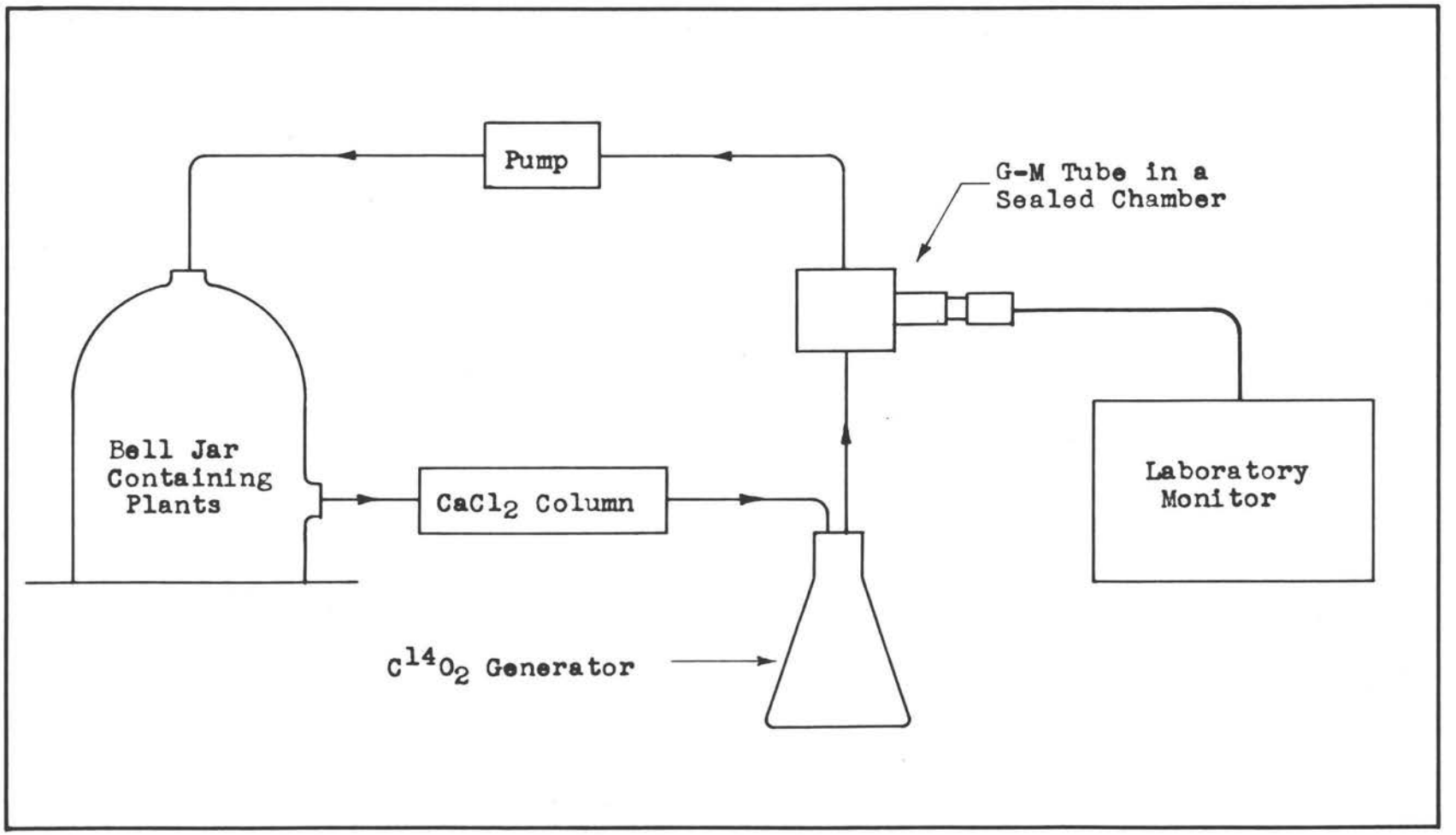
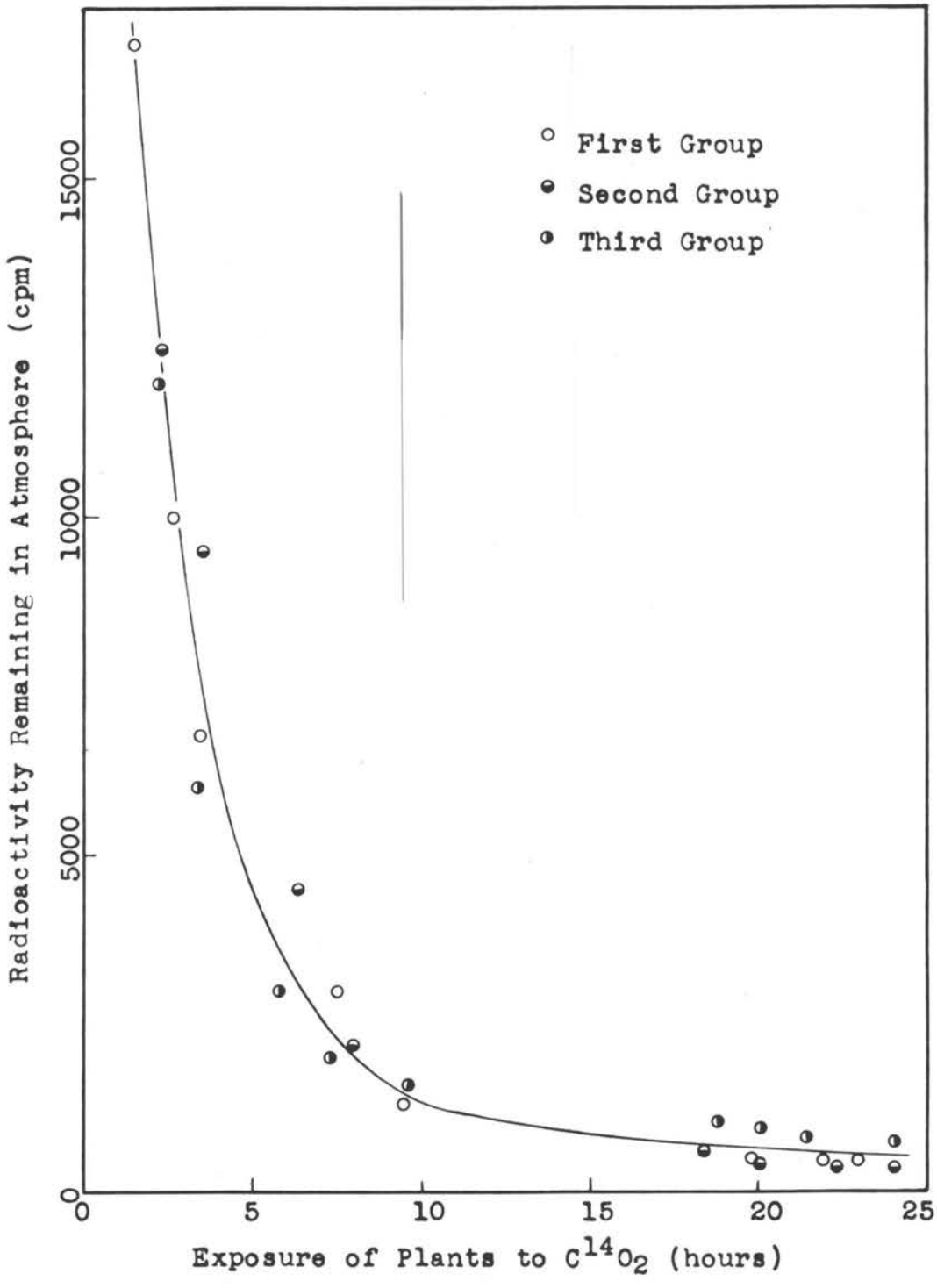


Figure 1 Block Diagram of the Sealed System Used for $C^{14}O_2$ Absorption

Figure II Absorption of Radioactive Carbon Dioxide by Bean Plants



water and evaporation to dryness *in vacuo*. The hydrolysate was made up to 100 milliliters and the residue was removed by passing through a dry filter.

The acidic compounds were separated from the neutral and basic compounds by means of ion-exchange columns of Amberlite IR-4B. Several glass columns 16 millimeters in diameter were filled to a height of 25 centimeters with resin in the basic form. The columns were backwashed, charged with four percent ammonium hydroxide, and washed with distilled water until neutral to pH indicator paper.

The corresponding pairs of treated and control samples were run simultaneously to reduce the errors due to differences in operation. An aliquot of each solution was allowed to pass through a resin column at the rate of approximately one drop every three seconds. When the last of the solution reached the top of the resin bed, the column was washed with distilled water, allowing no break in the continuity of the liquid phase. This washing was continued until no activity was detected in the effluent. Four percent hydrochloric acid was passed through the column to elute the acidic compounds (including aspartic and glutamic acids) from the resin. The radioactivity of each effluent was

checked by counting half milliliter aliquots that had been evaporated to dryness in cup planchets. The excess hydrochloric acid was again removed from the effluent by repeated addition of water and evaporation to dryness, *in vacuo*. The samples were then made up to 25 milliliters.

Paper chromatography was used to gain further separation of the amino acids. Aliquots of the solutions were applied quantitatively as spots or lines to strips of Whatman No. 1 filter paper (1 inch by 22 inches), maintaining a diameter or width of about 0.75 centimeters during application. A stream of warm air was used to speed evaporation. Only a small amount of heat was used as it was noted that overheating seemed to fix some of the amino acids to the paper so that they would not travel when the chromatogram was developed.

These strips were developed in phenol saturated with water for about 18 hours by the descending method, then air-dried for at least 12 hours. Small chromatographic chambers holding six or seven strips at a time were used. Each sample was run in triplicate and the results were averaged.

The observed R_f values varied from 0.23 to 0.29 for aspartic acid and from 0.31 to 0.38 for glutamic acid.

Although the Rf values varied between runs, the ratio between the R_f values of the two acids remained essentially the same. Block (2, p.67) has shown that aspartic and glutamic acids have R_f values of 0.19 and 0.31 respectively, in aqueous phenol solution. It is assumed that the higher values obtained in this study were contingent upon the fact that the amino acids were in the form of the hydrochloride salts. The literature R_f values were obtained by chromatographing the pure amino acids.

A standard solution was made up containing one milligram per milliliter each of aspartic and glutamic acids, and equivalent amounts of hydrochloric acid were added to form the salt. A micro-syringe was calibrated with mercury and found to deliver 10 microliters with an accuracy of ± 3% and 50 microliters with an accuracy of ± 0.1%. The micro-syringe was used to apply varying amounts of the standard solution to strips which were run with the unknown, treated and control strips.

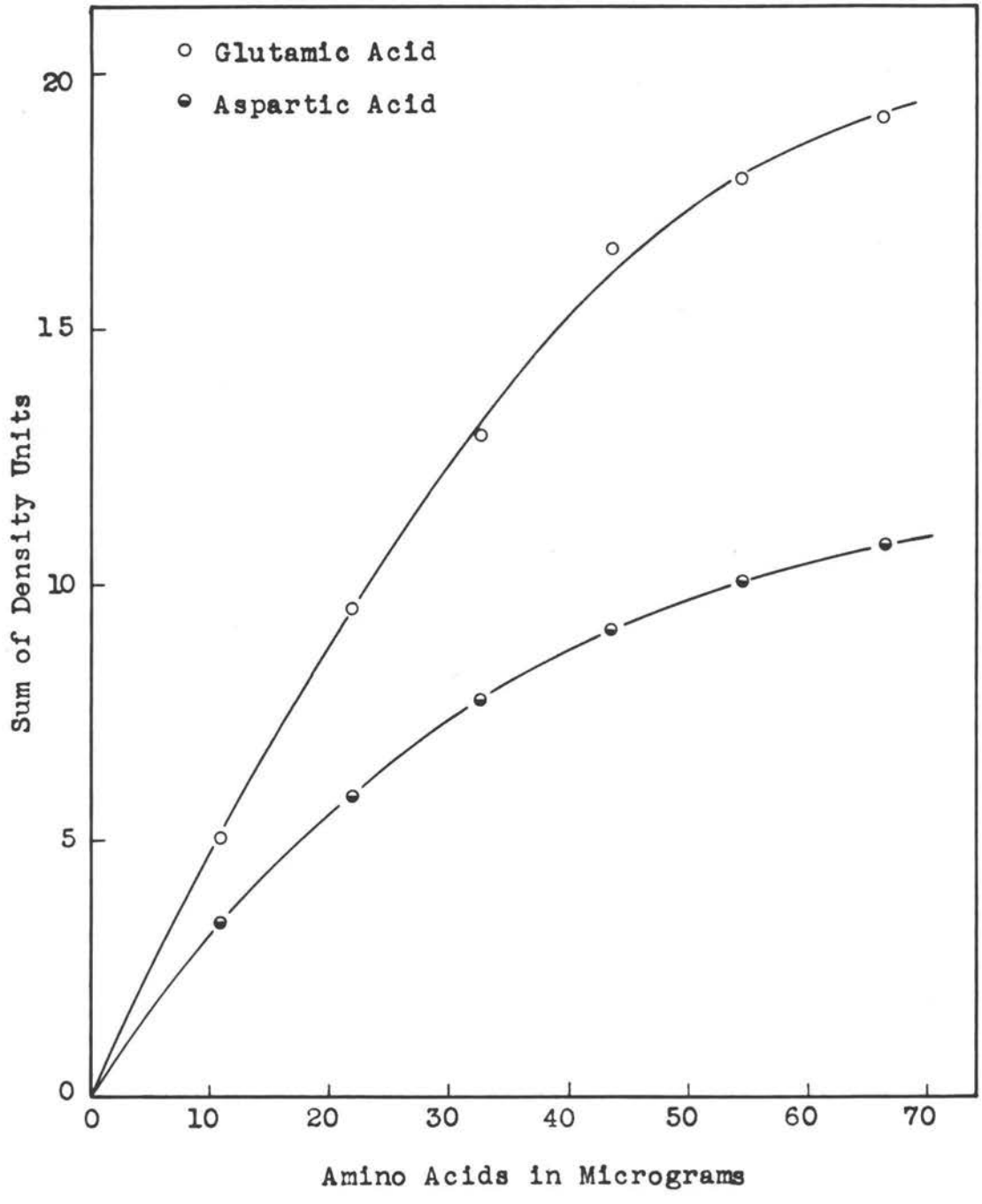
After the radioactivity of the unknown strips had been measured, quantitative determination of the amino acids present was made by color development with ninhydrin solution. The method which proved to be the most applicable was similar to that of Block (1, pp.1327-1332).

The strips were sprayed with 0.25% ninhydrin in isopropyl alcohol. This process was repeated on the reverse side when the strips were almost dry. The strips were heated in an oven at 100° C. for five minutes then taken out and allowed to stand one to two hours for full color development. The color then remained essentially unchanged for several hours if the strips were kept in a dry, dark place.

The relative color density was measured with a Photovolt Electronic Densitometer, model 525, using a blue filter. With a slit-width of one millimeter the spots were scanned at increments of one millimeter. The density readings were then added to give a figure representing the amount of the amino acid in that spot. A standard curve (Figure III) was determined and plotted for each amino acid in the range of 10 to 70 micrograms. Each value was determined in triplicate. The concentrations of the amino acids in the samples were then obtained by reference to this curve.

The spectrophotometric methods of Moure and Stein (11, pp.367-388) and Fouden (7, pp.327-333) were also tried but they proved to be quite tedious and gave unsatisfactory results.

Figure III Comparison of Densitometer Readings to Amounts of Aspartic Acid and Glutamic Acid



RADIOACTIVITY MEASUREMENTS

Weighed aliquots (10 milligrams) of each sample were oxidized by the "dry" combustion method using a standard combustion train (18, pp.82-121). After putting the platinum boat containing the sample in the combustion tube, another boat containing glucose was put in the tube, three to four inches behind it. The glucose was combusted immediately following the combustion of the plant sample to flush all the radioactive carbon dioxide from the tube. The carbon dioxide was quantitatively absorbed in carbonate-free sodium hydroxide solution and precipitated as barium carbonate. This was mounted on thin copper planchets and the radioactivity was measured with a thin mica window Geiger-Müller counter (1.9 milligrams per square centimeter) connected to a Tracerlab "Utility Scaler".

The background count of this instrument was found to be consistently in the region of 19 to 22 counts per minute. All planchets were counted for 25,600 counts which gave a maximum of one percent statistical error for all samples. A self-absorption curve for barium carbonate was determined and plotted. All measurements were corrected to zero thickness of barium carbonate

in the usual manner (3, pp.27-32).

After the chromatograms were developed and dried the radioactivity of the amino acids was determined. The paper strips were counted at centimeter intervals through a one centimeter slit. The Geiger counter was placed as close as possible to the strip to increase the counting efficiency. The values were corrected for self absorption and for paper absorption by a method similar to that used in the barium carbonate counting. Several one centimeter sections of the paper chromatograms containing various amounts of radioactivity were combusted, and the radioactivity was redetermined as barium carbonate. These comparison values were plotted to determine a correction curve.

There was approximately ten percent statistical error in the counting of the paper strips. This error was less when the triplicate values were averaged.

RESULTS AND DISCUSSION

The rate of absorption of radioactive carbon dioxide is shown graphically in Figure II. Approximately ninety percent of the radioactivity was absorbed by the bean plants in the first eight hours of exposure, with the remainder being absorbed at a much slower rate.

The amount of carbon 14 incorporated into each plant sample is given in Table I. It is interesting to note that the 2,4-D treated plants absorbed much less radioactive carbon dioxide than did the control plants. Furthermore this difference in absorption, which is shown in Table I as the Ratio of Total Plant Activity, is relatively the same for all three experiments.

These results, which indicate that 2,4-D treatment reduces the rate of photosynthesis, agree closely with the work reported by Freeland (8, pp.621-628).

The results of the chemical analyses are given in Table II. The entries are the average of triplicate runs on each sample. The left side of the table gives the amount of aspartic and glutamic acids in each plant section. Since the number of plants in each experiment was small and the weights of the plant parts varied somewhat, the percentage of amino acids in each section was calculated to obtain a more comparable result. These data are shown on the right side of Table II.

In the first day samples, the percentages of aspartic acid and glutamic acid are essentially the same in both control and treated plants. As shown in Figure IV, the leaves of the control plants showed a sharp decrease in the amount of both amino acids in the

TABLE I

The Effect of 2,4-D Treatment on Photosynthesis in Bean Plants as Determined By Radioactive Analyses of Plant Tissues.

Sample*	Wt. of Sample (mg)			Specific Activity** (c/m/mg)			Total Activity (c/m)			
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Total Plant
1st Day										
Treated	403.1	254.1	207.4	3,697	5,534	4,442	1,490,261	1,406,189	921,271	3,817,721
Control	236.3	231.9	219.9	5,181	11,225	10,803	1,224,270	2,603,078	2,375,580	6,202,928
Ratio T/C				0.714	0.493	0.411	1.217	0.540	0.388	0.615
3rd Day										
Treated	222.3	370.7	300.4	1,981	12,567	4,394	440,376	4,658,587	1,319,958	6,418,921
Control	291.2	356.6	231.6	10,504	11,271	13,963	3,058,765	4,019,239	3,233,831	10,311,835
Ratio T/C				0.189	1.115	0.315	0.144	1.159	0.408	0.622
7th Day										
Treated	238.2	400.1	238.1	4,082	5,131	7,996	972,332	2,052,913	1,903,848	4,929,093
Control	325.2	374.3	380.4	5,140	7,387	9,394	1,671,528	2,764,954	3,573,478	8,009,960
Ratio T/C				0.794	0.695	0.851	0.582	0.742	0.533	0.615

* 1st Day - 4.3 mg $BaCO_3$ - 0.0206 mc/mg
3rd Day - 6.3
7th Day - 5.1

** The activity was measured by a thin mica window G-M Counter (1.9 mg/cm^2).

TABLE II

The Effect of 2,4-D Treatment on the Contents of Aspartic and Glutamic Acids in Bean Plants.

Sample	Total Amount of Amino Acids in Plant Sections (ug)				Percent Amino Acid in Plant Section ($\times 10^{-1}$)					
	Aspartic Acid		Glutamic Acid		Aspartic Acid			Glutamic Acid		
	Treated	Control	Treated	Control	Treated	Control	T/C	Treated	Control	T/C
Leaf										
1 Day	553	625	461	485	2.67	2.84	0.94	2.22	2.21	1.00
3 Day	700	232	731	207	1.75	0.67	2.82	1.83	0.55	3.33
7 Day	179	340	244	280	0.75	0.89	0.84	1.02	0.74	1.38
Stem										
1 Day	2863	2551	1219	898	11.27	11.00	1.02	4.80	3.87	1.24
3 Day	3489	3854	1140	1442	9.41	10.81	0.87	3.08	4.04	0.76
7 Day	1606	3273	610	1130	4.01	8.74	0.46	1.53	3.02	0.51
Root										
1 Day	396	233	480	289	0.99	0.99	1.00	1.20	1.22	0.98
3 Day	169	384	-	724	0.76	1.32	0.58	-	2.49	-
7 Day	362	503	448	1542	1.52	1.55	1.02	1.88	4.74	0.40
Total										
1 Day	3812	3409	2160	1672	4.41	4.95	0.89	2.50	2.43	1.03
3 Day	4358	4470	-	2373	4.88	5.08	0.96	-	2.70	-
7 Day	2147	4116	1302	2952	2.45	3.81	0.64	1.49	2.73	0.54

Figure IV The Effect of 2,4-D on the Concentrations of Aspartic and Glutamic Acids in the Leaves of Bean Plants

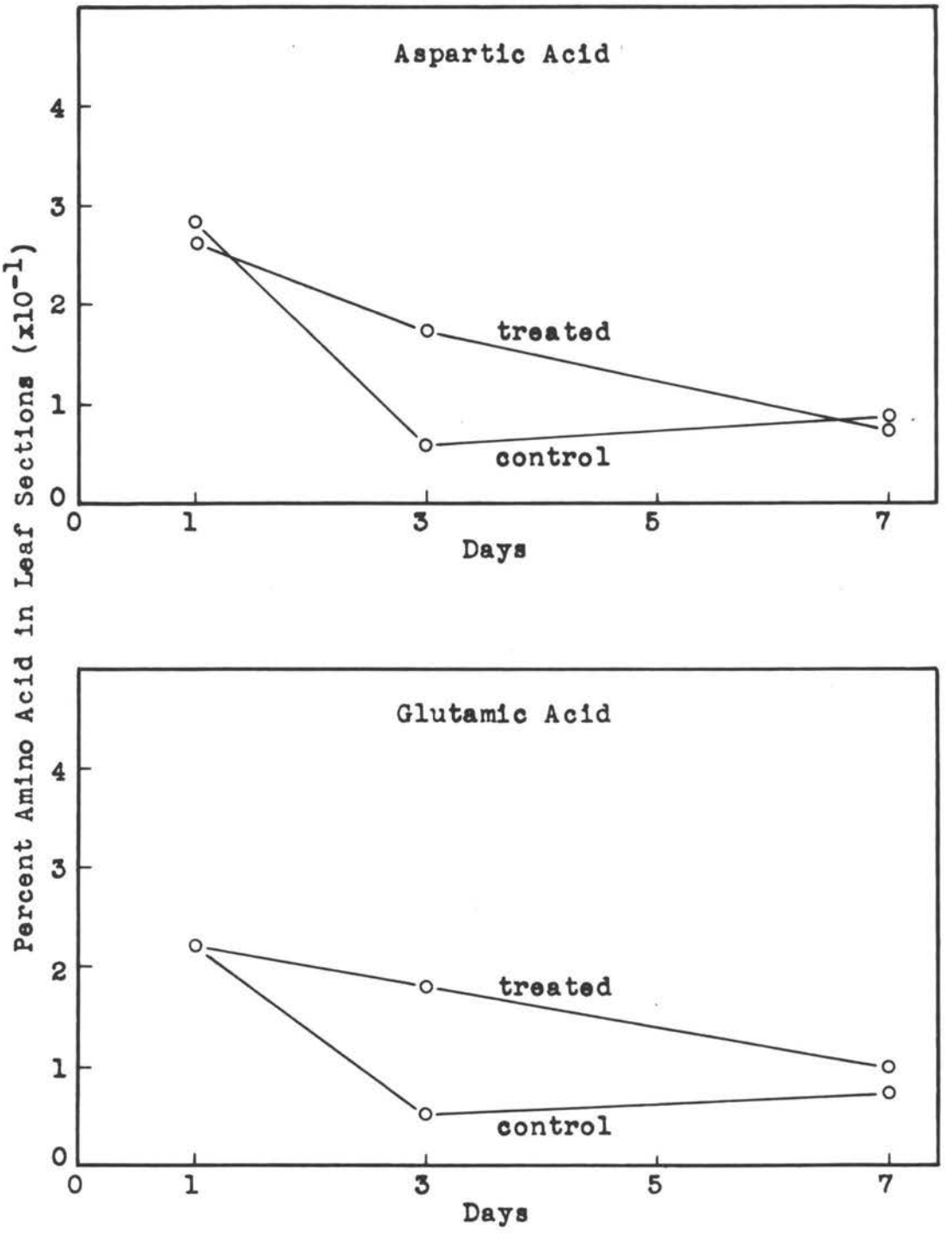


Figure V The Effect of 2,4-D on the Concentrations of Aspartic and Glutamic Acids in the Stems of Bean Plants

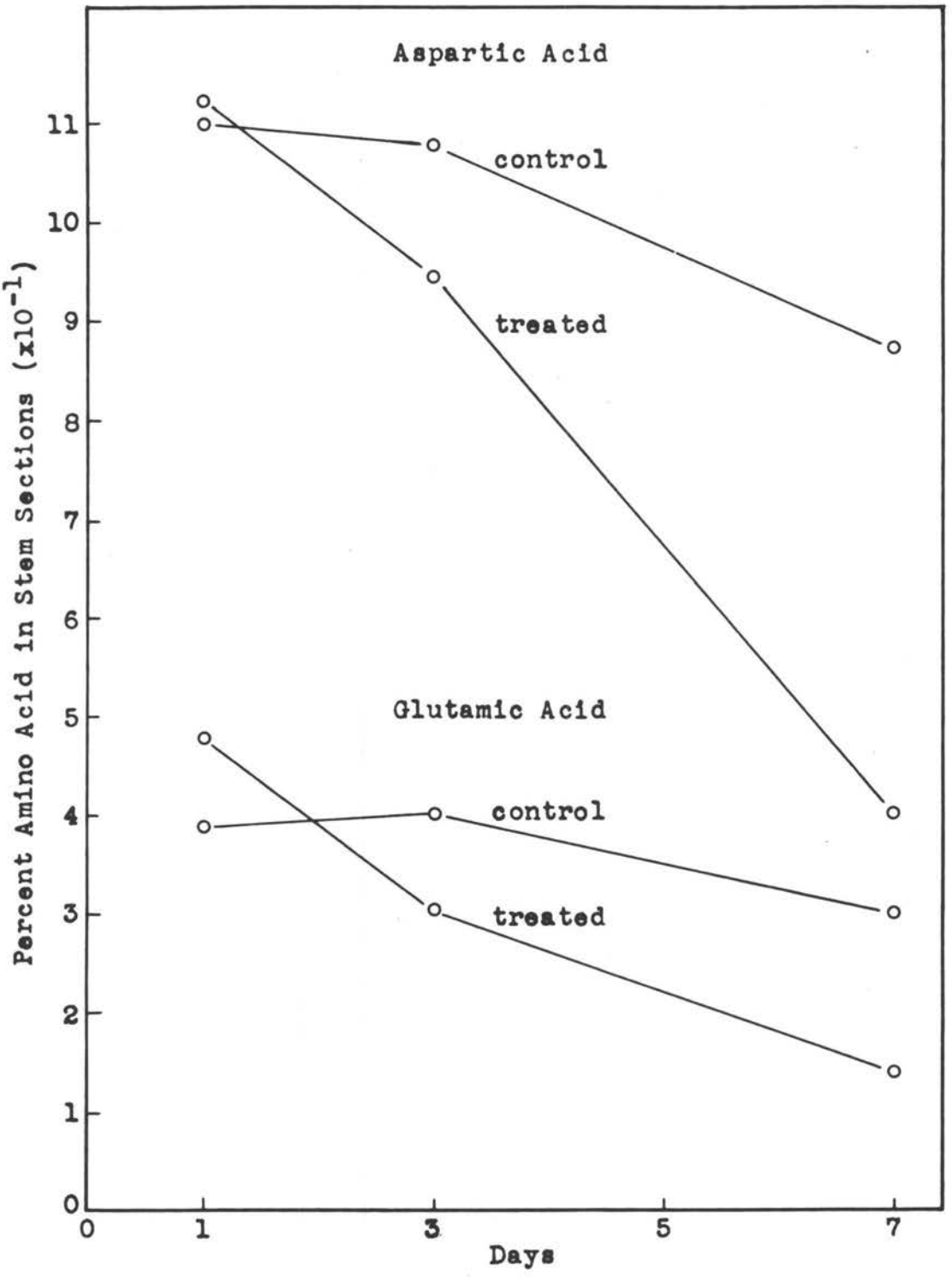
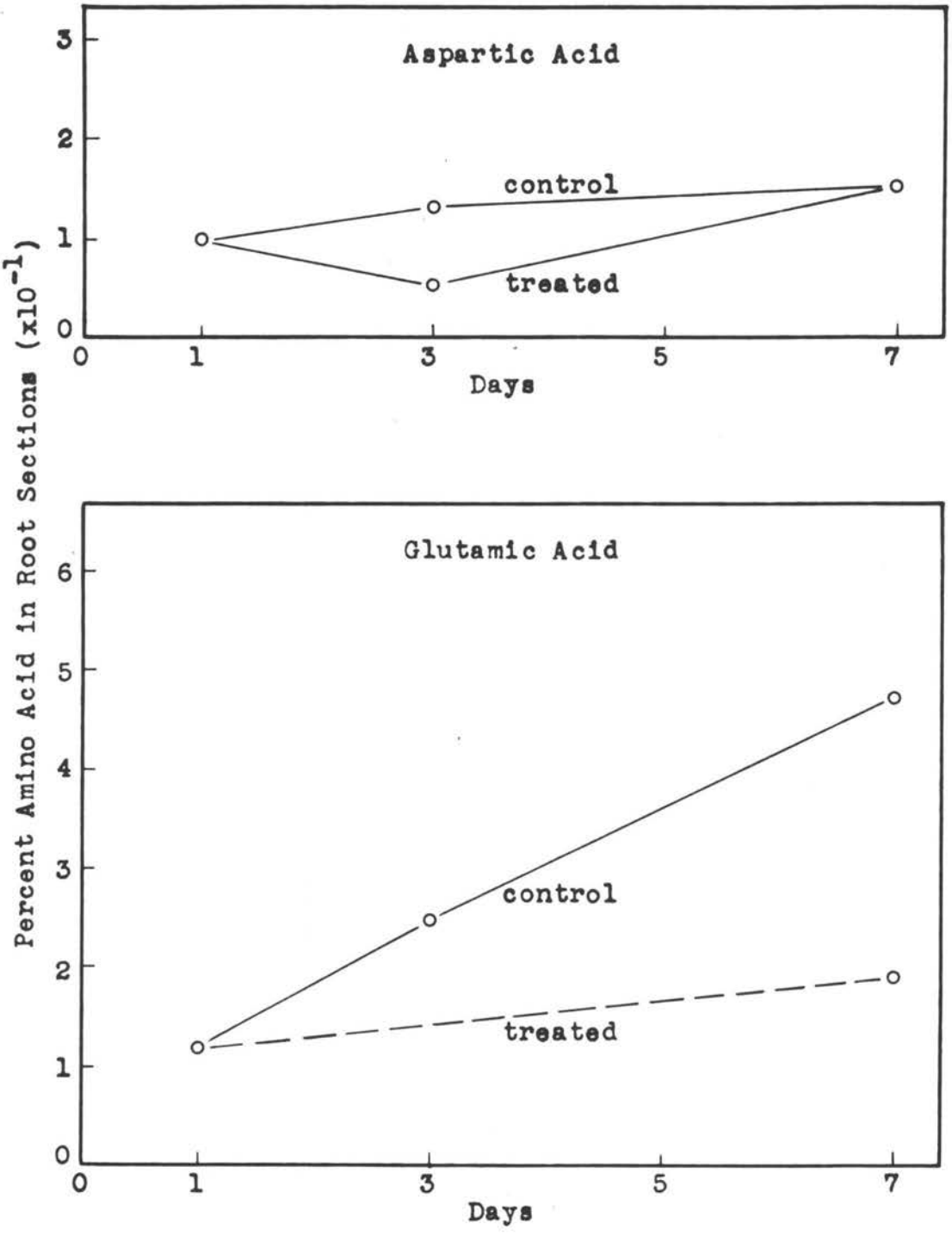


Figure VI The Effect of 2,4-D on The Concentrations of Aspartic and Glutamic Acids in the Roots of Bean Plants



third day sample and a slight increase in the seventh day sample as compared to the amount in the treated plants which gradually decreased to approximately the same value as the control. In Figure V a more significant difference may be observed. In the treated stem samples, the concentration of both aspartic and glutamic acids had decreased to 75-85 percent of the control values on the third day and to approximately 50 percent of the control values on the seventh day. In the root tissue, the concentration of both amino acids increased with age, and the 2,4-D treatment reduced only the accumulation of glutamic acid as shown in Figure VI.

In general, a decrease in protein content is noted as a plant grows older. The older tissue in a plant will also have a lower protein content than the younger, growing parts. The aspartic acid and glutamic acid contents of the control plants closely follow this general pattern.

Sell *et al.* (15, pp.295-299) and Weller *et al.* (19, pp.289-293) have reported the effect of 2,4-D treatment on changes in chemical composition of the leaves, stems, and roots of red kidney bean plants. From their more complete results on eleven amino acids they have suggested that 2,4-D treatment causes

a change in the character of the stem protein. The results of aspartic and glutamic acid determinations presented here, agree closely with their findings.

The results of the radioactive analyses are summarized in Table III. Each entry is the average of triplicate runs on each sample. On the left side of the table, the total radioactivities of aspartic and glutamic acids in each plant section are shown. Since different amounts of radioactive barium carbonate were used in the three experiments and since the rate of absorption of radioactivity was greatly reduced in the treated plants, the percentage of the total radioactivity which was found in each amino acid, was calculated. These percentages are shown on the right side of the table.

Percentagewise, the amount of carbon 14 incorporated into aspartic and glutamic acids was found to be greater in the treated plants than in the control plants. This difference was most pronounced in the stems. The same effect, but to a smaller extent, was noted in the roots with one exception. No satisfactory explanation can be given for the low activity of the treated roots of the third day sample or for the low activity of the amino acids in that sample. In the treated leaves, the

radioactivity of both amino acids was lower than the control values on the first day. The activity values for the leaves of the treated and control plants were about the same on the third day. The aspartic acid activity remained the same as the control value on the seventh day while glutamic acid activity was higher than the control value.

A simplified, schematic diagram showing the close interrelationship of photosynthesis, respiration (Kreb's Cycle), and protein metabolism is presented in Figure VII. Payne and coworkers (12, pp.142-150) used a similar diagram to explain their results in the study of the effect of 2,4-D on free amino acids in the potato tuber. They suggested that the decrease in amounts of the amino acids tested (excluding glutamic acid), following 2,4-D treatment, was due to the increase in activity of the specific oxidative deaminases and transaminases involved. They also suggested that there was an inhibition of the reaction of glutamic acid to α-ketoglutaric acid. With this explanation the nitrogen would be preserved (as glutamic acid or glutamine), and the increase of glutamic acid and the decrease of the other amino acids would be accounted for.

If the randomization of carbon 14 was unaffected

TABLE III

The Effect of 2,4-D Treatment on the Incorporation of Carbon 14 into Aspartic and Glutamic Acids in Bean Plants.

Sample	Total Activity of Amino Acids in Plant Section (c/m)				Percent Activity of Amino Acid to Total Plant Activity (x 10-1)					
	Aspartic Acid		Glutamic Acid		Aspartic Acid			Glutamic Acid		
	Treated	Control	Treated	Control	Treated	Control	T/C	Treated	Control	T/C
Leaf										
1 Day	1866	9531	2672	17961	0.49	1.54	0.32	0.70	2.90	0.24
3 Day	1521	2703	3296	4552	0.24	0.27	0.89	0.51	0.44	1.16
7 Day	1845	2645	9470	2334	0.37	0.33	1.12	1.92	0.29	6.62
Stem										
1 Day	21913	13635	19788	12120	5.74	2.20	2.95	5.18	1.95	2.66
3 Day	55827	18244	110591	16934	8.70	1.77	4.92	17.22	1.64	10.50
7 Day	9695	3732	69324	42719	1.97	0.47	4.20	14.06	5.53	2.64
Root										
1 Day	3121	1935	2792	2854	0.82	0.31	2.65	0.73	0.46	1.59
3 Day	724	3584	906	33155	0.11	0.35	0.31	0.14	3.22	0.04
7 Day	1846	2388	13941	12600	0.37	0.30	1.23	2.83	1.57	1.80
Total										
1 Day	26900	25101	25252	32935	7.05	4.05	1.74	6.61	5.31	1.24
3 Day	58072	24531	114793	54641	9.05	2.39	3.79	17.87	5.30	3.37
7 Day	13386	8765	92735	57653	2.71	1.10	2.46	18.81	7.19	2.63

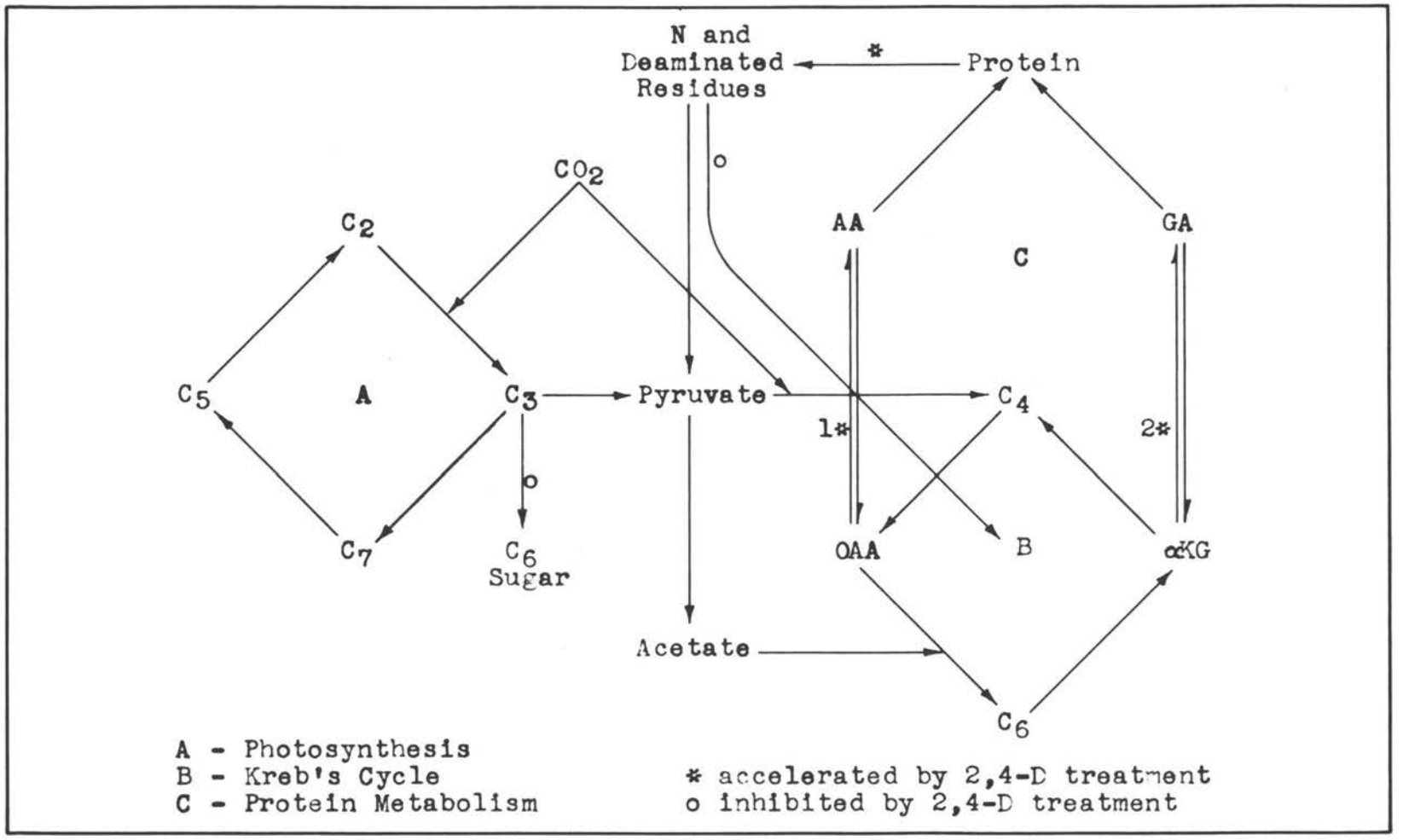


Figure VII Schematic Diagram Showing the General Relationship of Photosynthesis, Kreb's Cycle, and Protein Metabolism

in the treated plant, the amount of radioactivity incorporated into the amino acids should indicate the difference in the rate of synthesis during the experimental period. Since 2,4-D treatment increased the incorporation of carbon 14 into aspartic and glutamic acids, it is logical to conclude that the rate of synthesis of these two amino acids has been increased. However, the total quantity of these two amino acids was markedly decreased in the treated plants particularly in the seventh day samples. Therefore 2,4-D must increase the rate of oxidation or catabolism of these amino acids, and this increase must exceed the increase in the rate of synthesis.

This conclusion is supported somewhat by the works of Rebstock et al. (14, pp.639-643) and Freiburg (9, pp.674-675) who found that 2,4-D caused increases in proteinase and polypeptidase activities.

Since the carbohydrate and sugar reserves are readily depleted following 2,4-D treatment, as reported by several workers (15, pp.295-299; 16, pp.70-83; and 19, pp.289-293) and since the synthesis of aspartic and glutamic acids is increased as indicated by this work, it would seem that, during photosynthesis, more carbon 14 would enter the Kreb's cycle and nitrogen cycle

by common intermediates such as malic acid rather than into the carbohydrate cycle. As indicated in the schematic diagram, Figure VII, the ordinary reaction from C_3 to C_6 sugars in the photosynthesis process is greatly inhibited and further condensation from C_3 to C_4 units presumably is increased.

The increase in synthesis of aspartic and glutamic acids could probably be due to the increase in the action of the enzymes involved as shown in points 1 and 2 in Figure VII. The oxidation of aspartic and glutamic acids from the protein is also accelerated. The carbon fragments from this oxidation might not return to the nitrogen cycle for resynthesis as suggested by Payne and coworkers (12, pp.142-150) because the total amount of these two acids is greatly reduced during this seven day period.

Another possibility exists. If there is increased incorporation of carbon 14 into the Kreb's cycle in the 2,4-D treated plant, the higher amounts of radioactivity in the amino acids might result from the normal process of transamination. The decrease in total amounts of these amino acids could then be due entirely to an increased catabolism of the protein.

SUMMARY

It has been found that 2,4-D treatment markedly decreased the incorporation of radioactive carbon dioxide into the bean plant. The rate of inhibition in photosynthesis remained essentially constant over the seven day period after treatment.

Amounts of aspartic and glutamic acids were decreased in the treated plants being most pronounced in the stem tissue of the seven day plants. However, the incorporation of carbon 14 into these amino acids was far greater in the treated plants than in the control plants. Again the most pronounced effect was observed in the stems. The decrease in the total amount of these amino acids and the increased incorporation of carbon 14 were interpreted as an increase in synthesis of these amino acids with a more marked increase in the rate of oxidation of the amino acids. The net effect, then, is a slow decrease in the total amount of aspartic acid and glutamic acid.

BIBLIOGRAPHY

1. Block, Richard J. Estimation of amino acids on paper chromatograms. Analytical chemistry 22:1327-1332. 1950.

2. Block, Richard J., Raymond LeStrange, and Gunter Zweig. Paper chromatography, A Laboratory Manual. New York, Academic Press, 1952. 195p.

3. Calvin, Melvin et al. Isotopic carbon. New York, John Wiley and Sons, 1949. 376p.

4. Corns, W. G. Effects of 2,4-D and soil moisture on the catalase activity, respiration, and protein content of bean plants. Canadian journal of research 28C:393-405. 1950.

5. Crafts, A. C. Herbicides. Annual review of plant physiology 4:253-282. 1953.

6. Fang, Sheng C. The mode of action of labeled 2,4-dichlorophenoxyacetic acid (2,4-D) and similar agents. Part 2, 1953, The effect of 2,4-D, 2,4,5-T, and indoleacetic acid on nitrogen metabolism in bean plants. (Unpublished mimeographed report) Progress report to the Atomic Energy Commission on contract AT(45-1)-304 Department of Agricultural Chemistry, Agricultural Experiment Station, Oregon State College.

7. Fouden, L. Quantitative recovery and colorimetric estimation of amino acids separated by paper chromatography. Biochemical journal 48: 327-333. 1951.

8. Freeland, R. O. Effects of growth substances on photosyntheses. Plant physiology 24:621-628. 1949.

9. Freiberg, S. R. Effects of an exogenous growth regulator on proteolytic enzymes of the soybean plant. Science 115:674-675. 1952.

10. Freiberg, S. R. and H. E. Clark. Effects of 2,4-dichlorophenoxyacetic acid upon the nitrogen metabolism and water relations of soybean plants grown at different nitrogen levels. Botanical gazette 113:322-333. 1952.

11. Moore, Stanford and William H. Stein. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of biological chemistry 176:367-388. 1948.

12. Payne, Merle G., Jess L. Fults, and Ruth V. Hay. The effect of 2,4-D treatment on free amino acids in potato tubers. The American potato journal 29:142-150. 1952.

13. Payne, Merle G. et al. Protein content and specific gravity of red McClure potatoes increased by 2,4-D treatment. The American potato journal 30:46-49. 1953.

14. Rebstock, Theodore L. et al. Effect of 2,4-dichlorophenoxyacetic acid on proteolytic activity on red kidney bean plants. Plant physiology 27:639-643. 1952.

15. Sell, Harold M. et al. Changes in chemical composition at the stems of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Plant physiology 24:295-299. 1949.

16. Smith, Fredrick G. The effect of 2,4-dichlorophenoxyacetic acid on the respiratory metabolism of bean stem tissue. Plant physiology 23: 70-83. 1948.

17. Smith, Fredrick G., Charles L. Hamner, and Robert F. Carlson. Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2,4-dichlorophenoxyacetic acid. Plant physiology 22:58-65. 1947.

18. Steyermark, H. Quantitative organic microanalysis. Philadelphia, The Blakiston Company, 1951. 389p.

19. Weller, Lowell E. et al. Changes in chemical composition of leaves and roots of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Plant physiology 25:289-293. 1950.

20. Wort, D. V. Effects of non-lethal concentrations of 2,4-D on buckwheat. Plant physiology 26:50-58. 1951.