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This is to certify that, in accordance with the requirements of South Dakota State College for the Master of Science Degree, Mr. Clifford H. Hullinger has presented to this committee three bound copies of an acceptable thesis, done in the major field; and has satisfactorily passed a two-hour oral examination on the thesis, the major field, CHEMISTRY, and the minor field, ZOOLOGY.

Head of Major Department

August 21, 1948
Date

Head of Minor Department

Rep. of Graduate Committee

SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE NITROGEN AND CARBOHYDRATE METABOLISM OF THE CORN PLANT (*ZEA MAYS* L.)

by

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Submitted to Graduate Faculty

of

South Dakota State College of Agriculture and Mechanical Arts

in Partial Fulfillment of the Requirements for

the Degree of Master of Science

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Clifford H. Mullinger

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SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE NITROGEN AND CARBOHYDRATE METABOLISM OF THE CORN PLANT (*ZEA MAYS* L.)

Introduction

Since the introduction of 2,4-dichlorophenoxyacetic acid as an herbicide, considerable study has been given to the physiological action of this compound on various plants. Changes in metabolic rate have been reported by many workers (1,2,3). These changes have been measured by the respiration of tissue slices, by changes in the carbohydrate and nitrogen constituents of the plant, and by changes in the water content.

Smith et al. (4) found a decrease in starch-dextrin reserves, an increase in total sugars, a decrease in total nitrogen in leaves, and an increase in total nitrogen in stems and roots when bindweed was treated with 2,4-D. It was suggested that the altered respiration rate might be an indication of some physical abnormality. When the Red Kidney bean was treated with 2,4-D, Smith (5) found a decrease in total nitrogen but little change in soluble nitrogen. Rasmussen (6) reported an increase in reducing sugar, but no great change in non-reducing sugar for the first 10 days of treatment, a decrease in polysaccharide, and little change in soluble nitrogen or protein nitrogen with increasing concentrations of 2,4-D on the dandelion. The annual morning glory showed little change in weight, an increase in sugars, and a decrease

in starch-dextrin when sprayed with the auxin (7). Erickson et al. (8) observed that the protein content of wheat seed increased when treated with 2,4-D.

In spite of the information presented above it is obvious that there is a great deal more to be learned about the effects of 2,4-D on the metabolism of plants. This thesis was undertaken to add to the existing knowledge of 2,4-D action and to determine if results observed for other plant species were also applicable to the corn plant.

Methods and Materials

Enough South Dakota single cross hybrid 105 x 107 Yellow Dent corn seed was taken to give 120 plants. The seed was planted in sandy gravel on June 10, 1948 and on June 26 the seedlings were transferred to 2 gallon, glazed, earthenware crocks filled with the nutrient solution developed by Trelease (9). Five plants were put in each crock and were held upright by threading them through rubber stoppers, packing cotton around the stems, and inserting the stoppers in paraffin-coated transite lids which fitted loosely on the crocks. The culture solution was changed weekly and iron as ferrous sulfate was replenished semiweekly. The crocks were placed on the greenhouse tables in two parallel rows of twelve each and moved twice a week so that they would make a complete circuit of the table every two weeks. This transfer was made so as to obviate to some extent the inequalities existing in lighting within the

greenhouse.

On July 10 the crocks were numbered and divided into four groups by lot. Group one was the control. Group two was treated with enough 2,4-dichlorophenoxyacetic acid solution to make the final concentration in the crocks 5 ppm; group three was made to 10 ppm. and group four to 20 ppm. of 2,4-D. The plants were treated at 1:00 p.m.

The plants were harvested at 9:00 a.m. on July 13 after 68 hours of treatment. The tops were cut off at the top of the rubber stopper, weighed, dried rapidly in a forced draft oven at 70° C., weighed, and ground. The roots were removed, and swung rapidly in a cheese cloth bag at the end of a cord to drive off the excess water, weighed, dried rapidly, weighed and ground.

Total nitrogen was determined by the salicylic acid-sulfuric acid method as described by Loomis and Shull (10). One half gram samples were weighed into 800 ml. Kjeldahl flasks, and 30 ml. of concentrated sulfuric acid, in which 1 g. of salicylic acid has been dissolved, were added. The mixture was allowed to react in the cold for 30 minutes with occasional rotation. Then 5 grams of sodium thiosulfate were added to reduce the nitro groups to amino groups. Sodium sulfate and copper sulfate were added as in the regular Kjeldahl procedure and the mixtures were digested 1½ times as long as it took the digest to clear. After cooling 400 ml. of water and 100 ml. of Greenbank's alkali solution were added, followed by distill-

ation of the liberated ammonia into 25 ml. of standard hydrochloric acid and titration of the excess acid with standard sodium hydroxide.

Nitrate nitrogen was estimated by the Devarda reduction method of Gerdel as modified by Olson (11). One gram sample was weighed into a 250 ml. erlenmeyer flask. About 100 ml. of boiling distilled water were added and the flask was placed on the steam bath for 2 hours. Approximately 125 ml. of water and 5 ml. of saturated neutral lead acetate were added and the mixture was allowed to cool. The solution was made up to 250 ml. in a graduated cylinder, shaken well and filtered. One hundred ml. aliquots of the filtered solution, 200 ml. of distilled water, 25 ml. of 5% sodium hydroxide, a small piece of paraffin, and a few glass beads were added to a Kjeldahl flask and the mixture was boiled for 30 minutes. After cooling to almost room temperature, 200 ml. of distilled water and 3 grams of Devarda's metal were added to the flask and the ammonia resulting from the reduction of the nitrate nitrogen was distilled over into standard hydrochloric acid solution. The distillate was made up to 250 ml. and 10 ml. aliquots were taken for nesslerization. The aliquot was placed in a 100 ml. volumetric flask, about 75 ml. of water and 5 ml. of Nessler's solution were added, and the contents were diluted to 100 ml. and thoroughly mixed. After 15 minutes the intensity of resulting color was determined with a

photoelectric colorimeter.

The method of Hassid (12) was used to determine the sugar content. One half gram samples were extracted with 80% alcohol for 6 hours. The extract was evaporated down to about 10 ml., and then a few ml. of water and 10 drops of saturated neutral lead acetate were added, followed by 20 drops of saturated disodium acid phosphate. A small amount of carbon was added to decolorize the solution and the preparation was filtered, the residue on the filter being washed several times with hot distilled water. The filtrate and washings were collected in 100 ml. volumetric flasks and made to volume.

Approximately one-half of the extract was transferred to an erlenmeyer flask and 3 drops of invertase solution and a few drops of toluene were added. This aliquot was kept overnight at room temperature.

Reducing sugars were determined on 10 ml. aliquots of the untreated (invertase-free) solution by mixing them with 5 ml. of alkaline ferricyanide and boiling for 15 minutes. Then 5 ml. of 5 N sulfuric acid were added and mixed by shaking, followed by the addition of 7 to 10 drops Setopaline C indicator. This solution, containing ferrous iron equivalent to the reducing sugars was titrated with a ceric sulfate solution that had been previously standardized against a standard glucose solution.

Total sugar was determined by applying the above method to the solution treated with invertase. The difference between the two values is equal to the non-reducing sugar or sucrose (in terms of glucose).

"True protein" nitrogen was determined on the residue left after the extraction with 80% alcohol. The alcohol was driven off in the drying oven and the residue was extracted repeatedly with boiling water, then filtered and the residue and filter paper were added to a Kjeldahl flask, digested with sulfuric acid, sodium sulfate, and copper sulfate for about 30 minutes and distilled as in the determination of total nitrogen.

Other nitrogen fractions were determined on aliquots of a water extract obtained by a slight modification of the methods of Vickery et al. (13). Samples of the tops (10 grams) and roots (5 grams) were weighed into erlenmeyer flasks and 150 ml. of water were added to each flask. The flasks were placed on the steam bath until they reached 70°C., kept at this temperature for about 20 minutes, and cooled. Then the mixture was added to a 200 ml. graduate, made to volume and then poured quickly into a large beaker. The mixture was filtered through a fluted filter paper using a diatomaceous silica filter-aid. To these extracts were added a few drops of chloroform, enough toluene to form a layer over the liquid, and the extract was stored at about 2° C.

Ammonium nitrogen was determined on aliquots of the extract on the same day of preparation and according to the method of Pucher et al. (14). An aliquot of 25 ml. was placed in the flask along with 20 ml. of phosphate-borate buffer and about 40 ml. of water. After the addition of 5 ml. of the borate-sodium-hydroxide mixture, the resulting ammonia was vacuum distilled (ca. 20 mm. Hg) at 40° C. The distillate was transferred to 100 ml. volumetric flasks and nesslerized as in the case of the nitrate nitrogen.

Glutamine (easily hydrolyzable) nitrogen was determined the next day in the same apparatus but using the partial hydrolysis method of Vickery et al. (13). Twenty ml. of phosphate-borate buffer were added to 10 ml. aliquots of the water extract and the mixture was kept in a boiling water bath for 2 hours. The original ammonium nitrogen in the sample plus the ammonia resulting from the amide nitrogen of glutamine was determined in the same manner as for ammonium nitrogen.

Total amide plus ammonium nitrogen were determined according to the method of Vickery et al. (13) To 10 ml. aliquots of the extract, 2 ml. of sulfuric acid (6 N) were added and the mixture was placed in a boiling water bath for 3 hours. The acid was partially neutralized by the addition of 10 ml. of 1 N sodium hydroxide and the original ammonium nitrogen plus the ammonia formed by the hydrolysis of the amide groups was estimated by the method outlined for ammonium.

The asparagine amide nitrogen values were obtained by

subtracting the ammonium and glutamine amide nitrogen from the total amide nitrogen plus ammonium.

The Van Slyke apparatus was used for the estimation of alpha amino nitrogen. Aliquots of 5 ml. were taken for the determination. The first step was the displacement of air from the apparatus by nitric oxide. This was carried out by washing the system repeatedly with the reaction products of acetic acid and sodium nitrite. The sample was introduced into the system and the nitrous acid reacted with the alpha amino groups to give molecular nitrogen. The excess nitric oxide was absorbed by shaking it with alkaline potassium permanganate and the remaining nitrogen was measured in a gas burette. The procedure is described by Morrow and Sandstrom (15). Since these determinations were made without pretreatment of the extract, a correction factor for 25 percent of the ammonia nitrogen[1] and 80 percent of the glutamine amide nitrogen (13) was applied.

Basic nitrogen was determined according to the method of Umbreit and Wilson (16) on 20 ml. aliquots of the extract. Enough trichloracetic acid was added to make the solution up to 3% trichloracetic acid. Since no precipitate formed filtration was unnecessary and 1.1 ml. of 20 N sulfuric acid and 2 ml. of phosphotungstic acid reagent were added. The mixture was warmed on a steam bath for several minutes and then stored for 48 hours at 2° C. The precipitate was filtered and washed. The basic nitrogen phosphotungstic was

[1]Unpublished data from this laboratory indicates that 25 per cent of ammonia nitrogen is recovered in Van Slyke procedure for determination of alpha amino nitrogen.

transferred to a Kjeldahl flask by dissolving the precipitate in about 10 ml. of 5% sodium hydroxide. The digestion and distillation was carried out as in the total and "true protein" nitrogen determination and the distillate was made to volume (250 ml.). Ten ml. aliquots were nesslerized in 100 ml. volumetric flasks.

To find the total water-soluble nitrogen in the extracts 10 ml. aliquots were treated according to the methods of Pucher et al. (17). The aliquots were transferred to Kjeldahl flasks and 20 ml. of water, 10 ml. of 18 N sulfuric acid and 3.0 grams of reduced iron powder were added. After a 30 minutes reduction period the contents in the Kjeldahl flask were refluxed for 5 minutes and digestion and distillation were done as in the case of total nitrogen.

The total water-soluble nitrogen minus the sum of the determined nitrogen fractions is tabulated as the undetermined nitrogen.

Since glutamine and asparagine each have one amide and one amino group the nitrogen in glutamine and asparagine is equal to twice the amide nitrogen determined. In this report these fractions are given as the glutamine and asparagine nitrogen and this value includes both the amide and amino nitrogen. The total alpha amino nitrogen minus the amino nitrogen in these two amides is equal to the residual alpha amino nitrogen and is so reported.

Results and Discussion

Within 24 hours after treatment with 2,4-D leaves were wilted and some stem curvature was noted. After 48 hours all the leaves were wilted and all the stems were bowed. No consistent difference was noted in the three treated groups of plants as some individuals were only slightly bent while others in the same crock were drooping at 180° angles. Taylor (2) noted that Kidney Bean plants were also erratic in this respect and that the epinastic responses were not clearly relatable to the concentration applied.

There was no further change apparent in the remainder of the test period except a yellowing of some of the leaves. None of the leaves had dried at the time of harvest.

High temperatures throughout the test period probably accounted for the increased toxicity of 2,4-D noted in the experiment. Hamner and Tukey (18) noted that 2,4-D had more effect in hot weather than in cool. The reported temperature was above 90° F. on each day of the test and it was of course warmer in the greenhouse.

The treated roots compared closely to the "much inhibited, non-fibrous, non-elongated, discolored, bulbous-tipped root, system, with abnormal numbers of adventitious roots" described by Taylor (2).

A decrease in fresh weight of the treated plants indicates

that the plants were being dehydrated or that their growth was being retarded. Since this decrease was accompanied by similar decreases in both dry weight and percent of water it was apparent that both effects were in evidence. (Table I.) These results are in conflict with those reported for the bean plant by Brown (1) who found that "on an over all basis, however, the treated plants had higher percentages of moisture than the untreated ones."

Weaver (3) observed that the fresh weight of the tops of Red Kidney bean decreased when treated with 2,4-D and Taylor (2) noted the same effect in corn plants. It should be noted that all concentrations used in this experiment were apparently lethal in strength and the results reported here could be in direct conflict to lighter and possibly stimulating treatments.

The results of the carbohydrate analyses (Table I, figures 2 and 3) agree quite well with values previously reported (4,6,7). As these workers have concluded that 2,4-D stimulates the break down of the starch-dextrin reserves there is no reason to feel that the same effect is not shown here, but the analysis on this project did not include tests for any carbohydrate reserves.

The results of analyses for the various nitrogen fractions are shown in Tables II and III and in figures 4 through 11. Table II gives the percent of the nitrogen fractions

based on dry weight and Table III shows them on the basis of percent of total nitrogen. Figure 4 is an area graph showing the relative amount of the water-soluble nitrogen fractions based on percent of total nitrogen. The other figures show the effects of the 2,4-D on the individual nitrogen fractions.

Total nitrogen (figure 5) decreased with the 2,4-D treatment. This decrease was reflected in a large part by the loss in "true protein" nitrogen (figure 6) and a small gain in water-soluble nitrogen (Table II). This could be indicative of the proteolysis previously reported (4,5, and 6). The increase observed by Erickson et al.(8) applied to the wheat kernel only and might not be true of the entire plant. It should be noted that there was a slight increase in "true protein" and total nitrogen in the roots but no consistent change in the water-soluble nitrogen.

Further analyses of the water-soluble extract revealed the following information. Ammonia nitrogen (figure 7) showed little change in the tops but increased sharply in the treated roots. Glutamine nitrogen (figure 8) and asparagine nitrogen (figure 9) increased in both the treated roots and tops. A similar sharp increase in residual alpha amino nitrogen (figure 10) was noted. Determination of the basic nitrogen which includes polypeptides, and peptide

nitrogen, the basic amino acids (arginine, histidine, lysine), etc. showed only a slight increase. Evidently most of the increase in alpha amino nitrogen comes from those amino acids containing no basic nitrogen.

A decrease in nitrate nitrogen (figure 11) was very evident especially in the roots. Nitrate nitrogen in the roots was more than halved in the case of the treated plants. This decrease of nitrate could be explained by a number of factors. The damage to the roots might inhibit the absorption of nitrate ion or favor the absorption of ammonium ion over the nitrate ion. The auxin might also stimulate the formation of alpha amino acids at the expense of nitrate ions.

Subtracting the sum of the determined nitrogen fractions from the total-water-soluble nitrogen provides a measure of the unidentified nitrogen. The gain in undetermined nitrogen constituents in the tops accounts for about one-half of the gain in water soluble nitrogen. In the roots the undetermined nitrogen decreases and its loss parallels loss in the water-soluble nitrogen. In other words, the sum of the determined nitrogen fractions in the roots is fairly constant since the loss in nitrate nitrogen is largely offset by the increase in residual alpha nitrogen.

Obviously the effects of 2,4-D are too complex

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in character to enable one to attribute all the chemical and physical changes to one factor. Tukey et al.(20) advanced the idea that increased respiratory activity might be a clue to physiological abnormalities. The mere depletion of food reserves in itself is a doubtful cause of death. While it is difficult to say what amounts of carbohydrate and protein are necessary for a plant to live, variations in these constituents during the growing season have been recorded (20) which are as extreme as some of the variations shown to take place in the treated plants of this study. Rasmussen (6) points out that dandelions died before food reserves were exhausted.

The variation in water content of the treated plants seems hardly sufficient to cause death. The group of plants treated with 5 ppm. showed severe responses yet had only 0.7 per cent less moisture than the control in the tops and only 2.4 per cent less moisture in the roots.

In studying the changes in nitrogen fractions as recorded in Table II and III it is apparent that there was a decrease in both the primary and final components of protein synthesis in the tops with a loss of about 1.0 per cent in true protein and a gain of about 0.4 per cent in soluble protein nitrogen for a net loss of 0.6 per cent in total nitrogen. That difference could come from more rapid absorption of nitrogen from the culture solution in the control or by loss of nitrogen from the roots of the treated plants. While it seems

unlikely that the group of control plants would gain as much as 0.6 per cent of their dry weight in nitrogen in 68 hours, it is necessary to consider this possibility in light of the statements of Hoaglund (21). The nutrient solutions were changed on July 9 and 24 hours later the 2,4-D was added. This change would have the effect of aerating the solution and, as Hoaglund pointed out, would accelerate the absorption of nitrate ions. If this were the case then the 2,4-D must inhibit the absorption of nitrate ions from the solution.

Another possibility is the loss of nitrate ions by the treated plants to the nutrient solution. Normally the cells of the root are able to retain ions against a concentration gradient but 2,4-D might interfere with this property. Several workers (2,6, 19) have observed histological changes in root tissue following treatment with 2,4-D. Tukey et al.(19) suggested that one of the effects of 2,4-D might be the disorganization and rupture of rhizome and root cortex. It is possible that this damage to root tissue and its subsequent effect on absorption might be one of the factors in the herbicidal action of 2,4-D. This approach undoubtedly deserves further study.

A third possibility of nitrogen loss from plant tissue is the escape of molecular nitrogen into the air through the reaction of alpha amino nitrogen with nitrite nitrogen derived as an intermediate in nitrate assimilation by plants. No tests were made to confirm this possibility, but Pearsall

and Billimoria (22) observed losses in nitrogen in leaves of the daffodil under special experimental conditions and advanced this hypothesis to explain the nitrogen loss. Vickery et al. (23) in their discussion of the data of Pearsell and Billimoria note that the latter did not provide chemical evidence for the formation of nitrite, either from nitrate or from ammonium ion, in their experimental material.

Further study is required before any conclusion can be made relative to these speculations about the loss of nitrogen from plant tissues.

Perhaps the most interesting field for further study is suggested by the sharp increase in amino acids as shown by the rise in alpha amino nitrogen content. This increase could come, as has already been pointed out, from increased assimilation of nitrate ion, increased proteolysis, or both. If protein was catabolized in the treated plants with a corresponding increase in the amino acids, one would expect an increase in the intermediates of proteolysis, especially the peptides and polypeptides, which would be included in the basic nitrogen determinations (figure 1). Since these intermediates do increase slightly it appears proteolysis is a result of 2,4-D treatment. Further study of tables II and III show the loss in "true protein" will more than account for the gain in the water-soluble nitrogen fractions.

Another possibility that the results of this experiment do not preclude is that 2,4-D might act to block the formation of new protein. The loss in "true protein" in the treated plants could be explained by the studies with heavy nitrogen (24, 25) which show that protein in the growing plant is continually being broken down and resynthesized. If resynthesis is inhibited, the total amount of protein would decrease.

One apparent point of exception to the above suggestions the lack of ammonia increase in the tops and the slight increase of ammonia in the roots can be explained by the findings of Viets el al. (26) in their experiments with ammonium nutrition of corn. Even when the plants were in ammonium solutions so concentrated that the plants were severly damaged, ammonia nitrogen did not increase in the tops of the corn until after injury symptoms appeared in the plants and ammonia nitrogen had accumulated in the roots.

Only further investigation will determine the merit of the above suggestions but the data compiled here may help other investigators in their efforts to determine the primary effect of 2,4-D.

Summary

The mechanism of herbicidal action of 2,4-dichlorophenoxyacetic acid was investigated by means of chemical analysis of carbohydrate and nitrogen fractions of four groups of 30 corn plants each, treated with 0 ppm., 5 ppm., 10 ppm., and 20 ppm., of 2,4-D respectively.

Decreases in fresh and dry weight and in percent of moisture are recorded and discussed.

Changes in the value of sugar fractions are noted and related to previous work on the effect of 2,4-D on carbohydrates in plants.

The following changes in the values of the nitrogen fractions in plants treated with 2,4-D are tabulated and discussed:

1. Ammonium nitrogen increased slightly in the tops and to a greater extent in the roots.
2. Nitrate nitrogen decreased markedly in both tops and roots.
3. Glutamine and asparagine nitrogen increased in both tops and roots.
4. Alpha amino increased very rapidly in tops and roots.
5. Basic nitrogen showed a slight increase in both tops and roots.
6. Undetermined nitrogen increased in tops and decreased in the roots.
7. Water soluble nitrogen increased in tops and decreased in the roots.
8. True protein nitrogen decreased in the tops and showed no consistent change in the roots.
9. Total nitrogen decreased in the tops and showed a slight increase in the roots.

Possible reasons for the decrease in total nitrogen and the increase in alpha nitrogen are noted and discussed in terms of the data available.

Suggestions as to the possible mechanism of the herbicidal action of 2,4-D are presented for consideration.

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TABLE 1

		Tops				Roots			
	Concentration of 2,4-D	0 ppm.	5 ppm.	10 ppm.	20 ppm.	0 ppm.	5 ppm.	10 ppm.	20 ppm.
Weight in grams	Fresh weight of 30 plants	722	365	557	291	220	148	148	138
	Dry weight of 30 plants	46	26	54	34	12	12	13	10
	Percent of moisture	93.6	92.9	90.5	88.4	94.5	91.9	91.2	92.7
Based on per cent of dry weight	Total Sugar	.84	2.75	2.79	2.93	.96	.72	.80	.80
	Reducing Sugar	.75	2.25	2.32	2.12	.92	.64	.70	.67
	Non-reducing Sugar	.09	.50	.47	.81	.04	.08	.10	.13

TABLE II

Concentration of KH_2PO_4	Tops			Roots			
	5 p.p.m.	15 p.p.m.	20 p.p.m.	0 p.p.m.	5 p.p.m.	15 p.p.m.	20 p.p.m.
Nitrogen Fraction Based on Percent of Dry Weight							
Ammonium Nitrogen	.038	.038	.035	.047	.115	.117	.122
Nitrate Nitrogen	.020	.032	.025	.021	.421	.324	.194
Glutamine Nitrogen	.046	.052	.033	.038	.201	.141	.125
Asparagine Nitrogen	.214	.232	.234	.105	.096	.204	.122
Residual Am. Nitrogen	.471	.458	.571	.321	.503	.515	.786
Basic [1] Nitrogen	.432	.470	.427	.457	.662	.601	.637
Total	1.45	1.45	1.60	1.46	1.44	1.57	1.80
Undetermined Nitrogen	.51	.65	.64	.80	.40	.55	.80
Water-soluble Nitrogen	1.96	3.08	2.24	1.26	1.94	1.92	2.00
True Protein Nitrogen	1.74	1.65	1.58	2.54	1.79	1.90	1.80
Total	3.70	3.73	3.84	4.30	3.65	3.82	3.80
Total Nitrogen	5.74	5.80	5.80	4.42	5.83	5.72	5.98

[1] These values for basic nitrogen were not included in the calculated totals.

TABLE III

	Concentration of 2,4-D.	Nitrogen Fractions Based on Percent of Total Nitrogen											Total Nitrogen percent dry wt.
		Ammonium Nitrogen	Nitrate Nitrogen	Glutamine Nitrogen	Asparagine Nitrogen	Residual Am. Nitrogen	Basic Nitrogen[1]	Total	Undetermined Nitrogen	Water-soluble Nitrogen	True Protein Nitrogen	Total	
Tops	0 ppm.	.837	20.83	.858	2.38	8.16	10.34	33.09	8.76	41.85	57.4	99.25	4.42
	5 ppm.	.955	18.34	1.228	5.74	12.59	11.55	38.81	13.59	52.40	46.5	98.90	3.74
	10 ppm.	1.00	17.15	1.368	6.10	12.05	12.37	37.66	17.07	54.73	43.4	98.13	3.80
	20 ppm.	.921	18.28	1.988	6.16	15.02	11.24	42.18	16.76	58.94	41.5	100.14	3.80
Roots	0 ppm.	1.676	26.28	2.166	4.76	5.05	14.32	40.25	18.57	58.82	48.8	107.12	3.40
	ppm.	3.136	12.05	5.66	2.38	16.63	18.48	39.87	11.24	51.11	49.7	100.81	3.60
	10 ppm.	3.14	10.59	3.87	5.26	13.84	16.20	36.92	14.69	51.61	51.0	102.61	3.72
	20 ppm.	3.58	10.70	5.11	3.32	21.41	17.31	41.57	12.77	54.34	48.9	103.24	3.68

[1]These values for basic nitrogen were not included in calculating totals.

Figure 1

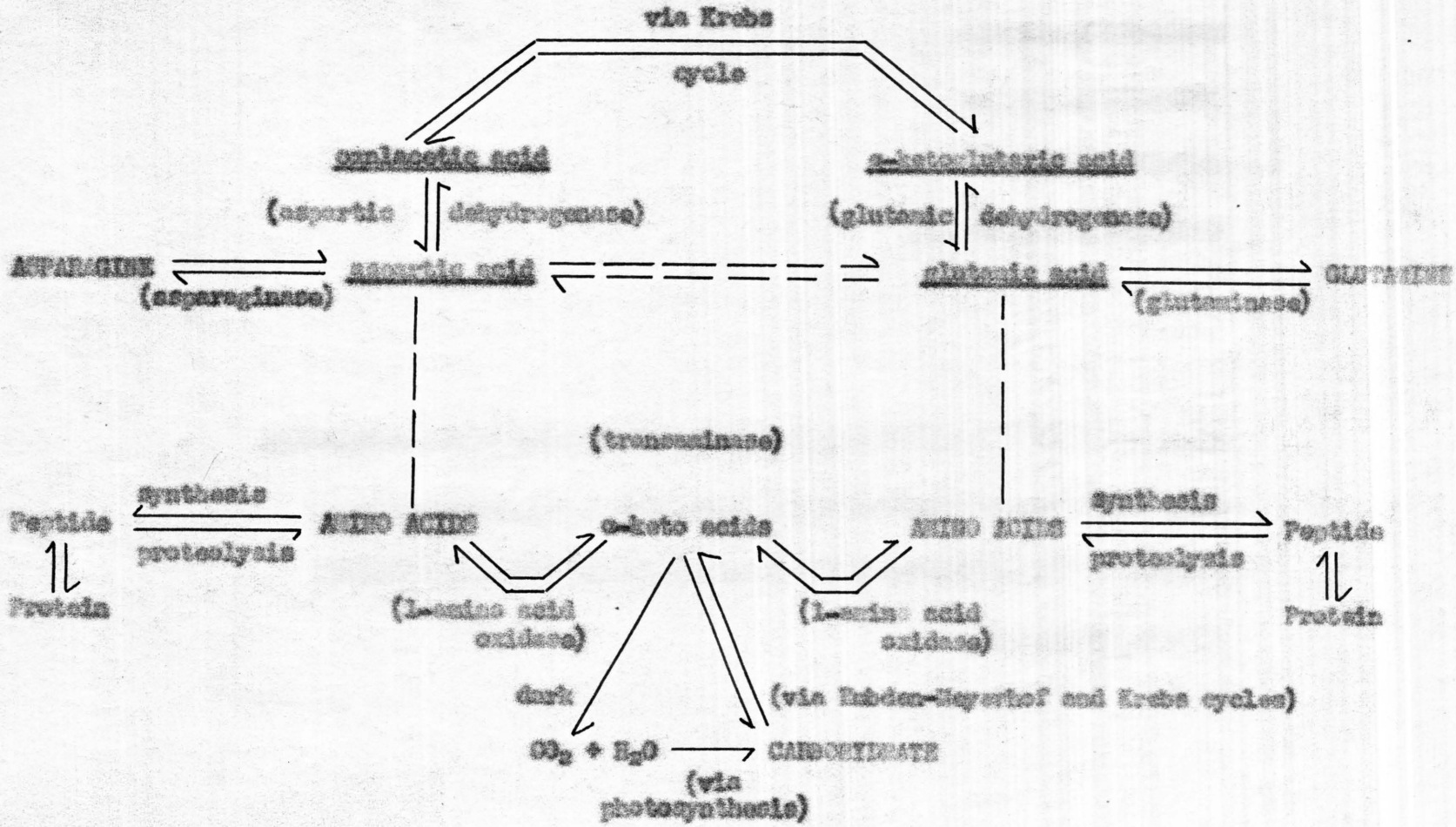
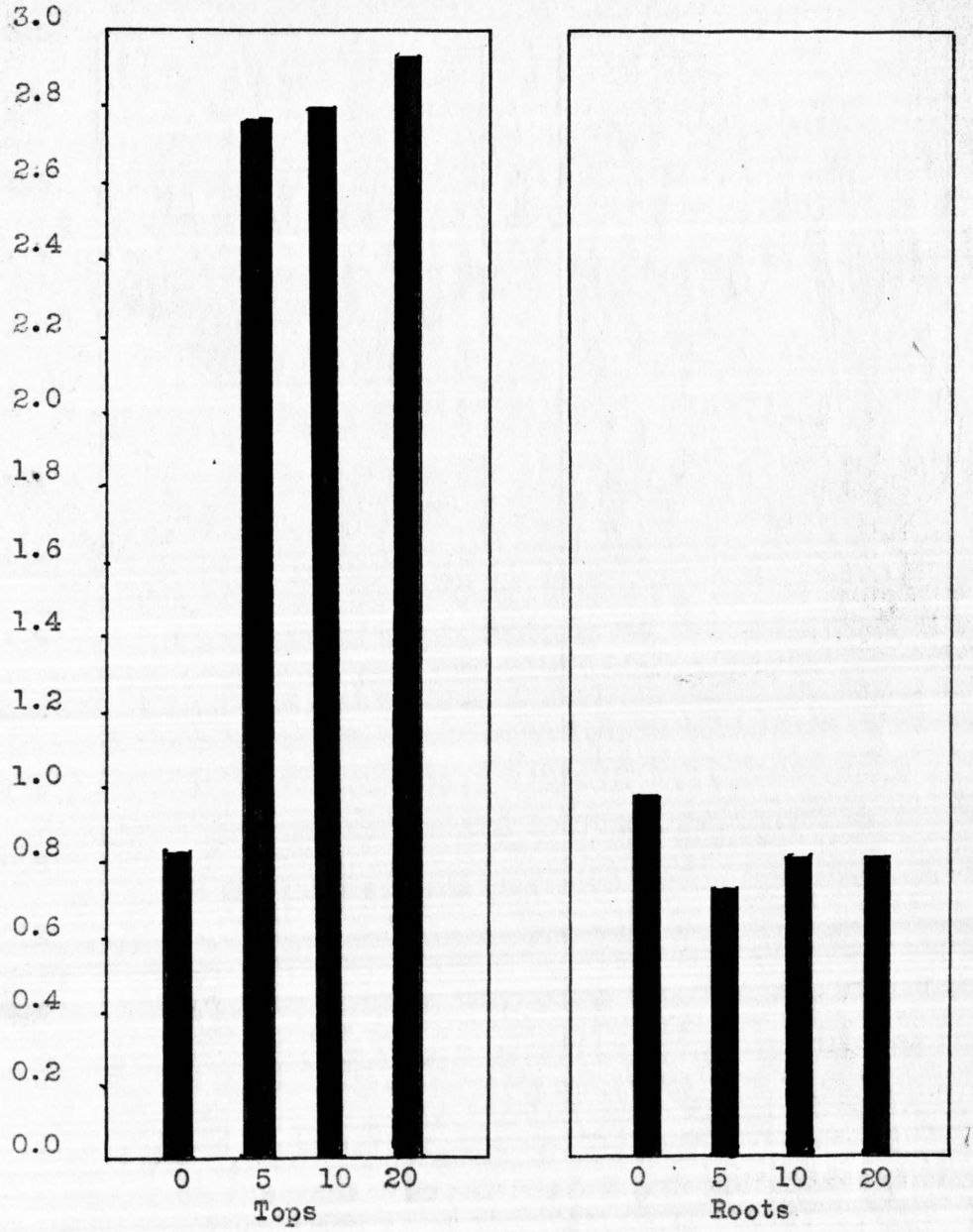

via Krebs
cycle
oxaloacetic acid
α-ketoglutaric acid
(aspartic dehydrogenase)
(glutamic dehydrogenase)
ASPARAGINE
(asparaginase)
aspartic acid
glutamic acid
GLUTAMINE
(glutaminase)
(transaminase)
synthesis
Peptide
proteolysis
AMINO ACIDS
α-keto acids
AMINO ACIDS
synthesis
Peptide
proteolysis
Protein
Protein
(L-amino acid
oxidase)
(L-amino acid
oxidase)
dark
(via Embden-Meyerhof and Krebs cycles)
$CO_2 + H_2O$
CARBOHYDRATE
(via
photosynthesis)

FIGURE 2

TOTAL SUGAR

Percent of Dry Weight



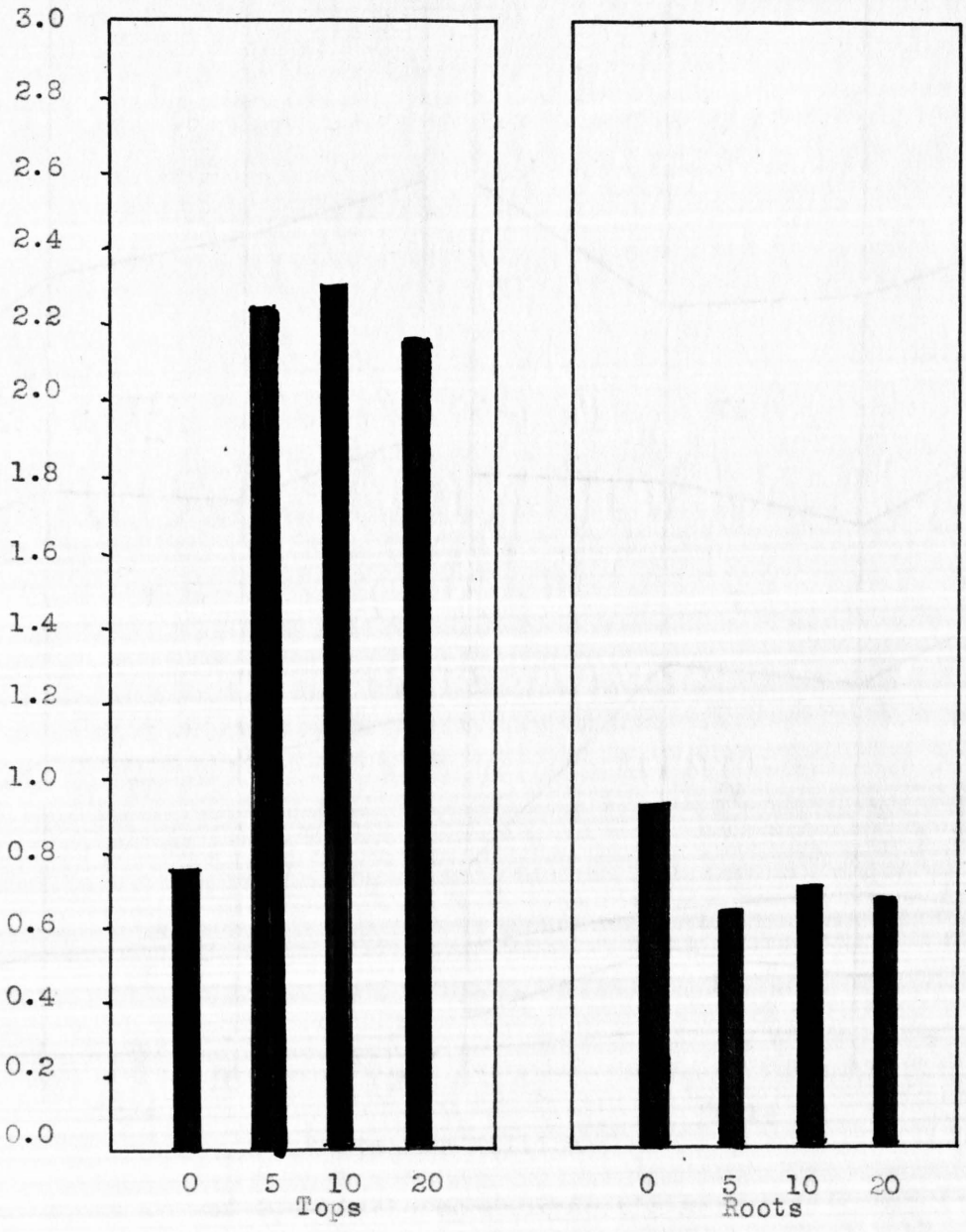
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 3

REDUCING SUGAR

Percent of Dry Weight

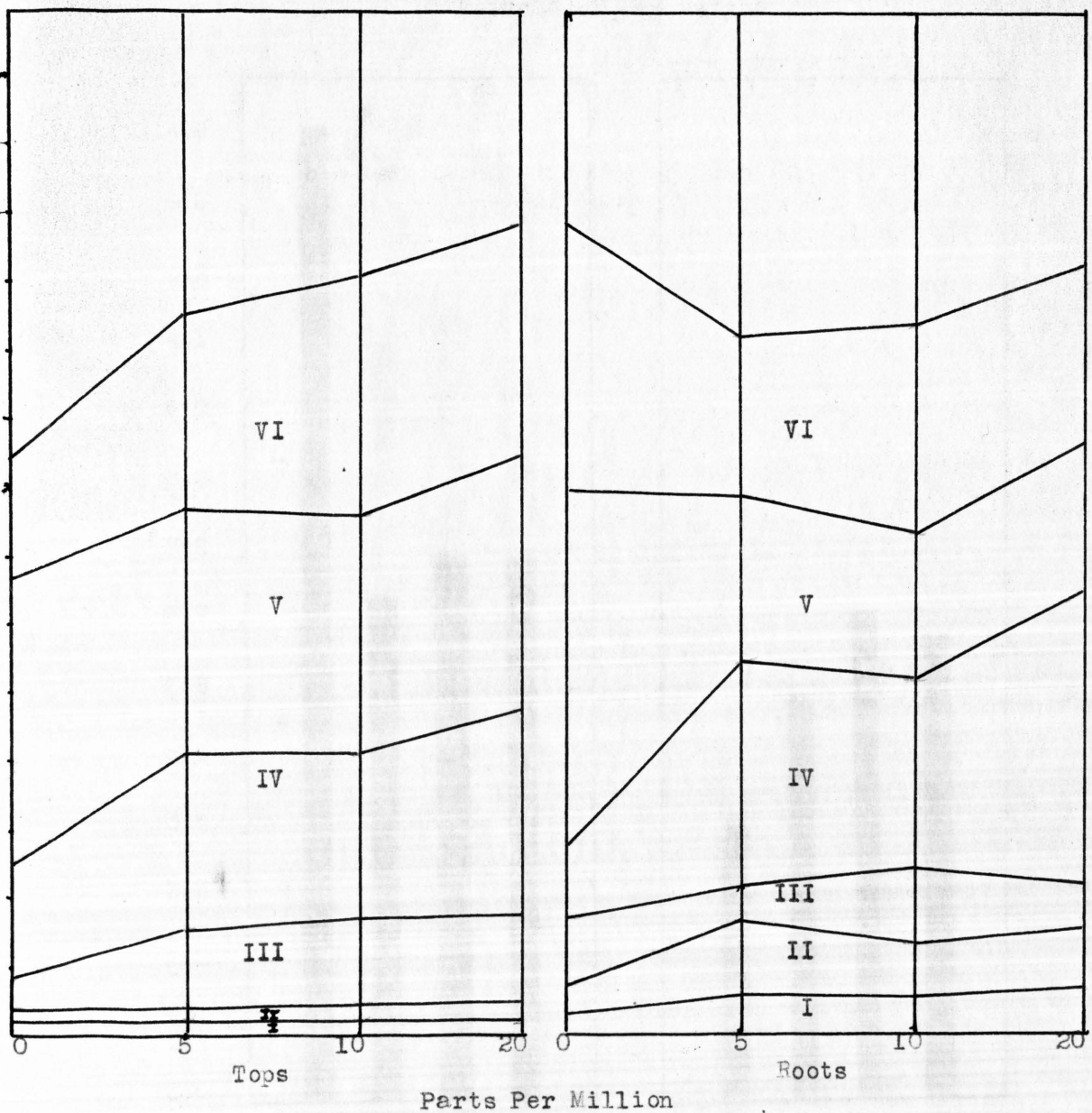


Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 4

Relative strength of water-soluble nitrogen fractions based on per cent of total nitrogen



Parts Per Million

of 2,4-Dichlorophenoxyacetic acid

Legend:

I. Ammonia Nitrogen

II. Glutamine Nitrogen

III. Asparagine Nitrogen

IV. Residual Amino Nitrogen

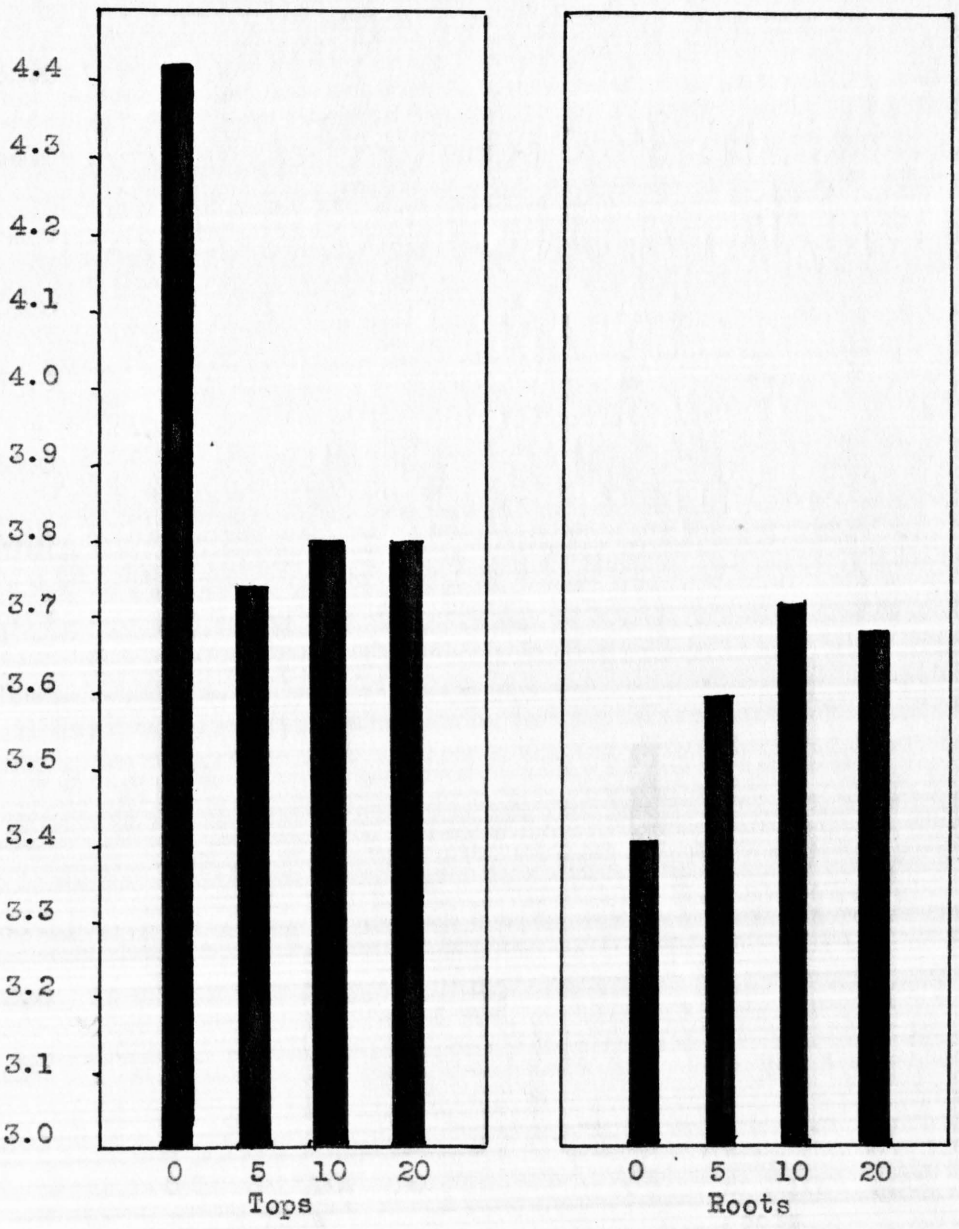
V. Nitrate Nitrogen

VI. Undetermined Nitrogen

FIGURE 5

TOTAL NITROGEN

Percent of Dry Weight



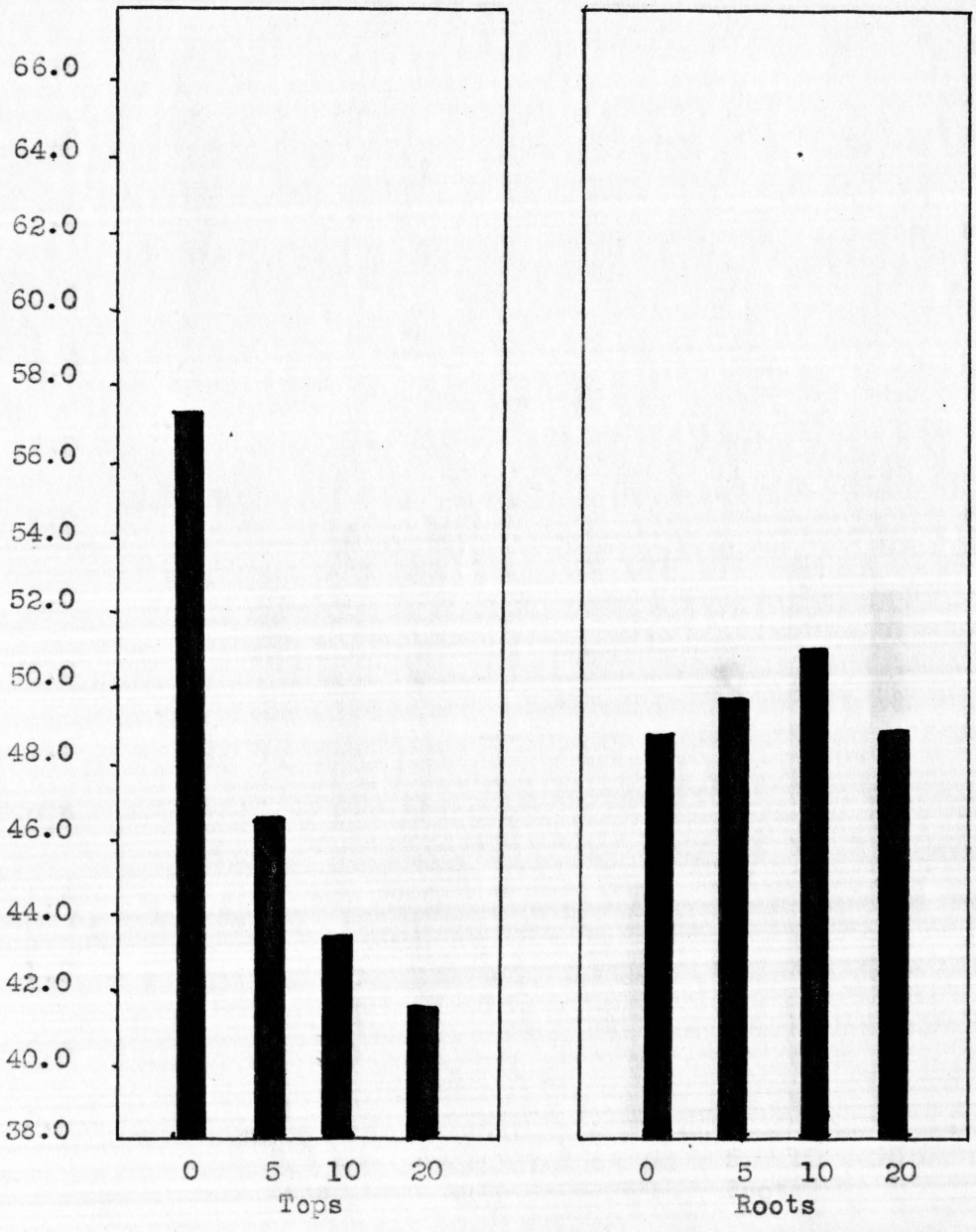
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 6

TRUE PROTEIN NITROGEN

Percent of total Nitrogen



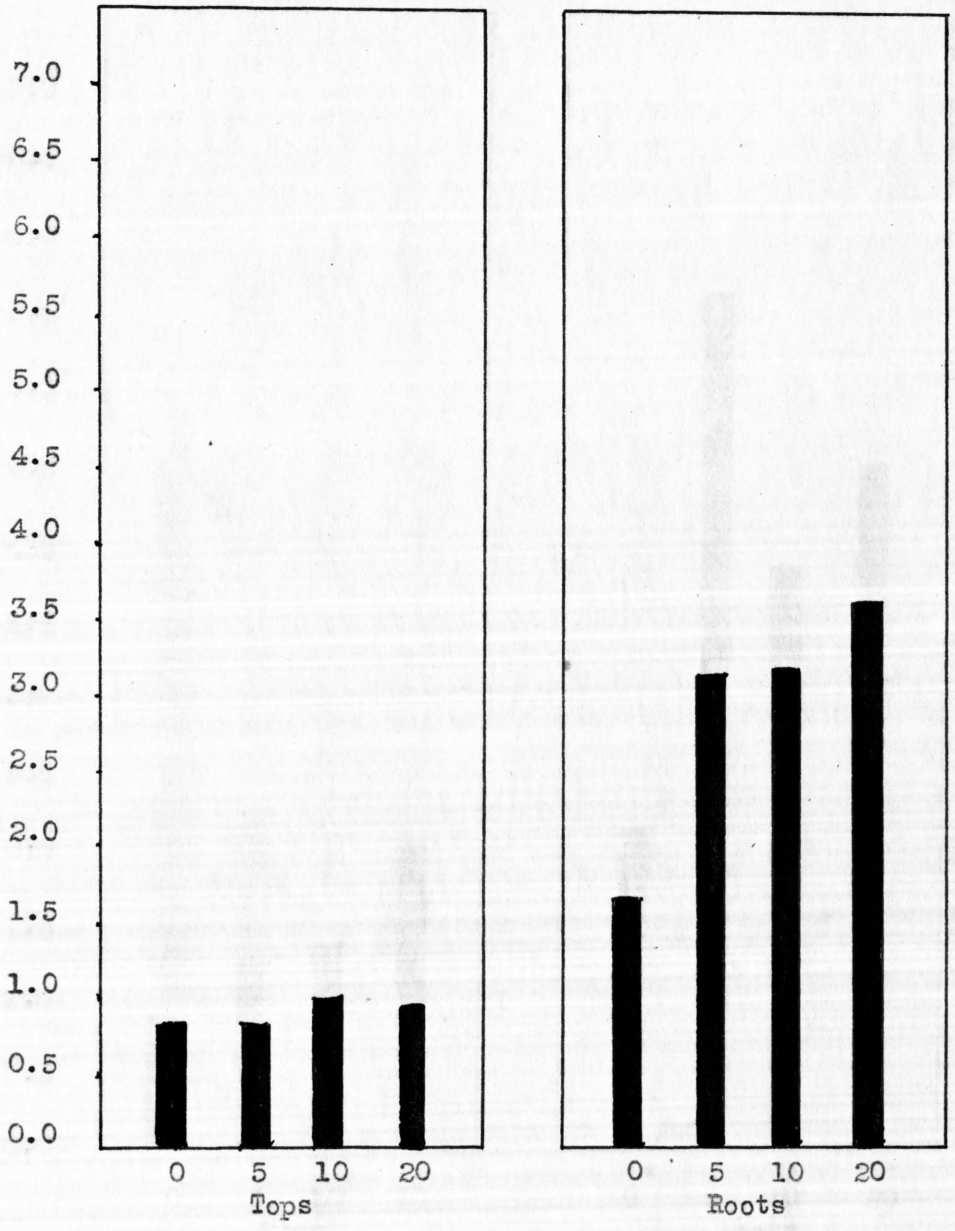
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 7

AMMONIA NITROGEN

Percent of total Nitrogen



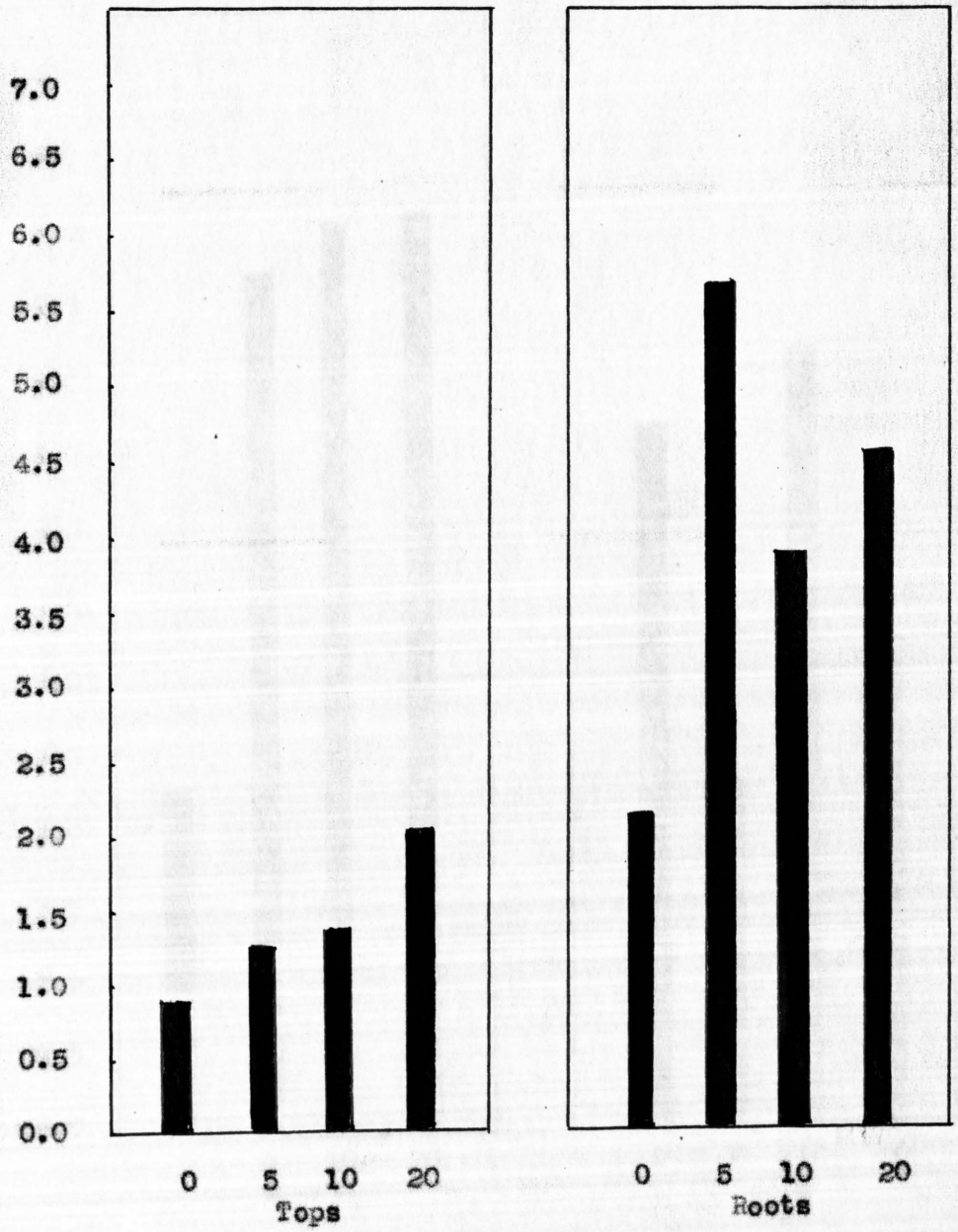
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 8

GLUTAMINE NITROGEN

Percent of total Nitrogen



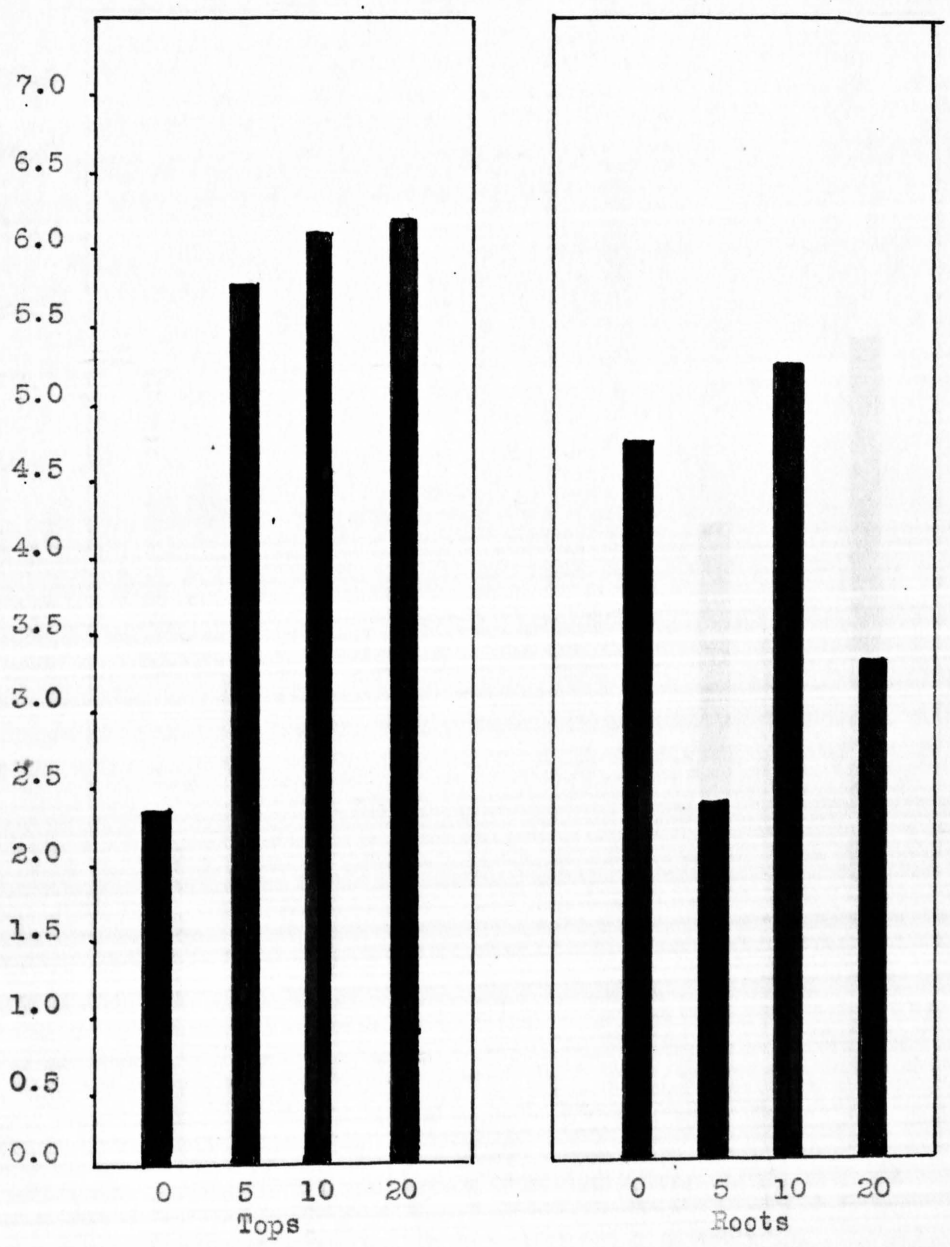
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 9

ASPARAGINE NITROGEN

Percent of total Nitrogen



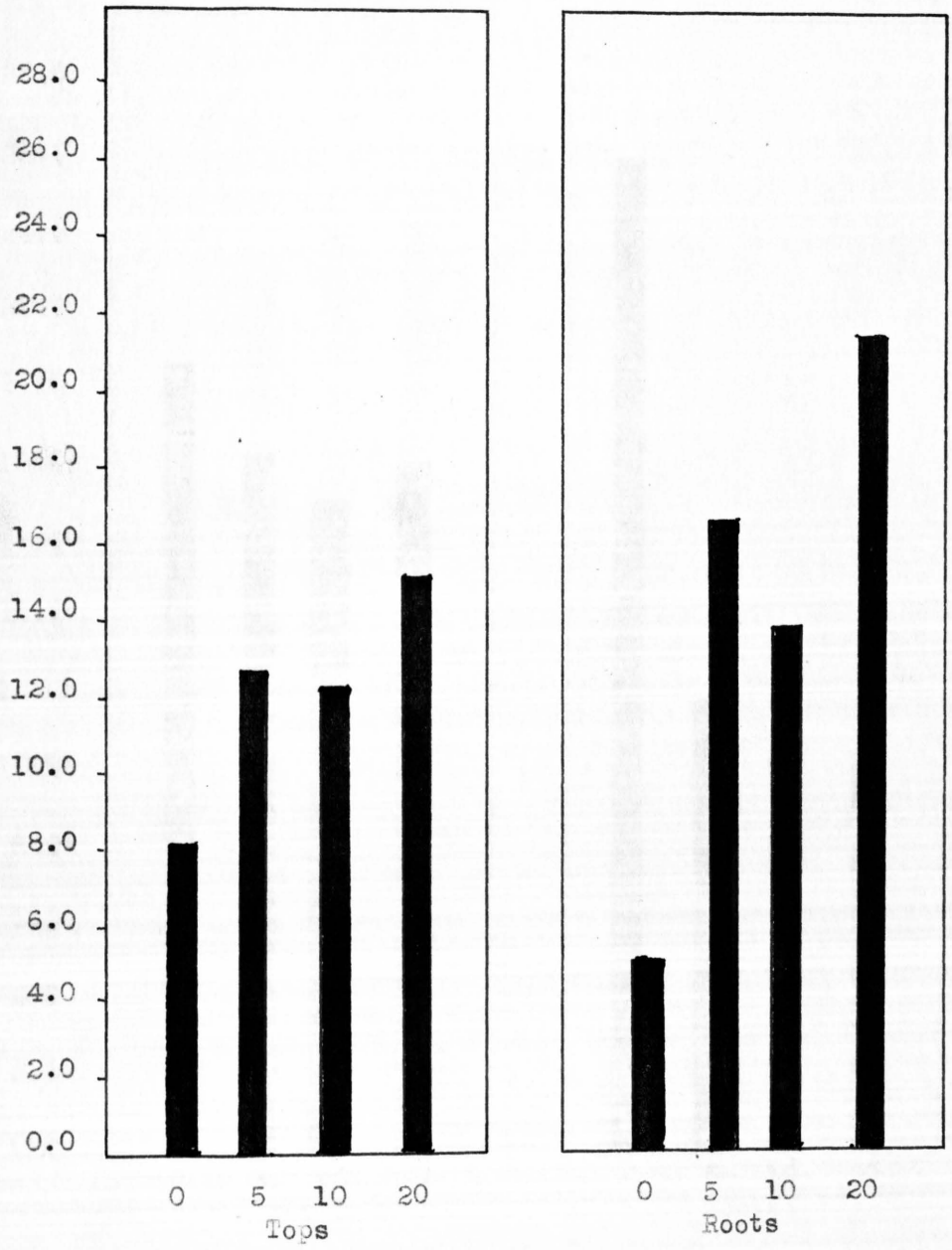
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 10

RESIDUAL AMINO NITROGEN

Percent of total Nitrogen



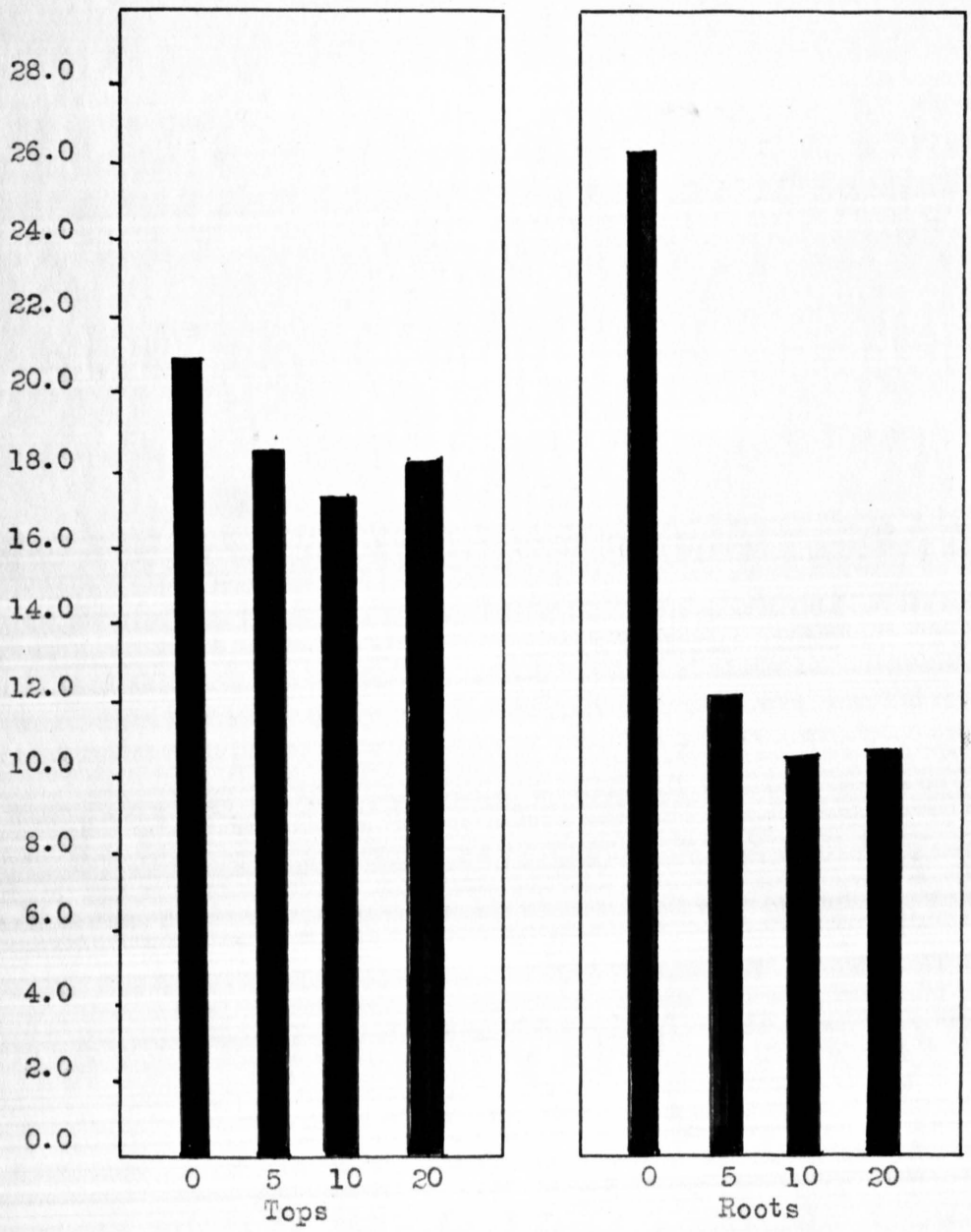
Parts per Million

of 2,4-dichlorophenoxyacetic acid

FIGURE 11

NITRATE NITROGEN

Percent of total Nitrogen



Parts per Million

of 2,4-dichlorophenoxyacetic acid