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GRADUATE COLLEGE

EFFECTS OF PLANT GROWTH REGULATORS ON AMINO ACID METABOLISM IN OAT LEAVES

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EFFECTS OF PLANT GROWTH REGULATORS ON AMINO ACID METABOLISM IN OAT LEAVES

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EFFECTS OF PLANT GROWTH REGULATORS ON AMINO ACID METABOLISM IN OAT LEAVES

CHAPTER I

INTRODUCTION

Plant growth regulators have been shown to affect many diverse processes in plants. Considerable effort has been expended in investigating the influence of auxins on carbohydrate, nitrogen, high energy phosphate, and cell wall metabolism in the hope of determining which facet of the metabolism is affected by auxin. However, the mechanism of the action is still obscure.

Freiberg and Clark (4) established that the nitrogen distribution pattern in soybean plants whose roots were exposed to 2,4-D (2,4-dichlorophenoxyacetic acid) was greatly affected, but the total nitrogen content was unchanged.

Yasuda et al (21) showed that treatment of potato plants with 2,4-D prior to harvest increased the protein content of the tubers.

According to Wort (19) it was reported by Rasmussen and Lawrence that low rates of 2,4-D application to Canada thistle caused significant increases in free amino acids and protein in the roots.

Akers and Fang (1) found that 2,4-D treated bean plants showed a reduction in photosynthetic fixation of $C^{14}O_2$. They also noted that a de-

crease in the aspartic acid and glutamic acid content of both stems and roots of bean resulted from the application of 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).

Vernon et al (14) applied C^{14}-labeled acetate to pea root tissues and noted that the dual effects of IAA (indoleacetic acid) and 2,4-D upon the growth of roots were closely associated with their influence on acetate utilization.

Rohrbaugh (9), studying the effects of plant growth regulators on the metabolism of oat plants, reported that the labeling of several metabolic intermediates during photosynthetic fixation of $C^{14}O_2$ was affected considerably by pretreating the leaves with these substances. He also observed that the effects of IAA and 2,4,5-T appeared to be significantly different at the same concentration. Some of the equilibria involving alanine, aspartate, glutamate, glycine, and serine were affected (10).

The present study made use of carbon-14 as a tracer in an attempt to determine differences in amounts and specific activities of certain free and protein-bound amino acids resulting from treatment of oat leaves with growth regulators.

CHAPTER II

MATERIALS AND METHODS

Victory oats (Avena sativa) were planted and grown in soil in an air conditioned growing room at 70-75° F. A bank of six "cool white" 40 w fluorescent lamps provided a light intensity of 800-2000 ft-c at the leaf surface of the seedlings.

Two weeks after planting, seedlings with uniformly expanded second leaves were selected. These leaves were then cut under water leaving approximately 1 cm of sheath on each leaf. After blotting away the excess water, each leaf was weighed. The terminal end of the blade was trimmed away until the remaining part of each leaf weighed the same for a single experiment.

The cut ends of the sheaths were then placed in vials containing the pretreatment solutions which consisted of distilled water (control), 5×10^{-4} M potassium chloride (KCl-control), 5×10^{-4} M potassium salt of indoleacetic acid (IAA), and 5×10^{-4} M potassium salt of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The leaves in their respective solutions were placed under a bank of six 30 w fluorescent lamps which provided an irradiance of 2000 ft-c at the level of the leaves. Air was circulated over the surface of the leaves during pretreatment.

At the end of the four hour pretreatment period, the vials contain-

ing the leaves and solutions were transferred to photosynthesis chambers. Each photosynthesis chamber consisted of an 80 ml glass test tube, a stopper through which passed a curved glass side arm, and a serum vial cap. Each chamber was placed in a water bath the temperature of which was maintained at 20 ± 1° C. Eight 150 w reflector flood lamps, four on each side, provided an irradiance of approximately 2500 ft-c on both surfaces of the leaves.

An aliquot of sodium bicarbonate ($NaHC^{14}O_3$) equivalent to 50 uc of C^{14}, and a drop of methyl red indicator were placed in the reservoir of the side arm. After closing the side arms with serum vial caps, a 10 per cent solution of acid (H_2SO_4) was injected into the reservoir by means of a hypodermic needle on a syringe. Enough acid was injected to liberate the $C^{14}O_2$ from the $NaHC^{14}O_3$ as indicated by methyl red. After 20 or 30 minutes of photosynthesis the leaves were removed from the chambers and plunged into boiling 85 per cent ethanol. They were boiled for approximately 3 minutes and then allowed to stand in the alcohol solution overnight. After removing the leaf tissue from the alcohol extract, the tissue was boiled in distilled water for one hour. The tissue was then removed for storage until processing and the ethanol and water extracts were combined. The extracts were made to volume with 85 per cent ethanol and 10 ul aliquots in triplicate were plated on stainless steel planchets. These samples were dried and then counted with a mylar end-window G.M. tube and counter. The remaining leaf tissues when processed were dried in the oven at approximately 95° C for 24 hours. The total amount of C^{14} fixed into ethanol-water extracted materials was determined and recorded.

A known amount (3 mg fresh weight equivalent) of each extract was

spotted and dried on Whatman No. 1 filter paper (18 $\frac{1}{4}$ x 22 $\frac{1}{2}$ inches) for chromatography. The chromatograms were developed two-dimensionally with 71 per cent (by volume) phenol in water in the first direction and butanol, propionic acid, and water in the second direction (2). Radioautograms were made by placing the chromatograms on no-screen X-ray films (14 by 17 inches) for one to four weeks. The films were developed and the radioactivity of all spots found on the chromatograms were counted. The percentages of the total C^{14} fixed into these separate products were then determined.

The neutral and acidic amino acid mixtures were removed from the leaf extracts by ion exchange columns according to the method of Thompson et al (13).

In Experiment 1 these neutral and acidic amino acid mixtures were separated by paper chromatography. Each mixture was concentrated and spotted on Whatman No. 3 filter paper (18 $\frac{1}{4}$ x 22 $\frac{1}{2}$ inches). The chromatograms were developed as described above. Radioautograms were made as before. The quantitative estimation and specific activity (count per second per microgram) of each amino acid were determined as previously reported (7).

In Experiments 2 and 3, after the neutral and acidic amino acid mixtures were removed from other compounds in the leaf extracts by the method of Thompson et al (13), the individual amino acids were separated on an ion exchange column by the method of Moore et al (8). The identity of the individual amino acids was confirmed by co-chromatography with knowns. Then aliquots of 100 ul were taken from each 3 ml amino acid fraction and plated in triplicate on stainless steel planchets. These

were dried and then counted with a windowless counter. The total amount of radioactivity per 1 ml sample was calculated.

Duplicate 1 ml aliquots were also taken from each amino acid fraction and the amount of each amino acid was estimated by use of a ninhydrin reagent (3). The optical density of each sample was measured using a Spectronic 20 colorimeter at a wave length of 570 mu. The specific activity of the amino acid was then calculated for each sample.

The oven dried leaf tissues left after extraction with 85 per cent ethanol-water were hydrolyzed with 6 N HCl. The dried leaf tissues were placed in small sealed test tubes (ampules) with 5 ml of 6 N HCl. These ampules were autoclaved at 110° C for 24 hours. After the ampules had cooled to room temperature, they were broken and the materials were washed into test tubes. The black precipitated humins were separated from the hydrolysates by filtration, dried, and the amount of radioactivity in them was estimated. The filtrates were then evaporated to dryness and the residues were dissolved in distilled water. They were made to 1 ml volume with distilled water and the amount of radioactivity incorporated was determined. Aliquots of 10 ul were taken from each sample and plated on planchets in triplicate. These were dried and counted as before.

The total amount of C^{14} found in the hydrolysate and in the unhydrolyzed material of each sample was calculated.

The extraction, separation, and quantitative estimation of each amino acid in each hydrolysate of the later experiments followed the methods previously described (13,8). The specific activity of each amino acid was also calculated.

CHAPTER III

RESULTS

Experiment 1

In the first experiment oat leaves, each weighing 90 mg, were pretreated in either H_2O, 5 x 10^{-4} M KCl, 5 x 10^{-4} M IAA, or 5 x 10^{-4} M 2,4,5-T. The experiment was run in duplicate with four leaves in each vial. The pretreated leaves were allowed to fix $C^{14}O_2$ for 20 minutes. The amounts of C^{14} fixed into extractable, as well as into the hydrolyzable and non-hydrolyzable materials (humin) from the non-extractable fraction are shown in Table I.

In both replications leaves of the water and KCl controls fixed more C^{14} into ethanol-water extractable materials than did those pretreated with IAA or 2,4,5-T. The tissues pretreated with 2,4,5-T incorporated the least C^{14} into the ethanol-water extractable material, while the leaves pretreated with KCl incorporated the least C^{14} into the non-extractable material. The oat leaves pretreated with IAA fixed more C^{14} into the HCl-hydrolysates and humins than did the other leaves. Both IAA and 2,4,5-T pretreated tissues fixed more C^{14} into the hydrolysates and into the humins than did either control.

The percentage of total C^{14} found in separate metabolic intermediates during 20 minutes photosynthesis is shown in Table II.

The percentage of the total fixed C^{14} which was incorporated into alanine, aspartate, glutamate, and glutamine was greatly increased, that going into glycine was decreased, while that going into serine was not materially affected by 2,4,5-T pretreatment. Pretreatment with IAA produced similar but less marked effects on the labeling of alanine, glutamate, and glutamine, but apparently did not greatly affect the labeling of aspartate. The effect of IAA on the labeling of glycine and serine was similar to that of 2,4,5-T

The specific activities of the individual amino acids as determined after separation by column and paper chromatography appear in Table III.

These data show that the effect of 2,4,5-T on the specific activity of most of the amino acids analyzed is much more pronounced than is the effect of IAA. The specific activities of alanine, aspartate, glutamate, and glutamine were increased by pretreatment of 2,4,5-T. On the other hand the specific activities of glycine and possibly serine were decreased with such pretreatment. Pretreatment with IAA did not greatly affect the specific activity of alanine. It increased the specific activities of glutamate and glutamine, but it decreased those of aspartate, glycine, and serine.

Experiment 2

In the second experiment only 5×10^{-4} M KCl and 5×10^{-4} M 2,4,5-T were used as the pretreatment solutions. The experiment was done in triplicate, with 3 leaves each weighing 100 mg used in each vial. After pretreatment each vial was placed in a photosynthesis chamber, and photosynthesis was allowed to continue for 30 minutes.

The fixation of C^{14} in the ethanol-water extracted material, hydroly-

sate, and humin is shown in Table IV. These results confirm those found in Experiment 1. Pretreatment with 2,4,5-T resulted in less fixation of C^{14} into ethanol-water extractable materials but stimulated the incorporation of C^{14} into the HCl-hydrolysates and humins.

The percentage of the total C^{14} which was found in separate metabolic intermediates is shown in Table V. The results are similar to those observed in Experiment 1.

The specific activity determined for each amino acid is shown in Table VI. The same trends were observed as in Experiment 1. Pretreatment of tissue with 2,4,5-T increased the specific activities of alanine, aspartate, and glutamate and decreased the specific activities of glycine and serine, although these reductions were not significant.

The specific activities of amino acids in the HCl-hydrolysates of the non-extractable fractions appear in Table VII. Pretreatment of tissue with 2,4,5-T increased the specific activities of alanine, aspartate, and glutamate in the acid hydrolysates but reduced those of glycine and serine.

Experiment 3

In the third experiment the pretreatment and fixation period were the same as in Experiment 1, but no water-control was used.

The amounts of C^{14} fixed into the extractable and into the hydrolyzed and non-hydrolyzed fractions of the non-extractable materials are shown in Table VIII. The fixation of C^{14} into ethanol-water extracted materials, HCl-hydrolysates, and humins was similar to that shown by the results of Experiments 1 and 2.

The percentage of total C^{14} which was found in the separate metabolic

intermediates was determined by counting the radioactivity on the paper chromatograms. The results which are shown in Table IX in general confirm those of Experiments 1 and 2, but the percentage going into glycine was increased by the IAA and sharply reduced by 2,4,5-T. On the other hand, the percentage incorporated into serine was approximately doubled by the IAA and somewhat increased by 2,4,5-T. These results appear to be in the opposite direction from those noted in Experiments 1 and 2.

The extracts from the leaves receiving the same pretreatment were combined to obtain more materials for chemical analyses, and the specific activities of the amino acids were determined. The data appear in Table X.

Pretreatment of oat leaves with IAA or 2,4,5-T increased both the C^{14} incorporation into and the specific activities of alanine, aspartate, and glutamate, although the effect of 2,4,5-T was much more pronounced. The specific activities of glycine and serine were decreased by pretreatment with 2,4,5-T, but the specific activity of serine was slightly increased with IAA pretreatment while that of glycine was decreased.

The tissues remaining after extraction of each replicate with ethanol and water were not combined, and leaves from only one series were hydrolyzed with 6 N HCl. The specific activities of the amino acids from the hydrolysates are shown in Table XI. The results for 2,4,5-T confirm those of Experiment 2. The effects of pretreatment with IAA resembled those caused by 2,4,5-T but the changes of specific activities were not as great.

TABLE I

Effects of IAA and 2,4,5-T on $C^{14}O_2$ Fixation in Victory Oat Leaves during 20 Minutes Photosynthesis (Experiment 1)

4 Hours Pretreatment		Fresh Weight	Total C^{14}		
			Ethanol-water extract	HCl-hydrolysate	Humin
		mg	c/s	c/s	c/s
Water	a	360	62,120	1,028	244
	b	360	47,180	1,036	254
5×10^{-4} M KCl	a	360	65,120	756	275
	b	360	47,520	904	190
5×10^{-4} M IAA	a	360	42,540	1,888	461
	b	360	37,440	2,188	354
5×10^{-4} M 2,4,5-T	a	360	41,240	1,288	268
	b	360	37,760	1,052	270

TABLE II

Effects of IAA and 2,4,5-T on Labeling of Intermediates in Metabolism of Victory Oat Leaves during 20 Minutes Photosynthesis (Experiment 1)

Products	Percentage of total C^{14} incorporated into different products during 20 minutes photosynthesis after pretreatment for 4 hours							
	Pretreating Solution							
	Water		5×10^{-4} M KCl		5×10^{-4} M IAA		5×10^{-4} M 2,4,5-T	
	a	b	a	b	a	b	a	b
	%	%	%	%	%	%	%	%
Alanine	0.93	2.15	0.99	1.64	2.55	2.52	12.95	16.95
Aspartate	0.64	1.50	1.12	1.50	1.21	1.24	12.23	13.25
Glutamate	0.22	0.28	0.29	0.21	0.79	0.60	3.90	3.74
Glycine	35.31	33.81	37.01	34.81	10.04	8.27	7.14	5.47
Serine	13.03	14.69	12.14	12.62	10.00	8.20	12.23	12.12
Glutamine	trace	trace	trace	trace	1.37	0.95	1.93	1.58
Sucrose	15.42	9.38	11.96	13.37	24.20	30.98	9.86	14.43
Maltose	trace	trace	trace	trace	2.08	1.89	0.13	0.14
Citrate	0.25	0.21	0.33	0.23	0.34	0.33	1.27	1.05
Malate	4.85	5.50	5.51	5.33	3.31	3.65	10.06	8.86
Glycerate	4.97	5.52	4.62	4.80	1.76	1.52	2.52	2.36
Glycolate	0.50	0.46	0.43	0.65	1.00	0.84	0.30	0.35
Phosphates	19.64	22.49	21.43	20.76	34.39	32.53	20.02	13.85

TABLE III

Effects of Pretreatment with IAA and 2,4,5-T on the Labeling of Free Amino Acids in Victory Oat Leaves during 20 Minutes Photosynthesis in $C^{14}O_2$ (Experiment 1)*

4 Hours Pretreatment		Specific Activities c/s/ug					
		Alanine	Aspartate	Glutamate	Glycine	Serine	Glutamine
Water	a	54.6	13.8	2.0	995.3	478.5	39.0
	b	75.1	25.6	1.0	654.9	487.9	3.5
5×10^{-4} M KCl	a	29.8	26.9	1.9	768.7	427.0	15.7
	b	75.1	17.1	1.0	675.2	341.1	9.9
5×10^{-4} M IAA	a	76.8	8.8	8.2	360.1	347.0	97.9
	b	73.3	8.8	7.0	316.5	255.7	102.4
5×10^{-4} M 2,4,5-T	a	180.1	153.0	25.8	329.8	267.2	165.3
	b	362.1	97.9	49.5	210.6	347.4	318.3

*The amounts of amino acids used for quantitative estimation in these samples were as follows: 5.3 - 16.4 ug in alanine, 7.5 - 17.0 ug in aspartate, 17.4 - 38.3 ug in glutamate, 4.3 - 12.3 ug in glycine, 6.9 - 9.3 ug in serine, and 0.3 - 1.4 ug in glutamine.

TABLE IV

Effects of 2,4,5-T on the $C^{14}O_2$ Fixation in Victory Oat Leaves during 30 Minutes Photosynthesis (Experiment 2)

4 Hours Pretreatment		Fresh Weight	Total C^{14}		
			Ethanol-water extract	HCl-hydrolysate	Humin
		mg	c/s	c/s	c/s
5×10^{-4} M KCl	a	300	160,530	5,562	2,122
	b	300	137,260	4,581	1,665
	c	300	110,702	4,290	2,180
5×10^{-4} M 2,4,5-T	a	300	110,894	7,111	2,339
	b	300	112,490	5,037	2,045
	c	300	96,086	4,785	2,241

TABLE V

Effects of 2,4,5-T on Labeling of Intermediates in Metabolism of Victory Oat Leaves during 30 Minutes Photosynthesis (Experiment 2)

Products	Percentage of total C^{14} incorporated into different products during 30 minutes photosynthesis after pretreatment for 4 hours					
	Pretreating Solution					
	5×10^{-4} M KCl			5×10^{-4} M 2,4,5-T		
	a	b	c	a	b	c
	%	%	%	%	%	%
Alanine	0.40	0.50	0.36	3.34	3.89	3.37
Aspartate	0.60	0.66	0.80	4.61	5.26	4.75
Glutamate	0.35	0.31	0.29	3.64	3.89	3.77
Glycine	21.30	22.95	27.92	13.72	15.39	19.73
Serine	6.59	7.26	5.45	5.87	5.70	6.56
Glutamine	0.10	0.13	0.14	1.92	2.47	2.49
Sucrose	47.16	38.17	33.83	37.20	28.88	24.39
Maltose	0.30	0.22	0.26	0.60	0.35	0.62
Citrate	0.22	0.20	0.20	1.09	1.18	0.57
Malate	8.27	10.36	11.93	10.01	11.81	13.07
Glycerate	1.51	1.97	2.41	1.56	1.35	1.96
Glycolate	0.33	0.26	0.37	0.19	0.49	0.64
Phosphates	9.98	12.41	12.05	12.77	15.47	13.65

TABLE VI

Effects of Pretreatment with 2,4,5-T on Labeling of Free Amino Acids in Victory Oat Leaves during 30 Minutes Photosynthesis in $C^{14}O_2$ (Experiment 2)*

4 Hours Pretreatment		Specific Activities c/s/ug				
		Alanine	Aspartate	Glutamate	Glycine	Serine
5×10^{-4} M KCl	a	**	98.6	31.4	2148.7	850.0
	b	381.7	342.5	34.7	2802.2	1383.5
	c	117.5	408.3	30.0	2397.5	1309.5
5×10^{-4} M 2,4,5-T	a	632.3	492.4	164.4	1425.9	723.3
	b	1288.9	1100.0	330.0	2058.6	1138.5
	c	942.2	1055.9	166.2	1924.4	1059.4

*The amounts of amino acids used for quantitative estimation in these samples were as follows: 0.6 - 1.3 ug in alanine, 0.6 - 2.9 ug in aspartate, 4.3 - 7.3 ug in glutamate, 1.5 - 4.8 ug in glycine, and 1.4 - 3.7 ug in serine.

**No data were taken from this fraction.

TABLE VII

Effects of Pretreatment with 2,4,5-T on Labeling of Amino Acids in the HCl-hydrolysate of the Non-extractable Material of Victory Oat Leaves during 30 Minutes Photosynthesis in $C^{14}O_2$ (Experiment 2)*

4 Hours Pretreatment		Specific Activities c/s/ug				
		Alanine	Aspartate	Glutamate	Glycine	Serine
5×10^{-4} M KCl	a	3.4	2.2	**	12.6	12.5
	b	2.5	1.4	**	11.1	11.4
	c	2.3	1.4	0.0	11.7	11.0
5×10^{-4} M 2,4,5-T	a	3.6	**	0.5	7.0	9.7
	b	3.9	2.3	0.6	6.8	8.9
	c	3.9	1.8	0.6	6.2	9.0

*The amount of amino acids used for the quantitative estimation in these samples were as follows: 17.7 - 23.2 ug in alanine, 36.0 - 43.2 ug in aspartate, 5.4 - 31.0 ug in glutamate, 6.7 - 17.2 ug in glycine, and 11.0 - 13.7 ug in serine.

**No data were taken from these fractions.

TABLE VIII

Effects of IAA and 2,4,5-T on the $C^{14}O_2$ Fixation in Victory Oat Leaves during 20 Minutes Photosynthesis (Experiment 3)

4 Hours Pretreatment		Fresh Weight	Total C^{14}		
			Ethanol-water extract	HCl-hydrolysate	Humin
		mg	c/s	c/s	c/s
	a	360	199,150	1,665	1,049
5×10^{-4} M KCl	b	360	102,100	983	378
	c	360	75,600	1,682	546
	a	360	87,000	5,339	2,374
5×10^{-4} M IAA	b	360	77,100	1,682	867
	c	360	71,150	2,016	851
	a	360	86,450	1,909	1,343
5×10^{-4} M 2,4,5-T	b	360	51,450	1,346	533
	c	360	46,675	1,861	601

TABLE IX

Effects of IAA and 2,4,5-T on Labeling of Intermediates in Metabolism of Victory Oats during 20 Minutes Photosynthesis (Experiment 3)

Products	Percentage of total C^{14} incorporated into different products during 30 minutes photosynthesis after pretreatment for 4 hours		
	Pretreating Solution		
	5×10^{-4} M KCl	5×10^{-4} M IAA	5×10^{-4} M 2,4,5-T
	%	%	%
Alanine	0.96	1.74	13.98
Aspartate	0.61	0.96	11.94
Glutamate	0.24	0.54	4.43
Glycine	25.17	30.24	6.94
Serine	6.92	13.30	8.27
Glutamine	trace	trace	1.70
Sucrose	44.12	27.22	22.70
Maltose	trace	0.23	trace
Citrate	0.12	trace	0.76
Malate	4.08	5.36	8.57
Glycerate	2.20	3.29	1.55
Glycolate	0.40	0.73	0.12
Phosphates	12.43	14.43	16.25

TABLE X

Effects of Pretreatment with IAA and 2,4,5-T on Labeling of Free Amino Acids in Victory Oat Leaves during 20 Minutes Photosynthesis (Experiment 3)*

4 Hours Pretreatment	Specific Activities c/s/ug				
	Alanine	Aspartate	Glutamate	Glycine	Serine
5×10^{-4} M KCl	68.6	157.6	17.4	2617.5	1739.4
5×10^{-4} M IAA	635.9	411.5	43.4	2329.8	1879.5
5×10^{-4} M 2,4,5-T	900.8	881.8	106.8	1130.0	1255.1

*The amounts of amino acids used for the quantitative estimation in these samples were as follows: 2.7 - 9.7 ug in alanine, 4.0 - 18.0 ug in aspartate, 28.2 - 39.0 ug in glutamate, 4.6 - 18.5 ug in glycine, and 7.4 - 11.1 ug in serine.

TABLE XI

Effects of Pretreatments with IAA and 2,4,5-T on Labeling of Amino Acids in the HCl-hydrolysate of the Non-extractable Material of Victory Oat Leaves during 20 Minutes Photosynthesis (Experiment 3)*

4 Hours Pretreatment	Specific Activities c/s/ug				
	Alanine	Aspartate	Glutamate	Glycine	Serine
5×10^{-4} M KCl	0.8	**	0.5	8.3	10.9
5×10^{-4} M IAA	1.2	0.8	1.8	6.9	5.2
5×10^{-4} M 2,4,5-T	2.5	1.2	3.6	2.2	2.6

*The amounts of amino acids used for the quantitative estimation in these samples were as follows: 20.6 - 24.2 ug in alanine, 46.5 - 48.3 ug in aspartate, 3.1 - 14.3 ug in glutamate, 14.5 - 20.0 ug in glycine, and 16.3 - 18.3 ug in serine.

**No data were taken from this fraction.

CHAPTER IV

DISCUSSION

The incorporation of C^{14} into the alcohol-water extractable materials of oat leaves was decreased by both IAA and 2,4,5-T treatment. Rohrbaugh (10) and Akers and Fang (1) reported that the photosynthetic fixation of $C^{14}O_2$ in plants was reduced by treatment with growth regulators. Vernon et al (14) observed that C^{14}-labeled acetate uptake by pea root tissue was inhibited approximately 20 per cent by a concentration of 1×10^{-4} M 2,4-D.

In the present study the radioactivity incorporated in the alcohol-water insoluble residue was increased by IAA or 2,4,5-T treatment. Vernon et al (14) reported that the incorporation of C^{14}-acetate into the alcohol insoluble residue in pea root tissue was decreased with increasing time of treatment with IAA or 2,4-D. Sell et al (11) and Weller et al (16) found that there was a depletion of reducing and non-reducing sugars, a considerable reduction in starch and crude fiber, and a decrease in acid hydrolyzable polysaccharides in auxin treated stems. This was accompanied by an accumulation of both protein and amino acids. Only non-reducing sugars were depleted in the roots and leaves. It appears that the incorporation of C^{14} into the alcohol insoluble residue varies depending on the tissue and the time of auxin treatment used in the experiment.

In the earlier report (7), the determination of amino acids was ham-

pered throughout by ninhydrin sensitive materials on the chromatograph paper. In the present study in order to increase the final amount to be quantitatively estimated and thus reduce the percentage of error in the determination of amino acids on the paper chromatograms, a large aliquot of oat leaf extract was used in Experiment 1.

The separation of acidic and neutral amino acids from other extracted materials by the ion exchange columns according to Thompson et al (13) was very satisfactory.

Determination of the specific activities of neutral and acidic amino acids--alanine, aspartate, glutamate, glycine, and serine were important because the labeling of these amino acids is greatly affected by pretreatment with growth regulators prior to photosynthetic fixation of $C^{14}O_2$ (9).

The total amount of amino acids separated from the alcohol-water extracts (from 300 mg fresh weight of oat leaves) by the column was put on Whatman No. 3 filter paper. The resolution of the various individual labeled amino acids was good except for glycine and serine.

The method described by Moore et al (8) for separation of amino acids by means of an ion exchange column was very satisfactory. The amino acids were separated in large, easily estimated quantities and the specific activities of the labeled amino acids were more accurately determined.

Generally, the specific activities of the free amino acids--alanine, aspartate, and glutamate were increased by the treatments with IAA and 2,4,5-T. Rohrbaugh (10) reported that incorporation of C^{14} into alanine, aspartate, and glutamate when measured as a percentage of the total C^{14} fixed was generally increased by this treatment. Aker and Fang (1) also reported that the incorporation of C^{14} into aspartate and glutamate was 3

to 4 times greater in the 2,4-D treated plants. It appears that the application of growth regulators causes the rate of synthesis of these three amino acids to increase, and at the same time an increase in utilization occurs. The data also indicate that at least in most cases the pool sizes of these amino acids are not appreciably changed.

The specific activities of free glycine and serine were decreased by 2,4,5-T treatment. Rohrbaugh (10) reported that the incorporation of C^{14} into glycine and serine in oat leaves was decreased by IAA and 2,4,5-T. According to Wilkinson and Davis (18) and Wang and Waygood (15) glycine and serine are synthesized through glyoxylate formed by oxidation of glycolate in wheat plants. It is possible that the rates of synthesis of glycine and serine through these reactions are limited by treatment with 2,4,5-T.

It appears that some common factors regulate the rate of synthesis of these amino acids. The rate of utilization of the amino acid is regulated by its pool size which is in turn regulated by the rate of synthesis. The latter rate is proportional to some factor which may be common to all amino acids. This factor might be the amino acid precursors, enzymes, or coenzyme A. A higher activity of several Krebs cycle dehydrogenases (12) and other enzyme systems (20) may accelerate the formation of alanine, aspartate, and glutamate via the formation of pyruvate, oxaloacetate, and α-ketoglutarate. Leopold and Guernsey (6) proposed that auxin may act in plant growth and metabolism at least in part by reacting with coenzyme A to control the variety of synthetic and metabolic reactions in plants which are known to require coenzyme A.

The specific activity of protein-bound amino acids could be affected

by the increase in ribonucleic acid content (5,17) found in auxin treated plants. Several workers (11,19,21) reported that the protein content of plant tissues was increased by auxin treatment. Sell et al (11) reported that 2,4-D resulted in depletion of reducing and non-reducing sugars in the stems of bean plants, and also a decrease in other carbohydrate reserves including acid hydrolyzable polysaccharides. This decrease in carbohydrates was accompanied by a large increase in total protein. These authors suggested that their data indicated that the 2,4-D was causing a large part of the carbohydrates to be used in protein synthesis.

Since the changes of specific activities of protein-bound amino acids are similar to those of free amino acids, it seems that the utilization of these amino acids, perhaps via protein synthesis, is stimulated by these growth regulators.

CHAPTER V

SUMMARY

Victory oat plants (Avena sativa) were grown under controlled conditions of light and temperature. Two weeks after planting, selected leaves were pretreated in distilled water, KCl, IAA, and 2,4,5-T. The leaves were then permitted to fix $C^{14}O_2$ photosynthetically for 20 or 30 minutes. They were then extracted with ethanol and water. Alcohol-water insoluble residues were hydrolyzed with 6 N HCl. The total amounts of C^{14} fixed into ethanol-water soluble materials and into the HCl-hydrolysable and HCl-unhydrolysable material of the residue were determined. The percentage of the total C^{14} incorporated into the separate metabolic intermediates during 20 or 30 minutes was also determined.

The amino acids in the alcohol-water extracts and the hydrolysates were separated and purified by methods employing paper chromatography and ion exchange resins. The radioactivity and the amount of amino acid as estimated colorimetrically were measured and the specific activities of the amino acids determined.

These data show several trends.

1. The effects of 2,4,5-T are much more pronounced than those of IAA at the concentration of 5×10^{-4} M. These effects appear to be significantly different.

2. Oat leaves pretreated with IAA and 2,4,5-T fixed less C^{14} in the alcohol-water extractable substances, but tend to stimulate incorporation of C^{14} into the HCl-hydrolysable material and into humin.

3. Pretreatment of oat leaves with 2,4,5-T resulted in an increase in the specific activities of alanine, aspartate, and glutamate and a decrease in those of glycine and serine in both the alcohol-water extracts and the hydrolysates.

4. IAA pretreatment increased the specific activities of alanine and glutamate and decreased that of glycine in the alcohol-water extracts. The effect on the specific activities of aspartate and serine were varied. In the hydrolysates, the same trends were observed as in the hydrolysates from the leaves pretreated with 2,4,5-T.

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