CHANGES IN CHEMICAL COMPOSITION OF THE STEMS OF RED KIDNEY BEAN PLANTS TREATED WITH 2, 4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

It has been shown (**2**, **5**, **8**, **11**) that treatment of plants with 2, 4-D results in a reduction in carbohydrate content and an accumulation of nitrogen. Investigators (**4**, **6**,) have reported that some plants sprayed with solutions of 2, 4-D develop enlarged and proliferated tissues, noted primarily in the stems. It has also been observed that some plants which were sprayed with 2, 4-D and which developed proliferated tissue of this type were eaten more readily by animals (**7**). Since little is known about the chemical composition of this abnormal tissue, the work reported in this paper was projected to include the analysis for amino acids, ether extract, carbohydrates, ash and crude fiber. Results of chemical analyses are presented for the stems of untreated red kidney bean plants and for those treated with 2, 4-D.

Materials and methods

SAMPLES OF STEM TISSUE

Seeds of the red kidney bean were selected for uniformity of size and planted in 4-inch pots in the greenhouse. Each pot contained two plants that were treated when the first trifoliate leaf was expanding. Two replications of 200 plants each were used from which to obtain material of treated and non-treated plants (controls). Application of 2, 4-D was made by applying one drop (.05 ml.) of a 1000-p.p.m. solution to the base of the blade of one of the primary leaves (**6**). The plants were harvested six days after treatment at the time the stem tissue had proliferated considerably but yet showed no signs of necrosis. The material was dried according to the procedure given by LINK (**3**) and then segregated into the various parts. The hypocotyl, first internode, and leaf petioles were grouped together as stem tissue, and analyses were made of this material.

ANALYTICAL METHODS

Amino acids were determined microbiologically with the organisms *Lactobacillus arabinosus, Streptococcus faecalis* and *Leuconostic mesenteroides.* The media used in the various determinations was essentially the same as that described by SAUBERLICH and BAUMANN (**9**). Samples were prepared for assay according to the method of STOKES *et al.* (**12**), with the exception

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of the tryptophan sample which was prepared according to the method of WOOLEY and SEBRELL (**13**). The analyses for carbohydrates were made by the procedure outlined by SELL, JOHNSON and LAGASSE (**10**) and the nitrogen, crude fiber, ether extract and ash were determined by A. O. A. C. procedures (**1**).

Results and discussion

The results of the chemical analyses are summarized in tables I and II. Each entry is the average of two determinations on each of two separate samples.

The data in table I, based both on the percentage of amino acid or protein in the sample and on the milligrams per stem, show the following trends: The stems of the plants treated with 2, 4-D contain approximately twice as much protein as the stems of the non-treated plants. A similar observation was made by SMITH, HAMNER and CARLSON (**11**). The same trend as noted in the protein content was observed for leucine, isoleucine, valine, phenylalanine, histidine, arginine and threonine. The lysine and methionine content of the stems of the treated plants was approximately three times that of the controls. The tryptophan and asparic acid content were only slightly greater in treated than in non-treated plants.

AMINO ACID CONTENT OF THE STEMS OF RED KIDNEY BEAN PLANTS TREATED WITH 2, 4-DICHLOROPHENOXYACETIC ACID
(EXPRESSED ON A LIPIDE-FREE* DRY WEIGHT BASIS)

CONSTITUENT	NON-TREATED			TREATED		
	IN THE SAMPLE	PER STEM	IN CRUDE PROTEIN	IN THE SAMPLE	PER STEM	IN CRUDE PROTEIN
	per cent.	milligrams	per cent.	per cent.	milligrams	per cent.
Protein (NX6.25)	16.89	17.40		30.54	32.98	
Leucine	.72	.74	4.23	1.44	1.56	4.70
Isoleucine	.77	.79	4.53	1.56	1.68	5.11
Valine	.73	.75	4.28	1.75	1.89	5.21
Phenylalanine	.47	.48	2.80	1.04	1.12	3.39
Histidine	.43	.44	2.51	.76	.82	2.48
Arginine	.84	.87	4.94	1.49	1.61	4.82
Lysine	.51	.53	3.04	1.48	1.60	4.82
Tryptophan	.12	.12	.69	.17	.18	.55
Methionine	.07	.07	.38	.20	.22	.65
Threonine	.67	.69	3.96	1.14	1.23	3.73
Aspartic	1.48	1.52	11.31	1.88	2.03	6.37

* Substances soluble in diethyl ether.

In calculating the data in table I as per cent. of amino acid in the protein, the following results were obtained: The amount of histidine is almost the same in the stems of the treated plants as in the controls. The content of tryptophan, threonine, and arginine is slightly less in the stems of the treated plants than in the stems of those not treated. Some of the greatest differences in the quantity of amino acids in the stem tissue were found in the content of aspartic acid, lysine, valine, methionine and phenylalanine. It is noted that the stems of the non-treated plants contain 43.7 per cent.

more aspartic acid than the stems of the treated plants. However, the stems of the treated plants contain 17.9, 36.9, 41.5 and 17.4 per cent. more valine, lysine, methionine, and phenylalanine, respectively, than the controls.

The data presented on the basis of percentage of amino acid or protein in the sample and of milligrams per stem show that there is an accumulation of protein and amino acids in the stems of plants treated with 2, 4-D. When the data are expressed as per cent. amino acid in the crude protein, the results indicate that the character of the protein in the stems of the treated plants has changed as compared to that of the controls.

In table II, the stems of the plants treated with 2, 4-D show a depletion of reducing and non-reducing sugars. A similar observation has been noted by RASMUSSEN (**8**) in dandelion roots. SMITH *et al.* (**11**) and MITCHELL

CARBOHYDRATE, CRUDE FIBER, ETHER EXTRACT AND ASH CONTENT OF THE STEMS OF RED KIDNEY BEAN PLANTS TREATED WITH 2, 4-DICHLOROPHENOXYACETIC ACID (EXPRESSED ON A LIPIDE-FREE† DRY WEIGHT BASIS)

CONSTITUENT	NON-TREATED		TREATED	
	PER CENT.	MILLIGRAMS PER STEM	PER CENT.	MILLIGRAMS PER STEM
Reducing Sugar	1.66	1.71	.00	.00
Non-reducing Sugar	4.78	4.92	.00	.00
Starch	7.22	7.44	2.08	2.25
Polysaccharide*	12.16	12.52	10.09	10.09
Crude Fiber	30.34	31.25	19.99	21.59
Ether Extract (lipides, etc.)	1.64	1.69	2.26	2.44
Unsaponifiable Residue of the Ether Extract	.98	1.01	1.25	1.35
Fatty Acids (By difference) of Ether Extract	.61	.68	1.01	1.09
Ash	11.41	11.75	15.98	17.26

* Acid hydrolyzable residue from starch determinations.
† Substances soluble in diethyl ether.

and BROWN (**5**) observed simliar trends in bindweed and the morning glory. Considerable reduction in starch and a decrease in acid hydrolyzable polysaccharides were noted in stems of treated plants. Similar results have been obtained by other workers (**5, 8, 11**).

The depletion of reducing and non-reducing sugar and the utilization of the starch reserves and the large increase in protein in the stems of plants treated with 2, 4-D indicate that most of the carbohydrates are used in protein synthesis.

The quantity of crude fiber in the stems of the treated plants was considerably less than that of the controls. The decrease in acid hydrolyzable polysaccharide and crude fiber suggests that the simple sugars are not utilized to a great extent in the formation of structural constituents in the stems of plants treated with 2, 4-D. The amount of ash in the stems of the treated plants increased as compared to the non-treated plants. The accumulation of organic acids or an increase of free carboxyl groups in the protein molecule of the treated tissue may explain the increase in ash content.

The ether soluble substances (lipides, etc.) were slightly greater in the stems of the treated plants than in the controls. This same trend was noted in the contents of unsaponifiable material and fatty acid content in the ether extract. An increase in the amount of ether extract may be explained on the assumption that some of the carbohydrates are transformed into lipide material.

Summary

1. Both protein and amino acids accumulated in greater quantities in the stems of plants treated with 2, 4-D than in the stems of the non-treated plants. The amino acids, calculated as per cent. of crude protein, showed the greatest differences in the quantity of amino acids of the stem tissue to occur in aspartic acid, lysine, valine, methionine and phenylalanine. The variations in the content of amino acid indicate that the character of the protein is different in the treated stems than in the controls.

2. The reducing and non-reducing sugars were depleted in the 2, 4-D treated plants. The treated plants also showed a considerable reduction in carbohydrate reserves and a decrease in acid hydrolyzable polysaccharides. This decrease in sugar content and the tremendous increase in total protein suggests that a large portion of the carbohydrates is utilized in protein synthesis.

3. The amount of crude fiber decreased in the stems of 2, 4-D treated plants as compared to the controls. The decrease in crude fiber and acid hydrolyzable polysaccharides indicates that the simple sugars are not utilized in the formation of structural constituents in 2, 4-D treated plants. The ash content was greater in the stems of the treated plants than in the stems of the non-treated plants. This increase of ash may be due to an accumulation of organic acids or an increase in free carboxyl groups in the protein of the stems of the treated tissue.

4. The amounts of ether extract, unsaponifiable material and fatty acids of the ether extract were slightly greater in the stems of the treated plants than in those of the non-treated plants. These differences may be due to the conversion of some of the carbohydrates to lipide material.

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