Comparison of Changes in Nitrogenous Constituents Following Uprooting or 2,4-D Treatment of Bean Plants[1]

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NUMEROUS *in vivo* studies (Akers and Fang, 1956; Freiberg and Clark, 1955; Fults and Payne, 1956; Rakiten and Zemskaya, 1958; Weller et al., 1950; Wort, 1951) have shown changes in concentration of protein, or other nitrogen-containing compounds related to protein metabolism, in various parts of plants following treatment with 2,4-dichlorophenoxyacetic acid (2,4-D). That the injury and death of the plants can be attributed to these effects of 2,4-D is not necessarily the logical conclusion to be drawn. Many other changes in the constituents of plants follow 2,4-D treatments (Woodford, et al., 1958). The difficulty of distinguishing between primary effects and secondary or nonspecific effects has been discussed previously (Muzik and Lawrence, 1959).

If certain changes in composition resulting from 2,4-D treatment are found to be substantially the same as those resulting from treatment with presumably unrelated chemicals (Fults and Payne, 1956) which also damage the plant, or from damage associated with purely physical causes, such as drying, uprooting, etc., the presumption is strong (though not proven) that the changes result from the damage rather than directly from the chemical. In the present study, the changes in certain nitrogenous constituents in uprooted plants have been found to be very much like those in plants injured by 2,4-D.

Materials and Methods

Seeds of snap beans, *Phaseolus vulgaris* L. var. Black Valentine, were planted five to a pot. After three weeks, two plants in each pot were selected for uniformity, and the rest were discarded. Eleven pots were used for each plot, and there were three plots in each sampling time for each treatment. The pots were in a randomized block design in the greenhouse. Applications of 2,4-D were made at rates equivalent to 1, 2, and 4 pounds per acre as a foliage spray in water. An untreated group and an uprooted group were established. The latter were not treated with 2,4-D but were carefully dug out of the pots; the roots were washed clean of soil, and the plants were laid on

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the greenhouse bench. The roots of these were sampled at the same time intervals as the others. The plants were lighted 11 hours a day. The untreated plants and some of the treated plants came into flower during the course of the experiment.

The roots of three replicates each were sampled 1, 4, 11, and 18 days after treatment by digging up the plants and washing the roots. Surface water was blotted, and fresh weight per sample was determined. The roots were then chopped into small pieces, dropped into 70 per cent alcohol, and extracted 10 times in a Waring blendor with successive portions of 70 per cent alcohol. Determinations on the combined extracts were made of total protein (as alcohol-insoluble nitrogen by Kjeldahl) and free amino nitrogen (Rosen, 1957) on each of the three replicates, and individual amino acids (Lawrence, et al., 1960) on one of the replicates. An estimate of the dry weight of each sample was obtained from the sum of the air-dry weight of the residue from the alcoholic extraction and the dry weight of the alcoholic residue, determined by partial evaporation followed by lyophilization of an aliquot. Results are given on a per plant basis.

Results

Epinastic responses in the young leaves were visible on the first day after treatment, especially in the plants treated with the higher rates of 2,4-D. The plants treated with 4 pounds per acre were dead in 18 days. At this time the plants treated with 2 pounds per acre were very stunted and malformed, and those treated with 1 pound per acre apparently were recovering.

The changes that occurred in the protein content of these root samples can be assessed by referring to Table 1. The changes in free amino nitrogen

TABLE 1

COMPARISON OF PROTEIN LEVELS* IN ROOTS OF 2,4-D-TREATED BEAN PLANTS AND CONTROL PLANTS

Pounds 2,4-D per acre		*Days after treatment* 1	4	11	18
0	(untreated)	2.81	2.40	3.02	2.94
1		2.96	2.70	2.62	2.80
2		2.84	2.52	1.94	1.87
4		2.66	2.38	2.26	1.67
0	(uprooted)	2.93	1.97	1.95	2.05

*Mg. alcohol-insoluble nitrogen per plant. Mean value of 3 plots of 22 plants each.

per plant are shown in Table 2. When these changes are calculated on a dry-weight basis, the same trends are apparent, although somewhat less pronounced owing to a loss of dry weight for the two higher treatment rates and the uprooted samples. By the 18th day, this loss had amounted to 20 to 25 per cent as compared with the controls. (This type of observation shows the limitations of the dry-weight basis for reporting such data.)

The changes that were found in six of the free amino acids present in greatest amount are given in Table 3. Threonine, proline, pipecolic acid, and asparagine also were determined. Tyrosine was usually present, as were several unidentified spots. There were no significant differences in the relative amounts of the various amino acids in the different samples.

Discussion and Conclusions

Foliar treatment with 2,4-D appears to have caused a marked loss of protein in the bean roots; the loss increased with increasing severity of the treatment. The loss in free amino nitrogen was even more striking, although the untreated samples also showed some decrease of amino nitrogen, perhaps related to the physiology of flower formation. However, the decrease in protein and free amino nitrogen content of the roots of the uprooted plants was very similar to the losses after 2,4-D treatment.

The decreases occurring after uprooting were more abrupt and drastic, and the differences are statistically significant (as are also the differences resulting from the different levels of 2,4-D treatment). Loss of protein and free amino acids in the roots appears to be a usual consequence of injury and death, whether caused by 2,4-D treatment or uprooting. There is also an

TABLE 2

COMPARISON OF FREE AMINO NITROGEN LEVELS* IN ROOTS OF 2,4-D-TREATED PLANTS AND CONTROL PLANTS

Pounds 2,4-D per acre		*Days after treatment* 1	4	11	18
0	(untreated)	0.18	0.14	0.17	0.14
1		0.17	0.17	0.16	0.12
2		0.16	0.17	0.11	0.11
4		0.16	0.15	0.12	0.10
0	(uprooted)	0.21	0.16	0.03	0.02

*Mg. per plant. Mean value of 3 plots of 22 plants each.

evident similarity between the decreases of the individual amino acids in the roots of both the 2,4-D-treated plants and the uprooted plants.

These results, therefore, suggest that the changes in levels of these nitrogenous constituents in the roots following 2,4-D application at the rates of 1-4 pounds per acre to the foliage are not a specific response to the chemical.

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TABLE 3

COMPARISON OF LEVELS OF INDIVIDUAL AMINO ACIDS* IN ROOTS OF 2,4-D-TREATED PLANTS AND CONTROL PLANTS

Amino acid	*Pounds 2,4-D per acre*	*Days after treatment* 1	4	18
Aspartic acid	0	0.11	0.06	0.07
	1	0.07	0.11	0.04
	4		0.06	0.005
	Uprooted	0.14	0.05	0.007
Glutamic acid	0	0.12	0.05	0.09
	1	0.15	0.14	
	4		0.06	0.02
	Uprooted	0.08	0.04	0.01
Serine	0	0.05	0.02	0.02
	1	0.06	0.04	0.01
	4		0.02	0.003
	Uprooted	0.03	0.04	0.001
Alanine	0	0.03	0.02	0.03
	1	0.03	0.04	0.03
	4		0.02	0.009
	Uprooted	0.03	0.05	0.003
Valine	0	0.09	0.06	0.06
	1	0.16	0.06	0.03
	4		0.04	0.005
	Uprooted	0.07	0.09	0.005
Leucines	0	0.06	0.02	0.01
	1	0.06	0.07	0.02
	4		0.04	0.009
	Uprooted	0.04	0.04	0.005

*Mg. per plant.

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