LX. INVESTIGATIONS ON THE NITROGENOUS METABOLISM OF THE HIGHER PLANTS.

PART IV. DISTRIBUTION OF NITROGEN IN THE DEAD LEAVES OF THE RUNNER BEAN.

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IN a former paper [Chibnall, 1922, 1], the seasonal variations in the N content of the leaves of the runner bean, *Phaseolus vulgaris* v. *multifloris* (Scarlet Champion), were demonstrated and discussed. The period examined covered the first twenty weeks of the plant's life, from the seedling stage, until the leaves were showing signs of chlorophyll degeneration. Eleven samples were analysed, numbered consecutively "series 1-11."

The plants died during the 24th week, following two nights' hard frost, and the present communication deals with the distribution of N found in the dead leaves, which will be denoted henceforth as series 12. As picked from the dead plants they were shrivelled up and dry, whilst their green colour was masked by a brownish-red stain, probably due to iron salts, since it was easily washed away with cold water.

The results given later for series 12 have in all cases been compared with the corresponding ones for series 9, 10 and 11, the four together covering the latter half of the plant's life, from the 11th to the 24th weeks. During this period the plants were fully grown and pod formation was complete, so that the general metabolism would be, presumably, on the down grade. The tables given later show how remarkably stable is the general equilibrium between the nitrogenous substances in the leaf during this period, the changes, even at death, being of quite a small order. There is no evidence of any great withdrawal of N from the leaves before death, nor is there any accumulation of asparagine. Miyachi [1896] assumed that asparagine accumulates in the aged leaves of *Paeonia albiflora* since old leaves from dying plants, if placed under water for 14 days, become rich in this substance. In the present author's opinion this experiment merely demonstrates the presence of active enzymes in the old leaves. (Compare Chibnall [1922, 2], where observations on dried leaves suspended in water are recorded.)

The methods of manipulation and analysis were those used for dried leaves, as described by Chibnall [1922, 2].

The results can be summarised under the following headings: (1) There is no great withdrawal of N from the leaves to the stems or roots when the plant becomes aged [*vide* Czapek, 1920]. (2) The protein- and proteose-free water-soluble N of series 12 shows a slight increase, due chiefly to ammonia (as ammonium salts or "amide N of asparagine") and monoamino N. From the author's experiments on the changes in the leaf N due to low-temperature drying [Chibnall, 1922, 2], it would appear that this increase has taken place during the period of dehydration of the leaf on the plant. Towards the latter end of the plant's life the nitric N has fallen, as one would expect from the ageing of the root system, otherwise the equilibrium between the water-soluble nitrogenous products has remained very steady, indicating a period of low metabolic activity in the leaf. (3) The proteins in the leaf have undergone slight amidisation, a change that must be ascribed to the ageing of the leaf, since it was not observed in the proteins of leaves dried at low temperatures. The ratio of protein to non-protein N in the leaf has suffered no appreciable change with age or death.

Table I. *Showing the percentage dry weight and total N in the leaves of series 9-12.*

Series number	Age above ground in weeks	% dry weight	% of N per fresh weight	% of N per dry weight
9	11	15.51	0.675	4.35
10	14	16.31	0.724	4.44
11	20	16.13	0.622	3.86
12	24	91.6	—	3.60

Table II. *Showing the amounts of protein and non-protein N in the dead leaves (series 12).*

(In percentages of total leaf N.)

Colloidal protein extracted ...	...	...	29.22
Protein extracted by 1 % HCl ...	...	...	29.49
N in residue (protein?) ...	...	...	6.62
*Protein coagulated by ammonium sulphate ...	...	...	0.77
Total protein N ...	...	...	66.10
Proteose ...	...	...	6.28
Proteose and protein-free water-soluble N ...	...	...	22.10
Soluble in alcohol and ether ...	...	...	2.15
Total N accounted for ...	...	...	96.63

* In estimating proteoses by saturation with ammonium sulphate, a small amount of N, probably due to traces of coagulated protein, is not resolvable in water.

Table III. *Showing the water-soluble N in series 9-12.*

(In percentages of total leaf N.)

Series number	Total water-soluble N	Proteose N	Coagulated protein N*	Protein- and proteose-free N
9	21.57	4.35	—	17.22
10	22.75	4.57	—	18.16
11	22.41	4.38	—	18.03
12	29.15	6.28	0.77	22.10

* As in footnote for Table II.

Table IV. *Showing the distribution of the water-soluble N in series 9-12.*

Series number	Total protein- and protease-free N	(In percentages of total leaf N.)						
		Ammonia N	Amide N of asparagine	Nitric N	Humin N	Basic N	Mono-amino N	Other N
9	17.22	0.44	0.53	2.73	0.99	2.00	7.76	2.77
10	18.16	0.37	0.47	3.53	1.06	2.40	6.81	3.62
11	18.03	0.61	0.92	1.28	0.96	2.59	6.61	5.06
12	22.10	0.97	1.94	1.61	1.32	2.22	7.74	6.30

Table V. *Percentage distribution of N in the colloidal proteins of series 9-12.*

Series number	Amide N	Humin N	Basic N	Monoamino N	Other N
9	6.34	3.64	22.00	58.88	9.14
10	6.50	3.82	21.78	59.22	8.68
11	6.40	3.82	21.32	58.50	9.96
12	7.21	6.12	19.27	56.22	11.32
12*	8.81	6.67	19.27	51.90	13.35

* HCl extract of series 12.

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