The Effect of Fertilizers on the Levels of Nitrogen, Phosphorus, Protease, and Pectase in Healthy Tobacco Leaves

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Boiled or dried leaves have generally been used in obtaining the data already in the literature on the effect of fertilizer treatment on nitrogen and phosphorus levels in tobacco leaves (Vickery, Pucher, Wakeman & Leavenworth, 1940; Ward, 1942). In addition, much work has been done on cured material.

In the work reported in this paper methods of fractionation were such that sap and fibre fractions were obtained from fresh material. Nitrogen and phosphorus determinations were made on all fractions, and, in addition, the levels of protease and pectase were determined on some.

METHODS

Growing of the plants. Healthy tobacco (*Nicotiana tabacum* var. White Burley) was grown in pots in a heated glasshouse by the Plant Pathology Department. Potting material (1 kg./pot) was made up of 50% soil, 25% sand and 25% peat. Analyses of the mixture indicated that it was relatively deficient in N and P, but not lacking in K. Supplements of fertilizer, when added either singly or in combination, were at the rate of 2·8 g. $(NH_4)_2SO_4$, 0·75 g. $Ca(H_2PO_4)_2$ and 1·1 g. K_2SO_4/pot. The fertilizer was mixed with the soil when the plants were potted. In each experiment eight groups of five pots were used. The fertilizer treatment in the eight groups was: nil; N; P; N and P; K; N and K; P and K; N, P and K. Table 1 gives the cultural history of the plants and the method of sampling employed.

Harvesting of the plants. Usually the plants of half the fertilizer groups were harvested 3 days later than the others (the former having been used as controls for similar fertilizer treatments with virus-infected plants to be described in a subsequent paper). Plants were cut at soil level, adhering soil brushed off, and each plant was weighed to the nearest 0·1 g. In some experiments all the leaves were removed (Table 1), while in the others five leaves of corresponding position and age were removed from each plant. Finally the five lots of leaves in the group were pooled and weighed.

Fractionation. Within 1 hr. of harvesting the samples from each treatment were minced in a domestic meat mincer and the sap expressed by hand through madapollam. The residue after hand squeezing was reminced and again squeezed. The liquid obtained was termed 'crude sap' and the residue of fibrous material 'unwashed fibre'. Washings from the mincer were added to the unwashed fibre, and more water added to bring the total to about five times the volume of crude sap. This fibre suspension was squeezed through the cloth, and the fluid obtained called 'washings'. Washings and crude sap were kept separate, the volumes taken, and the fibre (washed fibre) weighed.

Crude sap was spun on a centrifuge for 15 min. at 3500 r.p.m. ($1500 \times g$), and the supernatant fluid decanted from the deposit of cell debris, starch and chloroplastic material. The supernatant fluid was called 'sap' and the deposit 'sap sediment'.

Analyses. (1) *Dry matter.* Measured portions of each fraction were dried overnight at 95–100° and weighed.

(2) *Nitrogen.* Total N was determined by a micro-Kjeldahl method using SeO_2-$CuSO_4$-K_2SO_4 (1 : 1 : 8) catalyst. Non-protein N was determined by analysis of the supernatant fluid obtained after the addition of an equal volume of 10% trichloroacetic acid. Non-protein N estimations were made on the washings in the first experiment, but not subsequently, as the results obtained showed that the washings were equivalent to diluted sap. For the purposes of calculation it was assumed that errors introduced by treating sap sediment and fibre N as protein N would not be large (Granick, 1938).

Table 1. *Cultural history of the tobacco plants*

Date potted	Date harvested	Sunshine (hr.)	Average weight of whole plant with full fertilizer supplement (g.)	Method* of sampling (leaves taken)
(1) 11. x. 46	30. xii. 46–2. i. 47	158–167	34·1	5
(2) 31. x. 45	21. ii. 46–26. ii. 46	196–222	92·0	5
(3) 4. ii. 46	26. iv. 46–2. v. 46	187–195	105·2	5
(4) 21. v. 46	11. vii. 46–16. vii. 46	290–333	144·5	All
(5) 31. vii. 46	23. ix. 46–26. ix. 46	228–238	88·0	All

* Five largest leaves on 17. xii. 46, 11. ii. 46 and 16. iv. 46, respectively in (1), (2) and (3) were marked, and harvested on the dates shown in the table.

(3) *Phosphorus* was determined by a modification of the method of Kuttner & Lichtenstein (1932).

(4) *Pectase* was determined on sap and on washed fibre (Na_2HPO_4 extract, pH 8) by the method previously described (Holden, 1946).

(5) *Protease* was determined on sap and washed fibre by the method previously described (Tracey, 1948).

Analysis of data. The data obtained were analyzed statistically by the methods described by Yates (1937). The results of this analysis are given in the form of main effects of N, P and K, and the interaction of N and P, other interactions being negligible. The treatment effect is one quarter of the difference between the sum of the values of the four sets receiving a treatment and the sum of the values of the four not receiving it, e.g. N effect = ¼(N + N and P + N and K + N, P and K) − ¼(0 + P + P and K + K). Thus if half the treatment effect is added or subtracted, according to sign, to or from the mean of all the treatments, the value for the leaves with that supplement is obtained. The standard errors given are for the treatment effects. Treatment effects greater than twice their standard error are regarded as significant. Extreme ranges and mean values of all treatments combined, and the means for no treatment group and N, P and K group separately, are tabulated. In all, 200 plants were harvested giving 40 results for groups of five plants. Figures for the two methods of sampling have not been separated. This affects the values quoted for total dry matter, N and P, but does not alter the significance of the fertilizer effect on these values.

RESULTS

Dry matter, nitrogen and phosphorus. The results obtained are given in Tables 2–4.

Protease. The results are given in Table 5. For the purpose of calculating the total protease of the leaves, it was assumed that the activity/g. protein N of the washings was the same as that of the sap, and that the sap sediment fraction had no activity. The figures for total protease/g. total protein N indicate

Table 2. *Variation in dry-matter content of tobacco plants*

	Extreme range	Mean of all treatments	Mean of untreated group	Mean of N, P and K group	Fertilizer effects: N	P	K	N and P	Standard error
Wet weight of whole plants (g.)	35·2–722·4	204·49	99·8	445·4	+98	+185	+35	+84	±21·9
Dry matter (% of wet weight of leaves)	7·1–16·8	11·9	11·7	12·2	−0·9	+ 1·3	+0·1	+0·2	± 0·33
Total dry matter (g.)	1·80–64·50	17·22	10·12	31·85	+6·37	+12·85	+1·22	+6·06	± 2·21
Dry matter (mg./ml. sap)	31·2–71·1	50·3	50·3	54·1	+3·0	+ 0·05	−0·6	+3·5	± 1·3
Fibre dry matter (% total dry matter)	42–65	51·2	49·6	51·4	−2·9	+ 4·8	+0·5	−0·2	± 1·32

Table 3. *Variation in nitrogen content of tobacco plants*

	Extreme range	Mean of all treatments	Mean of untreated group	Mean of N, P and K group	Fertilizer effects: N	P	K	N and P	Standard error
Total N (% of dry matter of leaves)	1·03–6·89	3·25	2·85	3·22	+1·90	− 0·94	−0·35	− 0·22	± 0·22
Total N (mg.)	75–2048	475	244·6	821·6	+425	+203	−45	+165	±66
N (mg./ml. sap)	0·94–4·52	2·20	1·86	2·23	+1·42	− 0·82	−0·22	− 0·34	± 0·51
Protein N (mg./ml. sap)	0·47–2·62	1·20	1·04	1·56	+0·63	− 0·08	−0·03	+ 0·16	± 0·16
Sap N (% of sap dry matter)	1·84–9·23	4·41	3·61	4·29	+2·72	− 1·60	−0·31	− 0·88	± 0·24
'Sap sediment' N (% of non-fibre N)	9·9–30·3	17·9	17·0	15·2	−3·0	+ 0·3	−0·5	+ 1·1*	± 1·23
Sap protein N (% of sap N)	33–82	57·2	57·6	69·2	−4·6	+11·8	+2·3	+11·1	± 1·95
Fibre N (% of total N)	29–59	42·1	42·4	45·0	−3·8	+ 9·2	+0·9	+ 3·3	± 1·55
Fibre N (% of fibre dry matter)	0·74–5·65	2·65	2·41	2·77	+1·35	− 0·37	−0·33	+ 0·17	± 0·19

* Appears to be a negative P and K effect of 2·9.

Table 4. *Variation in phosphorus content of tobacco plants*

	Extreme range	Mean of all treatments	Mean of untreated group	Mean of N, P and K group	Fertilizer effects N	P	K	N and P	Standard error
Total P (% dry matter)	0·09–0·52	0·245	0·14	0·34	+ 0·015	+ 0·204	− 0·022	+ 0·060	±0·018
Total P (mg.)	2·5–187·2	42·9	14·1	89·1	+18·8	+55·3	− 1·8	+18·0	±8·2
Sap P (% sap dry matter)	0·09–1·09	0·331	0·14	0·47	− 0·046	+ 0·361	− 0·045	− 0·049	±0·041
Fibre P (% total P)	26–58	42·1	48	39	+ 3·0	− 6·5	− 3·6	+ 4·4	±1·95
Fibre P (% fibre dry matter)	0·08–0·40	0·189	0·14	0·23	+ 0·039	+ 0·112	+0·043	+ 0·026	±0·0124

Table 5. *Variation in protease content of tobacco plants*

	Extreme range	Mean of all treatments	Mean of untreated group	Mean of N, P and K group	Fertilizer effects N	P	K	N and P	Standard error
Total protease (units/g. dry matter)	0·6–8·9	3·63	2·90	4·46	−0·30	+1·09	+0·36	+0·28	± 0·30
Protease (units/g. total protein N)	20–428	157	126·8	172	−86	+71	+25	+45	±18·9
Protease (units/ml. sap)	0·03–0·63	0·30	0·23	0·37	−0·097	+0·093	+0·059	−0·013	± 0·031
Protease (units/g. protein N in sap)	25–1040	310	209	255	−261	+124	+86	−125	±51
Protease (% in sap)	24–86	59	57·4	56·6	−8·4	−3·0	+3·1	−5·5	± 3·7
Protease (units/g. dry fibre)	0·8–12·4	3·00	2·34	4·18	+0·74	+1·12	−0·05	+0·72	± 0·42
Protease (units/g. fibre N)	22–262	117·5	98·2	145·8	−38	+57	+15	+0·9	±11·7

Table 6. *Variation in pectase content of tobacco plants*

	Extreme range	Mean of all treatments	Mean of untreated group	Mean of N, P and K group	Fertilizer effects N	P	K	N and P	Standard error
Fibre pectase (units/g. dry matter)	0·066–1·31	0·35	0·40	0·23	+0·17	−0·35	−0·01	−0·07	±0·06
Fibre pectase (units/g. N)	4·4–33·4	12·6	17·5	8·4	−1·1	−8·2	+1·3	+0·8	±1·7

the relative richness of the leaves in protease, and show that if all the leaf protein were hydrolyzed at the same rate as gelatin it would be completely destroyed by the leaf protease in 2–10 days (depending on the fertilizer treatment) at 40°.

Pectase. The effect of fertilizer treatment on the pectase content of tobacco leaves was mentioned briefly in a previous paper (Holden, 1946). In the present series of observations pectase was determined in sap and fibre. The results (Table 6) given do not include those for sap, because in every instance the values were low, and not more than 10% of the total pectase was present in the sap. Thus the earlier observation that the application of N and P together increased the percentage of sap-soluble enzyme was not confirmed. The fibre pectase is expressed as units/g. dry matter and units/g. N of fibre (Holden, 1946). The results of one experiment are not included as several values were missing.

DISCUSSION

The effects found are summarized in Table 7. In spite of seasonal variations in the size and composition of the plants it was found that significant fertilizer effects were obtained from the pooled data of all experiments. While the seasonal effects are of undoubted importance it was felt that the results obtained were not sufficient for discussion in this paper.

Table 7. *Summary of effects of fertilizers on tobacco plants*

Character	Change
Wet weight Total dry matter Total N Total P Fibre P as % fibre dry matter	Increased by both N and P
Dry matter as % wet weight Fibre dry matter as % total dry matter Fibre N as % of total N Protein N as % of total sap N Protease/g. protein N of sap Protease/ml. sap Protease/g. total protein N Protease/g. fibre N	Decreased by N Increased by P
Total N as % dry matter Sap N as % dry matter of sap Pectase/g. dry matter of fibre	Increased by N Decreased by P
Sap dry matter/ml. Fibre N as % dry matter of fibre Total N/ml. sap Protein N/ml. sap	Increased by N
Total P as % dry matter Sap P as % dry matter of sap Total protease/g. dry matter Protease/g. dry matter of fibre	Increased by P
Pectase/g. N of fibre Fibre as % total P	Decreased by P
'Sap sediment' N as % non-fibre N Sap protease as % total protease	Decreased by N

The obvious effects of both N and P supplements in making the plant grow to a greater total size explain their joint action in increasing wet weight, total N, and total P. In respect of many effects, N and P appear to act in opposition. This will be seen by inspection of the second section of Table 7 to be due to P increasing the proportion of fibre, mainly carbohydrate in nature, while N increases the proportion of protein and other nitrogenous compounds. The proportion of the total N that is soluble is increased by N as fertilizer, and similarly P increases the proportion of total P that is soluble. These results might be expected. The effects of N and P on enzyme levels are, however, of more interest. The increase of protein due to N seems to be coupled with a decrease in the enzyme responsible for its breakdown, while the increase of 'fibre' (presumably involving an increase in pectin) due to P is coupled with a decrease in the enzyme responsible for beginning the breakdown of part of the fibre. Unfortunately, the rigid interpretation of the results of experiments such as these is difficult, since the two fertilizers N and P affect the levels of all quantities measured. Consequently there is no constituent to which changes can be referred. Total dry matter and total protein N have been used in the calculation of protease levels. As the total dry matter may contain from 6 to 40% of its weight as nitrogenous compounds (if the conventional factor of 5·8 is used for conversion) and the protein level is obviously influenced by N as fertilizer, comparison of effects is difficult. The difficulty may be put in another way: both protein and carbohydrate may be laid down in excess of the normal requirements (whatever these are), and there is no way of determining the size of these 'stores' or, alternatively, of deciding whether too little of these compounds is present for 'health'. The amount of a substance present in a plant may be regarded as a reflexion of the balance between its synthesis and breakdown. The changes in enzyme levels described are compatible with the view that these enzymes, which have a destructive action *in vitro*, are not capable of synthesis *in vivo*, by reversal of their action, and that other synthetic systems are involved.

No significant effects of potassium were observed, presumably because the K content of the soil was such that plants without K supplement were not deficient.

SUMMARY

1. The effect of supplements of nitrogen and phosphorus on the nitrogen and phosphorus content of leaf fractions of healthy pot-grown tobacco has been determined. Experiments were carried out in five sets, each including eight different fertilizer treatments on groups of five plants.

2. The effect of nitrogen and phosphorus on the levels of two enzymes, pectase and protease, was also determined on the same material.

3. Phosphorus increases the non-protein, or carbohydrate, components of the leaf, while nitrogen increases the protein components.

4. Nitrogen increases pectase levels, while phosphorus decreases them. The reverse is true for protease. It is suggested that this may indicate the presence of different paths for the synthesis and breakdown of both pectin and protein.

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The Effect of Infection with Tobacco-Mosaic Virus on the Levels of Nitrogen, Phosphorus, Protease, and Pectase in Tobacco Leaves and on their Response to Fertilizers

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The effect of fertilizers on the levels of nitrogen, phosphorus, protease and pectase in healthy tobacco leaves has been described in a previous paper (Holden & Tracey, 1948). The plants used in getting the data given in this paper were a precisely similar series, but were infected with tobacco-mosaic virus.

Bawden (1943) and Wynd (1943) have reviewed the effects of virus infection on the metabolism of plants, and references to earlier work on tobacco infected with tobacco-mosaic virus can be found in their papers. The analyses described were made on tobacco plants supplied by Mr F. C. Bawden and Mr B. Kassanis, who were studying the effects of various fertilizer treatments on the susceptibility to infection and the multiplication of tobacco-mosaic virus. The data presented are restricted to a comparison of the nitrogen, phosphorus, protease and pectase levels in infected and healthy plants with different fertilizer treatments and the response to these treatments.

METHODS

Plants used. Tobacco (*Nicotiana tabacum* var. White Burley) plants were grown in pots in a heated glasshouse. The fertilizer treatments and levels were as described in the previous paper (Holden & Tracey, 1948).

Infection of the plants with tobacco-mosaic virus. In three experiments the plants were infected by rubbing five leaves with a virus preparation. These plants were harvested after 10–14 days, by which time only local virus multiplication had occurred. In the other two experiments the plants were infected when much younger, and grown for a period sufficient for the virus to spread systemically throughout the plant. The cultural history of the plants is given in Table 1.

Fractionation and analyses. The preparation of leaf fractions and their analysis has been described in the previous paper. pH determinations were made on spun sap using a glass electrode. Estimations of virus concentration were made on spun sap and washed fibre by Bawden & Kassanis (unpublished).

Analysis of data. The initial stages of the statistical analysis of the data were as described previously, except that the results from local and systemic infection were kept separate. The results for healthy controls were also analyzed, after these had been separated into groups corresponding to controls for local and systemic infection to eliminate seasonal differences. Standard errors for the means, and for the differences between means of healthy and infected plants, were calculated for both groups (systemic and local with their corresponding controls). A pooled standard error for the difference between fertilizer effects is given, as the individual standard errors were very similar.