NUTRIENT INTERACTIONS AND DEFICIENCY DIAGNOSIS IN THE LETTUCE

III. NITROGEN CONTENT AND RESPONSE TO NITROGEN

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Summary

Lettuce plants grown in sand culture, and receiving nitrogen, phosphorus, and potassium at five levels in all combinations, were analysed at different stages of growth for total, soluble, and nitrate nitrogen. An attempt was made to relate these analytical data to the subsequent response (in dry matter production) shown by the plants when a further amount of nitrogen was supplied.

The percentage of total nitrogen in the plant dry matter was generally increased by increasing levels of nitrogen or phosphorus supply, and by potassium deficiency. The increase in nitrogen content with phosphorus supply depended on the level of the other nutrients, being most marked where nitrogen supply was high, or potassium low. Changes in content of nitrate nitrogen were in general similar to those for total nitrogen, but much more marked.

The increase in dry weight of the plants as a result of supplementary nitrogen, supplied at 46 days from sowing, was closely related to the nitrogen and phosphorus content of the plants at earlier stages, not at all to their potassium content. Where total nitrogen content was used, the best forecast of response was obtained by sampling immediately before the supplementary nitrogen was applied; where nitrate analyses were used, the optimum time for sampling was two weeks earlier. In each case, data for phosphorus content provided valuable additional information as to the response potentialities of the plants. Determination of insoluble, or of total soluble nitrogen gave no advantage over total or nitrate nitrogen respectively. No clear preference could be expressed between older and younger leaves as sources of material for analysis, and nitrate determinations on midribs were disappointing. The relationship of response to the content of nitrogen and phosphorus could be fitted better by a curved than a plane surface.

I. INTRODUCTION

The principal purpose of the investigation described in the present series of papers was to study the relationship between the growth responses to nutrients applied to half-grown lettuce plants and the composition of the same plants at the time of application or earlier. Lettuce plants differing widely in nutritional status were obtained by sand-culture methods; five levels each of nitrogen, phosphorus, and potassium supply in all combinations were used. Samples of plants from some or all treatments were taken for analysis at 11, 22, 29, 37, and 44 days from sowing. At 46 days, additional amounts of nitrogen, phosphorus, or potassium were supplied each to one-quarter of the plants, while the rest received no further treatment. All plants were finally harvested at 98 days.

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Apart from the main purpose of the investigation, the experiment also provided abundant information on the effects of nutritional interactions on the growth and composition of the plants. The first two papers (Goodall, Grant Lipp, and Slater 1955; Goodall, Slater, and Grant Lipp 1957) thus dealt with the results in respect of dry matter and water content. The present paper presents the data for nitrogen content, and will then proceed to discuss the relationship between the responses to the nitrogen application at 46 days and the previous composition of the plants.

II. Materials and Methods

Details of the sand-culture and harvesting procedure have been given in the first paper of this series (Goodall, Grant Lipp, and Slater 1955).

For all the chemical analyses, the dry material was finely ground in a mortar, and portions of the powder were dried again at 100°C before analysis. The small amount of material available from many plants made it necessary to use semi-micro methods of analysis, and also prevented the determination of various nitrogen fractions which might have been of interest. Besides the total nitrogen content, it was possible in most samples also to determine the insoluble, soluble, and nitrate nitrogen. The analytical data are expressed as percentages of dry matter, except for nitrate nitrogen, which is given as parts per million.

Total nitrogen was determined by a form of the Kjeldahl method, described in detail by Slater (1952). The method was checked by frequent analysis of acetanilide: a consistent accuracy of 98–101 per cent. recovery of nitrogen was found, with duplicate determinations agreeing within 1 per cent.

Soluble nitrogen was extracted by shaking with water for 30 min—a procedure which gave similar values for insoluble nitrogen to those obtained by extracting with trichloroacetic acid, and did not (like the latter) cause loss of nitrate. Nitrate and total soluble nitrogen were both determined in the aqueous extract, the former by the phenoldisulphonic acid method (Ulrich 1948), the latter by the Kjeldahl method; details of procedure are given by Slater (1952).

Total nitrogen was determined on plants from 27 treatments in the 11- and 22-day harvests, the levels 2 and 4 of nitrogen, phosphorus, and potassium being omitted (see Goodall, Grant Lipp, and Slater 1955, p. 305). In the 29-, 37-, and 44-day harvests the analytical labour was reduced by omitting those treatment levels which seemed least likely to yield useful information (viz. N_4 and K_4). Replicate samples of plant material were combined for analysis. All available plants from the 98-day harvest were analysed, but so many plants receiving phosphorus levels P_1 and P_2 died before reaching this stage that it was necessary to omit these levels completely from the analysis of variance.

It will be recalled that, in the larger plants, the aerial parts harvested had been separated into younger and older leaves, and the latter further divided into midribs and laminas. These groups of organs were analysed separately. The data were too incomplete to provide a satisfactory basis for assessing statistically the effects of the treatments and their interactions, but were studied as a possible basis for estimating response.

For reasons explained before (Goodall, Grant Lipp, and Slater 1955) certain plants died before harvest. In a few other instances the amount of material was insufficient for chemical analysis. In all these cases "missing plot" values had to be fitted before proceeding to analyses of variance. The methods used are described by Slater (1952). One degree of freedom was subtracted for each fitted value in the analyses of variance.

III. Results

Table 1 presents analyses of variance for the per cent. total nitrogen in the plants at each harvest, including those harvested at 98 days and receiving no sub-treatment. The analyses of the nitrate data for earlier harvests are also presented

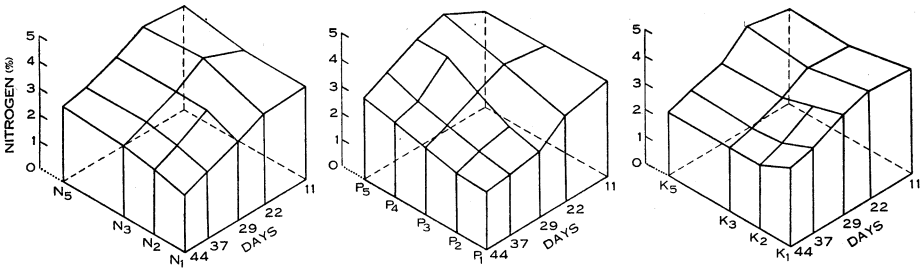


Fig. 1 Fig. 2 Fig. 3

Figs. 1–3.—Effect of nitrogen (Fig. 1), phosphorus (Fig. 2), and potassium (Fig. 3) supply on total nitrogen content at different stages of development.

in this table; nitrate analyses were not performed on the 98-day harvest. Table 2 gives analyses of variance for the effects of the three sub-treatments on nitrogen content at 98 days. Similar tables are not given for the effects of initial treatments and sub-treatments on soluble and insoluble nitrogen content, because their variation closely paralleled that in nitrate and total nitrogen respectively. The mean values of nitrogen and nitrate content corresponding with effects shown to be significant in the analyses of variance are presented in Tables 3–8, and, in appropriate cases, solid diagrams have been prepared (Figs. 1–10). Fuller data are available in the original thesis (Slater 1952) on which this paper is based.

(a) *Nitrogen Content as Affected by Initial Treatments*

(i) *Total Nitrogen*.—The total nitrogen content was highest in the young plants, and fell progressively as they grew (see Figs. 1–3). The data quoted for 11 days refer to the whole plants, and the presence of the roots may have been responsible for the fact that the nitrogen content at this time was lower than for the harvest at 22 days. As the solid diagrams show, the decline in nitrogen content with age was largely independent of treatment.

The nitrogen content of the plants at all stages of development increased with initial nitrogen supply (Fig. 1). But such effects were recorded for the higher levels of nitrogen supply only at the later harvests (if one excepts the anomalous results at 11 days). At 29 days, the maximum nitrogen content was already reached in the N_2 treatments, and at 37 days in the N_3 treatments; only from 44 days did the increase continue up to N_5. Since yield reached a maximum at N_3, these results represent marked "luxury consumption" at N_5. Phosphorus supply had the effect of increasing nitrogen content throughout the range tested, from the second harvest onwards (Fig. 2); the decrease in nitrogen content with time was more marked at higher than at lower levels of phosphorus supply. Potassium, unlike phosphorus, had the effect of decreasing nitrogen content (Fig. 3); this was, however, true only of the lowest potassium supply given (K_2)—higher levels of supply had no further effect.

Of the interactions which were significant when compared with the second-order interaction, that between nitrogen and phosphorus was the most consistent, being evident at each harvest (Fig. 4). The phosphorus–potassium interaction (Fig. 5) was significant only at 29, 37, and 44 days, and the nitrogen–potassium interaction not at all.

The general effect of the interactions between nitrogen and phosphorus supply (Fig. 4) was that the increase in nitrogen content with increased supply was much

TABLE 1

NITROGEN CONTENT AS AFFECTED BY INITIAL TREATMENTS: ANALYSES OF VARIANCE

Days from Sowing	Source of Variation	Degrees of Freedom	Mean Square	
			Total Nitrogen (% of dry matter)	Nitrate Nitrogen (p.p.m. of dry matter)†
11	N	2	0·799**	0·9200*
	P	2	0·003	0·0031
	K	2	0·596**	0·0013
	N×P	4	0·524**	0·0300
	N×K	4	0·077	0·0136
	P×K	4	0·135	0·0615
	Error	7	0·054	0·1031
22	N	2	1·571***	1·6922***
	P	2	3·059***	0·3342***
	K	2	0·375*	0·0392*
	N×P	4	0·350*	0·0762**
	N×K	4	0·061	0·0219
	P×K	4	0·137	0·0164
	Error	8	0·066	0·0073
29	N	3	1·534***	2·3917***
	P	4	8·711***	0·9052***
	K	3	1·480***	0·3276***
	N×P	12	0·297**	0·0956*
	N×K	9	0·073	0·0342
	P×K	12	0·251**	0·0602
	Error	36	0·075	0·0365

TABLE 1 (*Continued*)

Days from Sowing	Source of Variation	Degrees of Freedom	Mean Square	
			Total Nitrogen (% of dry matter)	Nitrate Nitrogen (p.p.m. of dry matter)†
37	N	3	1·144***	1·6076***
	P	4	3·543***	0·1791
	K	3	1·225***	0·4298**
	N×P	12	0·399**	0·1071
	N×K	9	0·136	0·0611
	P×K	12	0·348*	0·2159**
	Error	36	0·139	0·0709
44	N	3	2·058***	2·6257***
	P	4	2·097***	0·2101**
	K	3	1·903***	0·3633***
	N×P	12	0·484**	0·0777
	N×K	9	0·173	0·0468
	P×K	12	0·295*	0·0709
	Error	36	0·121	0·0467
98	N	4	18·991***	n.d.
	P	2	1·974**	
	K	4	1·935***	
	N×P	8	0·660*	
	N×K	16	0·266	
	P×K	8	0·115	
	Error	28	0·249	

* P=0·01–0·05. **P=0·001–0·01. ***P<0·001.

† Logarithmic transformation.

more evident at high than at low levels of phosphorus—though even at P_1 the effect was recognizable. This applied to all harvests except the first, where the aberrant figure for N_3P_1 was responsible for the significant interaction. Consequently the increase in nitrogen content with increasing phosphorus supply became more marked at higher nitrogen levels.

The interactions between phosphorus and potassium supply are illustrated in Figure 5. The principal feature was that the increase in nitrogen content with increasing phosphorus supply was more marked at the lowest potassium levels than elsewhere, i.e. the decline in nitrogen content with potassium supply was hardly evident at low phosphorus supply, but became clearer as the phosphorus supply increased.

(ii) *Nitrate Nitrogen*.—The general picture for nitrate nitrogen was similar to that for total nitrogen, but there were several differences. The most striking was the far wider range of values, the lowest nitrate contents being about 150 p.p.m.

and the highest 15,000 p.p.m.; this wide range made a logarithmic transformation necessary before analysis of variance. The overall mean nitrate content fell sharply from 22 days to 37 days, but not thereafter (Figs. 6–8). The mean content at 11 days was less than half that at 22 days—a difference which can hardly be attributed to

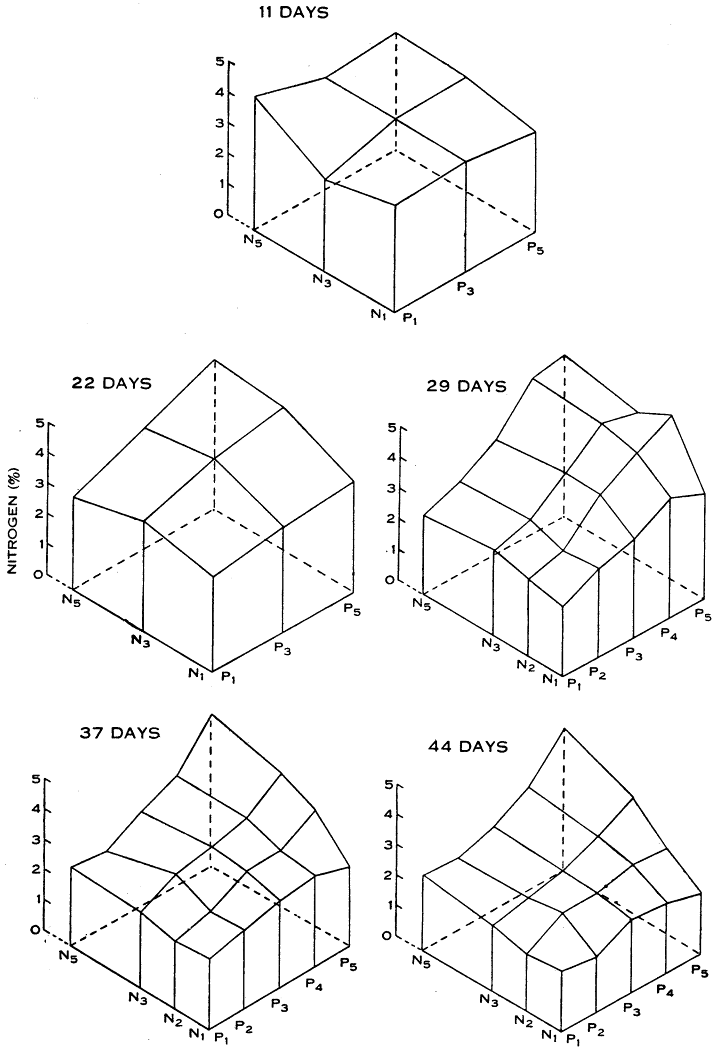


Fig. 4.—Interaction effects of nitrogen and phosphorus supply on total nitrogen content.

the presence of the roots in the former, but rather to the fact that, as shown below, uptake from the culture medium had barely begun at that stage.

The initial supply of nitrogen was consistently reflected in the nitrate content of the plants (Fig. 6), at all levels and at all stages of development. Increases in phosphorus supply, too, consistently caused increases in nitrate content from 22 days onwards (Fig. 7). Potassium supply decreased nitrate content (Fig. 8) as with total nitrogen, but no noticeable differences occurred among the various levels (K_2–K_5) of potassium supply.

As with total nitrogen, only the N×P and P×K interactions were significant. The interaction between nitrogen and phosphorus, though it reached significance

TABLE 2

TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AT 98 DAYS AS AFFECTED BY SUB-TREATMENTS: ANALYSIS OF VARIANCE

	Initial Treatments	Degrees of Freedom	Mean Square
Nitrogen sub-treatment effect		1	3·582***
Interaction with	N	4	1·361***
	P	2	0·048
	K	4	0·347*
	N×P	8	0·200
	N×K	16	0·286***
	P×K	8	0·395***
Phosphorus sub-treatment effect		1	0·019
Interaction with	N	4	0·726***
	P	2	0·643**
	K	4	0·223
	N×P	8	0·157
	N×K	16	0·197*
	P×K	8	0·462***
Potassium sub-treatment effect		1	1·653***
Interaction with	N	4	0·048
	P	2	1·628***
	K	4	1·472***
	N×P	8	0·356***
	N×K	16	0·305***
	P×K	8	0·371***
Error		276	0·104

$*P=0·01–0·05$. $**P=0·001–0·01$. $***P<0·001$.

only in the plants harvested at 22 and 29 days, showed a similar trend at 37 and 44 days too (Fig. 9). The increase in nitrate content with increasing phosphorus supply was much more marked at higher than at lower levels of nitrogen supply—indeed, at N_1 it was negligible.

As for the interaction between phosphorus and potassium (Fig. 10), this too was similar at all harvests after 11 days, though significant only at 37 days. It consisted in the fact that the decrease in nitrate content with potassium supply mentioned above was marked only at the higher levels of phosphorus supply.

(b) *Effects of Sub-treatments on Nitrogen Content*

Since nitrate analyses were not performed on the 98-day harvest, the effects of sub-treatments could only be studied on the total nitrogen content; as already mentioned, treatments including P_1 and P_2 were omitted from the statistical analysis because so many data for them were missing. The results of analysis of variance are presented in Table 2, the error term being obtained from the highest-order interaction for all sub-treatments taken together, whereas the interaction terms

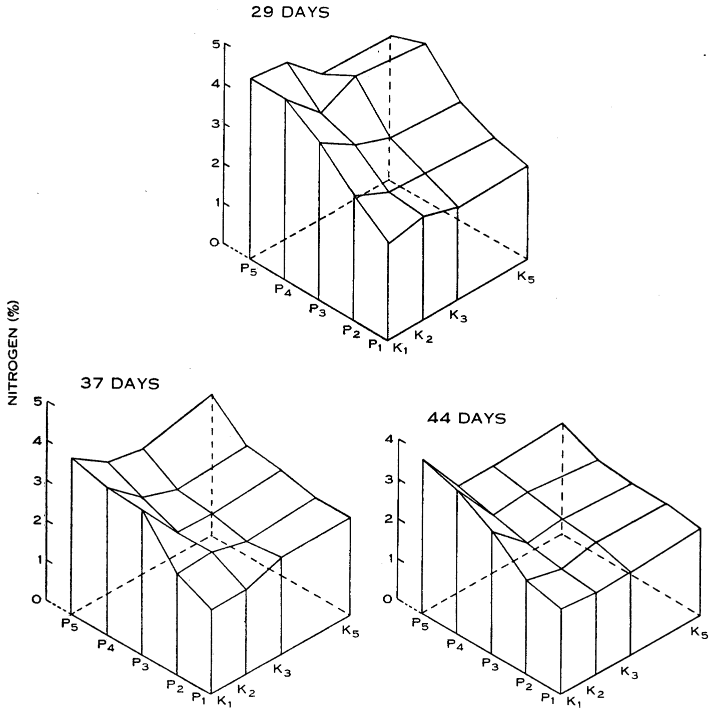


Fig. 5.—Interaction effects of phosphorus and potassium supply on total nitrogen content.

for each sub-treatment with the various initial treatments have been computed separately. The mean effects of the sub-treatments are shown in Tables 6–8, being expressed as the difference in total nitrogen content (per cent. of dry matter) between plants receiving the sub-treatment in question and plants receiving no additional nutrient supply.

(i) *Nitrogen Sub-treatment*.—The average effect of the nitrogen sub-treatment was to increase the nitrogen content of the plant by 0·31 per cent. This effect was more marked after low initial nitrogen supply, and negligible after high levels; it was unaffected by the initial supply of phosphorus (between the levels of P_3 and P_5). The initial supply of potassium affected the increase in nitrogen content as a result of supplementary nitrogen supply, in that this increase was substantial at low and high levels of initial potassium and smaller or negligible at intermediate levels; the large increase under conditions of low potassium supply might be explained as "luxury consumption" when growth was limited by the potassium level, but the differences between K_3, K_4, and K_5 are more difficult to account for.

TABLE 3

TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AS AFFECTED BY NITROGEN AND PHOSPHORUS SUPPLY

Days from Sowing	Nitrogen Levels	Phosphorus Levels				
		P_1	P_2	P_3	P_4	P_5
11	N_1	3·47	—	3·58	—	3·21
	N_3	3·00	—	3·68	—	3·66
	N_5	4·38	—	3·66	—	3·85
22	N_1	3·11	—	3·44	—	3·63
	N_3	3·58	—	4·32	—	4·68
	N_5	3·05	—	4·01	—	4·92
29	N_1	2·25	2·73	2·98	3·49	3·14
	N_2	2·44	2·65	3·53	4·07	4·55
	N_3	2·59	2·88	3·53	4·30	4·06
	N_5	2·48	2·79	3·33	4·37	4·59
37	N_1	2·21	2·44	2·69	2·79	2·44
	N_2	2·14	2·40	2·88	2·91	3·42
	N_3	2·34	2·78	2·98	3·19	3·82
	N_5	2·48	2·34	2·81	3·21	4·26
44	N_1	2·02	1·92	2·36	2·28	2·03
	N_2	1·94	2·48	2·52	2·71	2·59
	N_3	2·11	2·31	2·49	2·89	3·38
	N_5	2·39	2·30	2·59	3·08	4·04
98	N_1	—	—	1·97	1·92	1·55
	N_2	—	—	2·45	2·53	2·50
	N_3	—	—	2·66	3·30	3·32
	N_4	—	—	3·11	4·14	4·48
	N_5	—	—	4·66	4·71	5·10

Of the second-order interactions of nitrogen sub-treatment with initial treatments, that with initial nitrogen and phosphorus approaches significance, and consists in a tendency for the sub-treatment to decrease nitrogen content where initial nitrogen and phosphorus supply were high, while increasing it under other circumstances. The other second-order interactions are highly significant but rather obscure. The interaction with initial nitrogen and potassium seems to be attributable to the peculiar high positive value at N_5K_5; this resulted from a single very small plant with very high nitrogen content, and so may perhaps be ignored as fortuitous. In the interaction with initial potassium and phosphorus the most obvious element is the fact that the maximum effects of the sub-treatment on nitrogen

TABLE 4

TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AS AFFECTED BY PHOSPHORUS AND POTASSIUM SUPPLY

Days from Sowing	Potassium Levels	Phosphorus Levels				
		P_1	P_2	P_3	P_4	P_5
29	K_1	2·43	3·08	3·93	4·53	4·53
	K_2	2·59	2·71	3·41	3·69	4·44
	K_3	2·34	2·68	3·04	4·10	3·65
	K_5	2·39	2·57	3·00	3·92	3·61
37	K_1	2·12	2·57	3·62	3·71	3·95
	K_2	2·15	2·59	2·55	2·96	3·32
	K_3	2·47	2·35	2·55	2·67	3·20
	K_5	2·44	2·46	2·64	2·75	3·52
44	K_1	2·14	2·32	3·03	3·57	3·84
	K_2	2·05	2·11	2·27	2·48	2·69
	K_3	2·07	2·30	2·37	2·55	2·72
	K_5	2·20	2·29	2·28	2·37	2·78

content occurred when the supply of either potassium or phosphorus was high, while the other was low. This would suggest that the nitrogen content is most sensitive to changes in supply when growth is limited by lack of one other nutrient only.

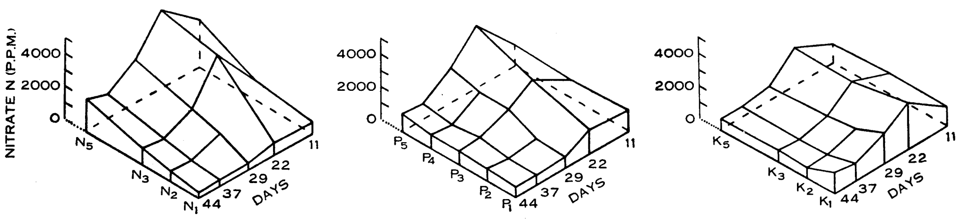


Fig. 6 Fig. 7 Fig. 8

Figs. 6–8.—Effect of nitrogen (Fig. 6), phosphorus (Fig. 7), and potassium (Fig. 8) supply on nitrate nitrogen content at different stages of development.

(ii) *Phosphorus Sub-treatment.*—The average effect of phosphorus sub-treatment on nitrogen content was negligible (—0·02 per cent.), but some of its interactions with the initial treatments were highly significant. At high initial nitrogen, phosphorus supplements increased the nitrogen content; high initial phosphorus, on the other hand (as might be expected), reduced the response of nitrogen content to added phosphorus.

The second-order interaction between phosphorus sub-treatment and nitrogen and potassium initial treatments was obscure and only just significant; it may probably be safely ignored. That with initial potassium and phosphorus supply was highly significant, and only in part accounted for by the aberrant value

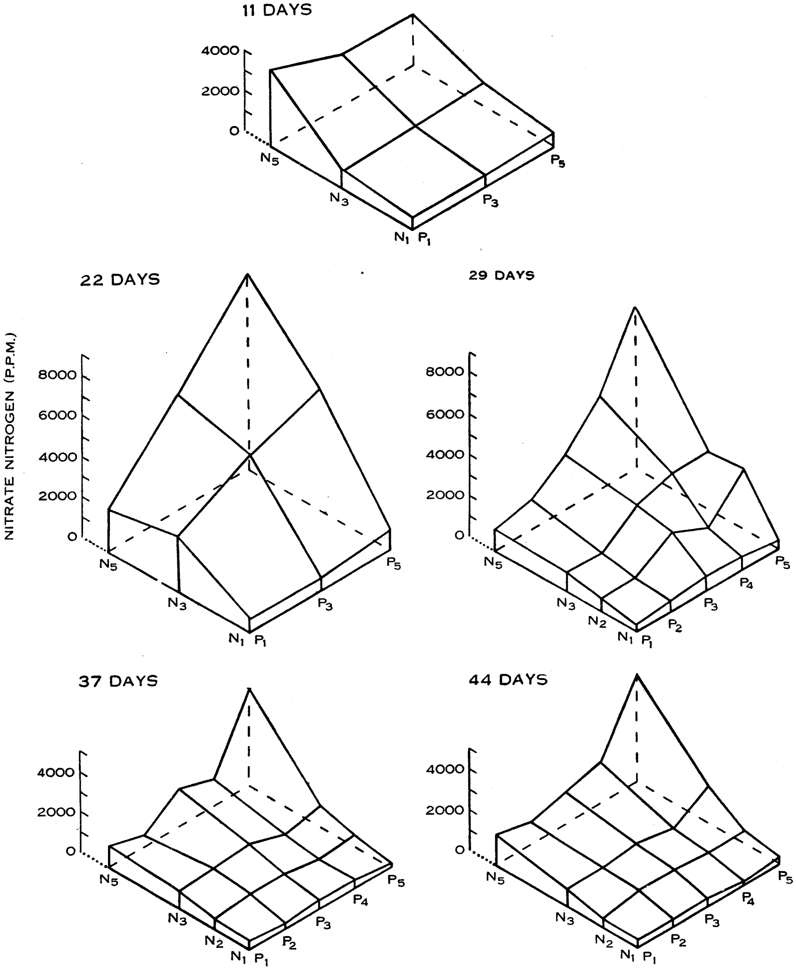


Fig. 9.—Interaction effects of nitrogen and phosphorus supply on nitrate nitrogen content.

for K_1P_3; it seems to have consisted in a reduction in the effect of initial phosphorus on the response (in nitrogen content) to phosphorus supplements at intermediate levels of potassium.

(iii) *Potassium Sub-treatment*.—The average effect of the potassium sub-treatment was to reduce the nitrogen content by 0·21 per cent. This was not dependent on initial nitrogen supply. Where potassium was supplied initially (K_2–K_5) the effect was negligible, but was very marked where the plants had been deprived of potassium; it was more noteworthy at higher than lower initial levels of phosphorus.

TABLE 5

NITRATE NITROGEN CONTENT (P.P.M. OF DRY MATTER) AS AFFECTED BY INITIAL NUTRIENT SUPPLY: GEOMETRIC MEANS

11 *Days from Sowing*

Mean Effects at Different Levels of Initial Treatments

N_1	668	P_1	1268	K_1	1156
N_3	1023	P_3	1183	K_3	1279
N_5	2802	P_5	1278	K_5	1238

22 *Days from Sowing*

Mean Effects at Different Levels of Initial Treatments

N_1	796	P_1	1592	K_1	2988
N_3	4172	P_3	2592	K_3	2243
N_5	4797	P_5	3861	K_5	2374

Interactions between Initial Treatments

	N_1	N_3	N_5
P_1	766	2624	2008
P_3	662	4642	5649
P_5	996	5957	9698

29 *Days from Sowing*

Mean Effects at Different Levels of Initial Treatments

N_1	413	P_1	648	K_1	1811
N_2	1201	P_2	721	K_2	1133
N_3	1676	P_3	1597	K_3	1065
		P_4	1659		
N_5	2697	P_5	2215	K_5	984

Interactions between Initial Treatments

	N_1	N_2	N_3	N_5
P_1	293	642	914	1022
P_2	349	678	806	1419
P_3	577	1803	2231	2797
P_4	551	1095	2814	4467
P_5	369	2907	2857	7852

TABLE 5 (*Continued*)

37 *Days from Sowing*

Mean Effects at Different Levels of Initial Treatments

N_1	331	P_1	574	K_1	1314
N_2	550	P_2	642	K_2	598
N_3	887	P_3	852	K_3	575
		P_4	647		
N_5	1526	P_5	970	K_5	604

Interactions between Initial Treatments

	K_1	K_2	K_3	K_5
P_1	577	474	653	609
P_2	734	900	492	523
P_3	2017	760	433	794
P_4	1615	603	497	363
P_5	2835	390	908	882

44 *Days from Sowing*

Mean Effects at Different Levels of Initial Treatments

N_1	298	P_1	574	K_1	1221
N_2	647	P_2	695	K_2	588
N_3	1075	P_3	818	K_3	719
		P_4	796		
N_5	2000	P_5	1176	K_5	750

Second-order interactions were also significant. The effect of initial phosphorus on the response to potassium supplementation (mentioned above) was most evident at the higher levels of nitrogen supply—i.e. at N_4 and N_5 the potassium sub-treatment increased nitrogen content at P_3 but decreased it at P_4 and P_5, whereas at lower levels of initial nitrogen supply the phosphorus supply had little effect. The decrease in nitrogen content as a result of the addition of potassium to plants short of this nutrient was particularly marked where the initial nitrogen supply had been abundant. The interaction of potassium sub-treatment with initial potassium and phosphorus supply is obscure and would seem to have little physiological meaning.

(c) *Nitrogen Uptake*

The analytical data for total nitrogen content and the mean dry weights were combined to determine the total quantity of nitrogen contained in the aerial parts of the plants at each harvest, or, at 11 days, in the whole plants. Only those treatments for which this information was available at each of the six harvests, viz. the levels 1, 3, and 5 of each nutrient, were analysed in this way.

At 11 days the mean amount of nitrogen in plants receiving the N_1 and N_3 treatments was 0·06 mg, the same as that in a single seed, but under the N_5 treatments there had been a slight uptake to give 0·08 mg per plant. Up to 44 days the most rapid uptake occurred in the N_5 treatments, provided phosphorus and potassium levels were favourable. The mean rate of uptake in the N_3 treatments was only a little lower, and increased relatively to that in N_5 during this period; at 98 days all but one of the plants receiving N_3 treatments contained more nitrogen than the corresponding N_5 plants.

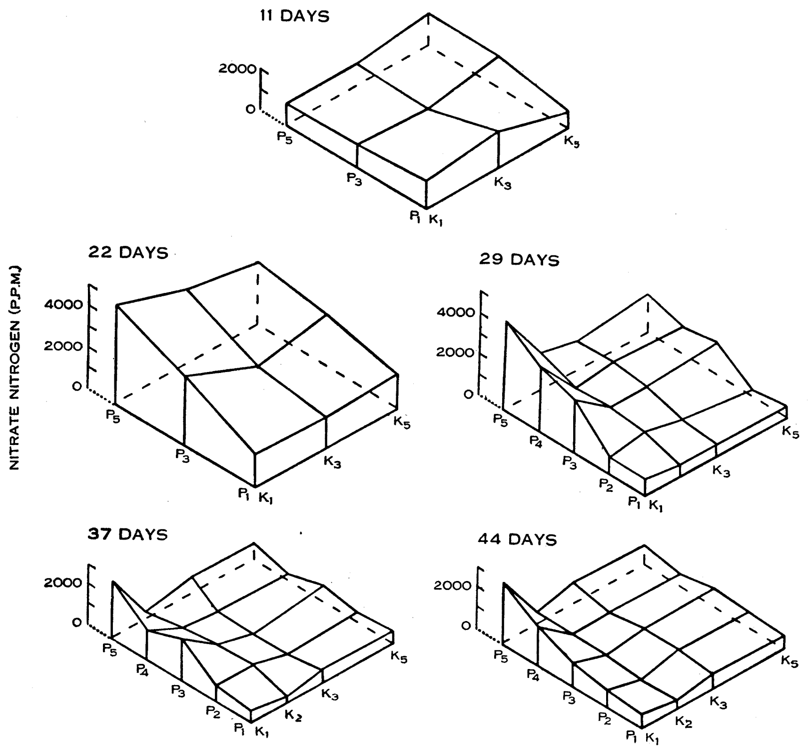


Fig. 10.—Interaction effects of phosphorus and potassium supply on nitrate nitrogen content.

The effects of the three sub-treatments on nitrogen uptake were well-marked. The plants which had been deficient in nitrogen or phosphorus and had been provided with a sub-treatment of the same nutrient recovered to such an extent that their total uptake rose to about half of the maximum figure, while in the potassium-deficient plants the effect of the deficiency was completely abolished by the potassium sub-treatment.

The initial supply of nitrogen to each pot was 50 mg, 500 mg, and 5000 mg in the N_1, N_3, and N_5 treatments respectively. The sub-treatment, applied at 46 days, consisted of 200 mg nitrogen. In the N_1 treatments, the total amount of nitrogen contained in the tops at 98 days reached a maximum of 19·4 mg; this represented nearly 40 per cent. of the supply, to which must be added an (unknown) amount in the roots, and some 5 mg (10 per cent. of supply) for plants removed from the pot at earlier harvests—i.e. more than half the nitrogen supplied was utilized in some of these treatments. At the N_3 level of nitrogen supply, more than 40 per cent. of the amount added was contained in plants harvested at 98 days, in some cases where phosphorus and potassium supply was abundant. So in these cases again more than half the nitrogen supplied would have been used; but at N_5 the

TABLE 6

RESPONSE OF TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AT 98 DAYS TO NITROGEN SUB-TREATMENT, AS AFFECTED BY INITIAL NUTRIENT SUPPLY

Average Effects at Different Levels of Initial Treatments

N_1	+0·94			K_1	+0·56
N_2	+0·50			K_2	+0·33
N_3	+0·24	P_3	+0·29	K_3	+0·03
N_4	−0·09	P_4	+0·38	K_4	+0·17
N_5	−0·04	P_5	+0·26	K_5	+0·46

Interactions between Initial Treatments

	N_1	N_2	N_3	N_4	N_5
P_3	+0·61	+0·32	+0·12	+0·12	+0·30
P_4	+1·35	+0·63	+0·18	−0·03	−0·24
P_5	+0·87	+0·55	+0·41	−0·37	−0·18
	N_1	**N_2**	**N_3**	**N_4**	**N_5**
K_1	+1·58	+0·67	+0·37	+0·58	−0·38
K_2	+0·80	+0·32	+0·62	−0·16	+0·08
K_3	+0·91	+0·61	−0·26	−0·29	−0·82
K_4	+0·96	+0·13	+0·23	−0·31	−0·18
K_5	+0·47	+0·77	+0·23	−0·29	+1·10
	K_1	**K_2**	**K_3**	**K_4**	**K_5**
P_3	+0·04	+0·40	+0·01	−0·09	+1·10
P_4	+0·76	+0·45	+0·25	+0·35	+0·07
P_5	+0·89	+0·14	−0·17	+0·23	+0·19

maximum absorption of nitrogen was less than 4 per cent. of the supply. The proportion of sub-treatment nitrogen absorbed in those treatments where its effect was greatest corresponds closely to the uptake from the initial N_3 treatments—about 100 mg, or 50 per cent.

(d) *Relationship between Growth Response to Nitrogen and Chemical Composition*

The data available on the chemical composition of the plants before the addition of the sub-treatments included the total, protein, non-protein, and nitrate nitrogen contents of the whole plants on five occasions, as well as less complete data on the composition of the portions, which could be analysed separately only in the larger plants. Data on phosphorus and potassium content were also available, having been determined as described by (Grant Lipp 1952). As explained previously, the plants receiving the N_4 and K_4 treatments were not analysed, so that analytical data were available for 80 treatments at 29, 37, and 44 days. All the plants which

TABLE 7

RESPONSE OF TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AT 98 DAYS TO PHOSPHORUS SUB-TREATMENT, AS AFFECTED BY INITIAL NUTRIENT SUPPLY

Average Effects at Different Levels of Initial Treatments

N_1	+0·08			K_1	−0·05
N_2	−0·10			K_2	+0·23
N_3	−0·48	P_3	+0·24	K_3	+0·05
N_4	+0·01	P_4	−0·14	K_4	−0·19
N_5	+0·38	P_5	−0·17	K_5	−0·16

Interactions between Initial Treatments

	N_1	N_2	N_3	N_4	N_5
P_3	+0·07	−0·11	−0·08	+0·52	+0·79
P_4	−0·01	−0·20	−0·49	−0·21	+0·21
P_5	+0·18	+0·02	−0·87	−0·29	+0·13
	N_1	N_2	N_3	N_4	N_5
K_1	−0·09	+0·10	−0·83	+0·49	+0·10
K_2	+0·41	−0·33	+0·12	+0·03	+0·60
K_3	+0·08	+0·41	−0·64	+0·01	+0·40
K_4	+0·53	−0·19	−0·94	−0·18	+0·14
K_5	−0·53	−0·37	−0·32	−0·32	+0·66
	K_1	K_2	K_3	K_4	K_5
P_3	+0·81	+0·31	−0·23	−0·10	+0·40
P_4	−0·74	+0·39	+0·28	−0·09	−0·54
P_5	−0·21	+0·01	+0·10	−0·40	−0·34

had been harvested at 11 and 22 days were analysed, giving data for 27 treatments on each of these occasions.

The response associated with each treatment was given by the difference between the dry weights for the nitrogen sub-treatment and its control at 98 days, the differences being corrected for differences in size at the time of applying the sub-treatment, as described earlier (Goodall, Grant Lipp, and Slater 1955). The responses, expressed in milligrams, are shown in Table 9.

The relationship between the responses measured in this way and the nitrogen, phosphorus, and potassium content of the plants was examined, using analytical data for each of the five harvests between 11 and 44 days, by the method of multiple regression.

Where the purpose was to compare different sets of independent variables (e.g. analyses on different occasions) as bases for estimation of response, it would clearly be inappropriate to use different ranges of treatments. Accordingly, only those treatments were used in which a complete set of data for responses and for analyses on each of the occasions under comparison was available. For samples taken at 29, 37, and 44 days, these numbered 70, but if the earlier harvests were to be compared as well only 20 treatments could be included. This, of course, greatly reduced the precision with which regressions could be calculated, and accordingly both bases of computation (for 20 and for 70 treatments) were used for the later harvests.

Table 8

RESPONSES OF TOTAL NITROGEN CONTENT (PER CENT. OF DRY MATTER) AT 98 DAYS TO POTASSIUM SUB-TREATMENT, AS AFFECTED BY INITIAL NUTRIENT SUPPLY

Average Effects at Different Levels of Initial Treatments

N_1	−0·09			K_1	−0·99
N_2	−0·26			K_2	+0·02
N_3	−0·20	P_3	+0·18	K_3	−0·05
N_4	−0·30	P_4	−0·52	K_4	−0·13
N_5	−0·19	P_5	−0·29	K_5	+0·11

Interactions between Initial Treatments

	N_1	N_2	N_3	N_4	N_5
P_3	+0·09	−0·31	+0·12	+0·55	+0·49
P_4	−0·42	−0·28	−0·51	−0·53	−0·57
P_5	+0·05	−0·20	−0·22	−0·92	−0·18

	N_1	N_2	N_3	N_4	N_5
K_1	−0·72	−0·80	−0·37	−1·49	−1·46
K_2	+0·28	+0·03	−0·30	−0·12	+0·20
K_3	+0·11	+0·39	−0·19	+0·25	−0·82
K_4	+0·00	−0·52	−0·06	−0·28	+0·19
K_5	−0·14	−0·41	−0·01	+0·13	+0·96

	K_1	K_2	K_3	K_4	K_5
P_3	−0·69	+0·71	+0·08	−0·11	+0·93
P_4	−1·56	−0·39	−0·40	+0·01	−0·27
P_5	−0·72	−0·26	+0·17	−0·31	−0·34

In no case was the inclusion of analytical data for potassium found to increase the precision with which response to nitrogen could be estimated, over that obtainable from the nitrogen and phosphorus content alone. Accordingly, potassium content has been ignored. On the other hand, where the multiple regression as a whole was significant, the contributions from the two other independent variables (nitrogen and phosphorus content) were both individually significant. In Tables 10–13 these two regression coefficients are quoted in mg/l per cent. change in nutrient content, together with their significance individually; the significance of the multiple regression as a whole is also presented in the form of a variance ratio.

TABLE 9

GROWTH RESPONSE TO NITROGEN (MG)

Nitrogen Levels	Potassium Levels	Phosphorus Levels				
		P_1	P_2	P_3	P_4	P_5
N_1	K_1	+15	−557	+314	−505	+876*
	K_2	−1	−151	+2130	+5171	+2844
	K_3	−42	−254	+1515	+5933	+1357
	K_5	+38	−233	−740	+2429	+3196
N_2	K_1	−1	+130	+1874*	+96	−362
	K_2	−122	+30	+597	+2197	+1257
	K_3	+122	−106	−554	−1485	+5389
	K_5	+74	+35	+144	+3410	+2718*
N_3	K_1	+2	−104	+243	+131	−263
	K_2	−12	−201	−84	+265*	−3199
	K_3	−24	−102	−1198	−338	+29
	K_5	−119	+199	+154	−5078	−587
N_5	K_1	+1	−5	+13	−209	+119
	K_2	−75	+7	+114	+842	+2601*
	K_3	+2	−7	+727*	−705	−1864
	K_5	+28	+93	−145*	−3016	+885

* Fitted values, excluded from regression calculations.

(i) *Total Nitrogen.*—Where the total nitrogen content of the aerial part of the plant, together with its phosphorus content, was used to estimate the response (Table 10), it was found that the accuracy of estimation increased as the plant grew older. At 44 days, the regression reached very high significance and accounted for nearly a third of the total variation in response.

(ii) *Nitrate Nitrogen.*—Where data for nitrate nitrogen were used in place of total nitrogen content (Table 11) the significance of the regression was much less for analyses of the later harvests but reached a maximum in the intermediate harvests (29 and, perhaps, 22 days). At 29 days the response could be estimated almost as accurately from the nitrate content as it could from the total nitrogen content at 44 days.

(iii) *Organic Nitrogen.*—Soluble organic nitrogen contents (soluble nitrogen minus nitrate nitrogen) were more uniform than other nitrogen fractions, so it was not

TABLE 10

REGRESSION OF RESPONSE ON TOTAL NITROGEN AND PHOSPHORUS CONTENT

	Age (days)	b_N	b_P	F for Multiple Regression
Analyses for 20 treatments on each occasion	11	−835	1515	1·1
	22	−995*	4874*	3·9*
	29	−1214**	5180**	5·9*
	37	−783	4100	1·7
	44	−984**	6346**	6·5**
Analyses for 70 treatments on each occasion	29	−1023**	5366**	4·8*
	37	−1362***	7385***	8·0***
	44	−1471***	6523***	10·4***

*P=0·01–0·05. **P=0·001–0·01. ***P<0·001.

expected that they would provide a particularly accurate prediction of response. The results of the regression, shown in Table 12, confirm this. The regression is

TABLE 11

REGRESSION OF RESPONSE ON NITRATE NITROGEN AND PHOSPHORUS CONTENT

	Age (days)	b_N	b_P	F for Multiple Regression
Analyses for 20 treatments on each occasion	11	−3100	405	1·0
	22	−2175**	3546*	4·4*
	29	−2181*	4273*	3·7*
	37	−1562	3278	†
	44	−1667	4520*	2·8
Analyses for 70 treatments on each occasion	29	−3512***	4866***	8·0***
	37	−2431*	4363*	3·6*
	44	−2540*	3822*	4·0*

*P=0·01–0·05. **P=0·001–0·01. ***P<0·001.

† Regression mean square less than error mean square.

only significant at 44 days, and then only at the 5 per cent. level. In view of these results obtained with data for 70 treatments, the corresponding regressions at 11 and 22 days with more limited sets of data were not computed.

(iv) *Insoluble Nitrogen.*—Most of the nitrogen in all plants was in the insoluble fraction, so there was a close correlation between this fraction and total nitrogen content. Only one regression was calculated for this fraction—that for 44 days, at which harvest the regression for total nitrogen reached the highest level of significance.

TABLE 12

REGRESSION OF RESPONSE ON CONTENT OF NITROGEN FRACTIONS AND PHOSPHORUS: 70 TREATMENTS

Fraction	Age (days)	b_N	b_P	F for Multiple Regression
Soluble organic nitrogen	29	−956	1499	1·2
	37	−1904	1953	2·6
	44	−2417*	2046	4·1*
Insoluble nitrogen	44	−1907***	6523***	7·5***

*P=0·01–0·05. ***P<0·001.

The results (Table 12) show that the substitution of insoluble for total nitrogen reduced rather than increased the significance of the regression.

(v) *Choice of Organ.*—The only harvest at which a fair number of the plants were large enough to obtain separated organs for analysis was that at 44 days, but even

TABLE 13

REGRESSION OF RESPONSE ON NITROGEN AND PHOSPHORUS CONTENT OF DIFFERENT ORGANS AT 44 DAYS

Fraction	Organ	b_N	b_P	F for Multiple Regression
Total nitrogen (32 treatments)	Older leaves	−1259*	6050	2·6
	Younger leaves	−1296*	4349	2·2
	Whole tops	−1319*	5969	2·8
Nitrate nitrogen (27 treatments)	Midribs	−452	1800	†
	Whole tops	−1219	5212	1·7

* P = 0·01–0·05.

† Regression mean square less than error mean square.

here most of the plants were too small for subdivision. The data available for comparison between organs are thus both rather limited in number and confined to larger plants, presumably with a more favourable nutritional status. Only two sample analyses will therefore be given. These were selected as being most likely to use the differences in nutrient content to the best advantage. For the comparison of older and younger leaves with the entire tops the total nitrogen content was used, but for the comparison of midribs with the tops the nitrate content was used, because this varied over a particularly wide range (0–20,000 p.p.m.).

The results of these analyses, shown in Table 13, suggest that, on the basis of these data, no preference between older and younger leaves, or between either organ and the whole tops, can be made; and that the nitrate content of the midribs is not closely correlated with response.

IV. Discussion

Among the treatment effects observed on nitrogen content, the general increase with increased nitrogen supply was to be expected; in fact, an increase in the proportion of a nutrient in plant dry matter with increased supply is usual and any exception calls for explanation. At higher levels of supply, though, the mean effects on nitrogen content are small. In accordance with general experience, the proportion of nitrate nitrogen is reduced at low levels of nitrogen supply—a greater proportion of the nitrogen taken up has been used promptly for protein synthesis. As for the effect of other nutrients, general experience is that, where increased supply of a nutrient increases growth, it results also in a decreased percentage content of other nutrients. This is true for the effects of potassium on nitrogen content in the present experiment—the increase from K_1 to K_2 effective in promoting growth is also effective in reducing nitrogen content. Such results are usually expressed in terms of "luxury consumption"—where potassium supply is limiting growth (K_1), nitrogen is taken up in excess of that which can be effectively used in growth, and additional potassium, by increasing growth, "dilutes" the nitrogen present. As usual with luxury consumption of nitrogen, the proportion of nitrogen in the form of nitrate increases. This effect of potassium supply on nitrogen content only shows itself where potassium is effectively limiting; it does not occur among the treatments with lower levels of phosphorus supply.

The effect of phosphorus supply on nitrogen content is very different, leading as it does to substantial increases. Since it was concurrently stimulating growth very markedly, nitrogen uptake was promoted in even greater proportion. Phosphorus is, of course, known to be involved in protein synthesis; but other factors are involved, for one finds the nitrate content increased by phosphorus supply even more than that of the other nitrogen fractions—i.e. the effects of phosphorus on nitrate uptake were even more marked than those on its reduction and utilization in protein synthesis. These effects of phosphorus on nitrogen content are more marked when nitrogen is in fully adequate supply—in which circumstances, of course, the phosphorus supply was able to exert its full influence on growth.

The wider range of variation in nitrate nitrogen than in total nitrogen content which is seen in these data has been very commonly observed. It rests on the fact that protein synthesis and the antecedent processes tend rapidly to use up all available nitrate when this is in short supply, but that when it is abundant it tends to be accumulated to some extent in the plant as a form of reserve. In accordance with this general rule, nitrate content also responds more markedly to the various nutritional treatments, whether they affect nitrogen uptake directly or indirectly.

In this experiment the effects of treatments on nitrate and total nitrogen content are remarkably similar, differing only in their magnitude.

The additional supplies of nutrients at 46 days had effects generally in line with those of the initial applications. The additional supply of nitrate generally increased the nitrogen content of the plants at 98 days; but where the plants already had a high nitrogen content (for instance with high initial nitrogen supply) this increase was small or negligible. Growth responses were also observed (Goodall, Grant Lipp, and Slater 1955) but clearly the nitrate uptake was stimulated more than the growth. Once again there is evidence that phosphorus favoured nitrogen uptake, for the plants which had received a high initial supply of phosphorus but little nitrogen showed a particularly marked increase in nitrogen content when supplementary nitrogen was supplied. Where potassium supply was limiting, the supplementary nitrogen led to increases in nitrogen content, presumably through luxury consumption.

The effects of supplementary supplies of phosphorus and potassium at 46 days are in general agreement with the effects of supplies of these elements at the outset. Extra phosphorus at 46 days increased the nitrogen content in phosphorus-deficient plants and in plants well supplied with nitrogen, in accordance with the marked interaction between the initial treatments with nitrogen and phosphorus. In potassium-deficient plants, potassium supply at 46 days reduced nitrogen content, especially where a substantial growth response was assured by adequate supplies of phosphorus and nitrogen.

Some of the results of the regression analysis are quite clear-cut. One of these is the progressive increase in significance of the regression of response on total nitrogen content up to the time of the supplementary application. This may be compared with the results of Crowther (1941), who found that the variation in different seasons in the yield of cotton in the Sudan was closely related to the nitrogen content of the seedlings within 2 weeks of sowing; the close correlation continued through the next month, but then decreased steadily. In comparing these results, however, it should be remembered that Crowther's differences in yield were mainly due to differences in accumulated nitrogen reserves and nitrification activity in the soil, and thus could be related only indirectly to the prospective responses to nitrogenous top-dressings applied during growth.

The high correlation of nitrogen response with nitrogen content occasions no surprise, but the importance of phosphorus content was unexpected. *Prima facie*, one would imagine that the *slope* of the nitrogen response curve might reflect the current nitrogen status only, the status in respect of other nutrients affecting the rate of change in slope rather than its initial value (the second rather than the first differential). This assumption cannot definitely be denied from the present data, for the supplementary nitrogen supply was far from infinitesimal; but it is made to seem unlikely. In this, the present work confirms the results of Lundegårdh (1941, 1943, 1951) who, it will be recalled, found that the response of oat plants in different localities to initial nitrogen supply was related to both nitrogen and phosphorus content of the control plants at flowering.

In contrast to phosphorus, potassium content seems irrelevant to the forecasting of response to nitrogen. This would not necessarily imply that potassium status has no influence on response to nitrogen (though this appears to have been the case in the present experiment—see Goodall, Grant Lipp, and Slater 1955); if potassium deficiency had been associated with a reduced response to nitrogen, as is often the case, this would have been reflected in an increased nitrogen content, enabling one to forecast the change in response potentialities without recourse to potassium analyses. Lundegårdh does not refer to any influence of potassium on the relationship between nitrogen content and nitrogen response; presumably it was missing from his results likewise.

TABLE 14

REGRESSION OF RESPONSE ON NITROGEN AND PHOSPHORUS CONTENT AT 44 DAYS: ANALYSIS OF VARIANCE

	Degrees of Freedom	Mean Square	F	P
Linear regression*	2	20831764	10·62	$<0\cdot001$
Quadratic terms†	3	6216438	3·17	0·01–0·05
Error	67	1961659		

$^*y = 2254-1280x_1+5866x_2$.

$^\dagger y = 4557-3981x_1+17292x_2+636x_1{}^2-3593x_2{}^2-3374x_1x_2$.

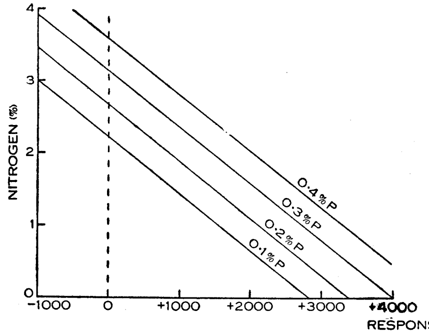
Lundegårdh showed the relationship between nitrogen content and nitrogen response, at all phosphorus levels except the lowest, as curvilinear; but he applied no tests to the deviations from linearity. That has been done for the present data. In the case of the analyses for total nitrogen and phosphorus of samples collected at 44 days (when the relationship was closest), an expression of the following form was fitted:

$$y = a+bx_1+cx_2+dx_1{}^2+ex_2{}^2+fx_1x_2,$$

where y is the response to supplementary nitrogen in milligrams, while x_1 and x_2 are the content of nitrogen and phosphorus (per cent. of dry matter) at 44 days. The results, as shown in Table 14, support Lundegårdh's view, though the improvement over the simple linear expression only just reaches significance.

The analyses for different nitrogen fractions do not suggest that any of them provides a better basis for forecasting response than the total nitrogen content, except that nitrate analyses may enable a satisfactory forecast to be given at an earlier date. At 29 days, nitrate analyses provided forecasts almost as good as those based on total nitrogen content at 44 days; and already at 22 days from sowing some information on the prospective response was available.

The analyses of different organs were unfortunately too few to enable a satisfactory test of their relative value as diagnostic material to be made. It would certainly seem that the difference in this respect between older and younger leaves was nothing like as great for nitrogen deficiency in lettuce as for potassium deficiency in barley (Goodall 1948). The failure of nitrate content of midribs to show a relation-



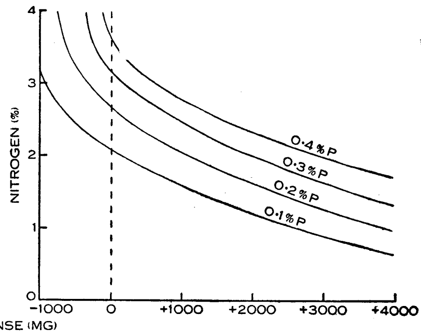


Fig. 11 Fig. 12

Figs. 11 and 12.—Relation between response to nitrogen and total nitrogen content at different phosphorus levels; analytical data for 44 days. Linear regression (Fig. 11); quadratic regression (Fig. 12).

ship with response to nitrogen is noteworthy and it is at variance with many recorded results (Carolus 1938; Harrington 1944; Ulrich 1942, 1946, 1948, 1950). It has often

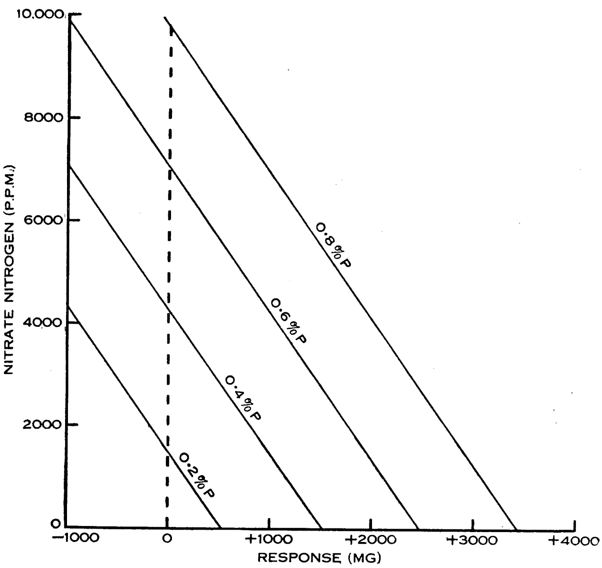


Fig. 13.—Relation between response to nitrogen and nitrate nitrogen content at different phosphorus levels; analytical data for 29 days.

been claimed (e.g. Emmert 1935) that this is a good index of current uptake by the roots and hence of the supplies of nitrogen available for synthesis and growth; if this were so, a close relationship with response would certainly be expected. It is possible that difference in current uptake of nitrate may have been masked to some extent by changes in the rate of nitrate reduction in the roots; the relative importance of different sites of nitrate reduction seems never to have been investigated in the lettuce.

To pass to practical conclusions regarding the nutrition of lettuce in the field would be beyond the scope of this paper, and would not be justified by the data. It may, however, be of interest to note the most clear-cut results as presented in Figures 11–13. These show the response as related to nitrogen content (total nitrogen at 44 days and nitrate nitrogen at 29 days), at four arbitrarily chosen levels of phosphorus content. In respect of total nitrogen content, both linear (Fig. 11) and quadratic (Fig. 12) expressions were used. It will be seen that positive responses were to be expected only where the nitrogen content did not exceed the values given in Table 15.

TABLE 15

NITROGEN CONTENT CORRESPONDING TO ZERO RESPONSE

29 Days from Sowing		44 Days from Sowing		
Phosphorus (%)	Nitrate Nitrogen (p.p.m.)	Phosphorus (%)	Total Nitrogen* (%)	Total Nitrogen† (%)
0·2	1520	0·1	2·22	2·09
0·4	4290	0·2	2·68	2·65
0·6	7060	0·3	3·14	3·16
0·8	9830	0·4	3·59	3·54

* Linear expression. † Quadratic expression.

It should be remembered that the samples for analysis were taken at fixed *times*, and not at corresponding stages of morphological or physiological development in the different treatments. With such a wide range of treatments, it would in fact have been almost impossible to find stages which could be said to correspond. Given a narrower range of conditions, sampling on the basis of developmental stage might be possible, and it would certainly be more practicable for advisory work under field conditions. It might then well be found that the relation of response to analytical data was closer for samples taken on this basis than samples at a fixed time.

V. ACKNOWLEDGMENTS

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VI. References

CAROLUS, R. L. (1938).—The use of rapid chemical plant nutrient tests in fertilizer deficiency diagnoses and vegetable crops research. Bull. Va. Truck Exp. Sta. No. 98. pp. 1531–56.

CROWTHER, F. (1941).—Studies in growth analysis of the cotton plant under irrigation in the Sudan. II. Seasonal variation in development and yield. *Ann. Bot., Lond.* (N.S.) **5**: 509–33.

EMMERT, E. M. (1935).—New methods for the determination of the availability of nitrogen and phosphorus to plants. *J. Amer. Soc. Agron.* **27**: 1–7.

GOODALL, D. W. (1948).—Studies in the diagnosis of mineral deficiency. IV. The mineral content of barley plants in relation to potassium deficiency. *Ann. Appl. Biol.* **35**: 605–23.

GOODALL, D. W., GRANT LIPP, A. E., and SLATER, W. G. (1955).—Nutrient interactions and deficiency diagnosis in the lettuce. I. Nutritional interaction and growth. *Aust. J. Biol. Sci.* **8**: 301–29.

GOODALL, D. W., SLATER, W. G., and GRANT LIPP, A. E. (1957).—Nutrient interactions and deficiency diagnosis in the lettuce. II. Effects of nutrition on water content. *Aust. J. Biol. Sci.* **10**: 57–65.

GRANT LIPP, A. E. (1952).—Mineral nutrition in lettuce with special reference to phosphorus and potassium. M.Sc. Thesis, University of Melbourne.

HARRINGTON, J. F. (1944).—Some factors influencing the reliability of plant tissue testing. *Proc. Amer. Soc. Hort. Sci.* **45**: 313–17.

LUNDEGÅRDH, H. (1941).—Die Tripelanalyse. Theoretische und praktische Grundlagen einer pflanzenphysiologischen Methode zur Bestimmung des Düngerbedürfnisses des Ackerbodens. *LantbrHögsk. Ann.* **9**: 127–221.

LUNDEGÅRDH, H. (1943).—Leaf analysis as a guide to soil fertility. *Nature* **151**: 310–11.

LUNDEGÅRDH, H. (1951).—"Leaf Analysis." (Trans. by R. L. Mitchell.) (Hilger & Watts Ltd.: London.)

SLATER, W. G. (1952).—Mineral nutrition in lettuce, with special reference to nitrogen. M.Sc. Thesis, University of Melbourne.

ULRICH, A. (1942).—Potassium content of grape leaf petioles and blades contrasted with soil analyses as an indicator of the potassium status of the plant. *Proc. Amer. Soc. Hort. Sci.* **41**: 204–12.

ULRICH, A. (1946).—Plant analyses as a guide in the fertilization of sugar beets. *Proc. Amer. Soc. Sugar Beet Technol.* **4**: 88–95.

ULRICH, A. (1948).—Plant analysis—methods and interpretation of results. In "Diagnostic Techniques for Soils and Crops." Ch. 6. pp. 157–98.

ULRICH, A. (1950).—Critical nitrate levels of sugar beet estimated from analysis of petioles and blades with special reference to yields and sucrose concentrations. *Soil Sci.* **69**: 291–309.