

NUTRIENT INTERACTIONS AND DEFICIENCY DIAGNOSIS IN THE LETTUCE

I. NUTRITIONAL INTERACTION AND GROWTH

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Summary

A sand-culture experiment with lettuces is described, having as its principal purpose the study of the relationship between the potential responses of plants to applications of nitrogen, phosphorus, and potassium fertilizers and the composition of their foliage. Plants were supplied initially with five levels of these nutrients in all combinations, samples of plant material were taken for analysis at various stages of development, and at 44 days from sowing additional quantities of nutrients were supplied to some of the cultures in order that their response potentialities might be determined. The present paper analyses the effects of the nutrient interactions on plant dry weight, further results being left to subsequent papers.

The most marked effects on growth were those of phosphorus supply, which resulted in increased dry weight throughout the range tested. Nitrogen supply gave an optimum-type curve, with a tendency for the optimum level to shift upwards with increasing phosphorus supply. The highest nitrogen supply level had a clearly adverse effect on growth. For potassium too there is a suggestion of an optimum-type curve, with an optimum shifting in accordance with phosphorus supply, but the plants were less sensitive to very considerable (30-fold) changes in potassium supply than in the case of either of the other nutrients. Within the sub-optimal range, the general effect of increasing the supply of one nutrient was to increase the proportional responses to the others. Calculation of relative growth rates indicated that the nutritional effects were spread through the growth period of the plants and not concentrated at any one stage of development.

The effects of nutrient applications at 44 days were in general conformity with the effects of initial applications. Plants whose initial treatment placed them in the sub-optimal range for a given nutrient, as indicated by the results from varying initial treatments, also responded, often very markedly, to a further application of this nutrient at 44 days.

The results of this experiment did not conform to the expectations from Mitscherlich's theories of the relation of plant growth to nutritional supply, but were in reasonable agreement with the "Resistance Formulae" of Maskell.

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I. INTRODUCTION

Much has been written in the past two decades on the use of chemical analysis of plant material as an aid in the diagnosis of mineral deficiency (see, for instance, Goodall and Gregory 1947). In most work on the subject, however, the purposes served have been qualitative. A disorder has been observed or suspected, and analysis of plant material has been performed in order to suggest or confirm a diagnosis of the disorder as due to deficiency of this or that nutritional element. In relatively few instances has there been an attempt to make the analytical data provide quantitative information as to the extent of the deficiency. Where "deficiency" is regarded as synonymous with the appearance of visual symptoms, there is, of course, no occasion for quantitative diagnosis by analysis—the quantitative indication is held to be provided by the severity of the symptoms. If, however, "deficiency" is considered to include any condition of a plant in which its "performance," however measured, suffers from the lack of a particular nutrient element and can be improved by an increase in its supply, no direct method of measuring the intensity of the deficiency is possible—there may well be no visually recognizable symptoms at all. If this meaning be ascribed to deficiency, then the most reasonable quantitative measure of deficiency is clearly the improvement in performance which may be expected to result from an increase in supply of the nutrient in question—a particular increase in supply which may be selected and specified arbitrarily. The resulting improvement in performance may be conveniently designated as "response," but can clearly be determined only by comparison between treated and untreated plants, and by allowing the disorder to run its course for at least one growing season. If chemical analysis could be used to estimate this potential response at an early stage in the plant's development, it would clearly be a much more valuable guide to fertilizer practice than any merely qualitative designation of deficiency.

Few investigators have attempted to establish a quantitative relationship between the response potentialities of plants and their chemical composition (e.g. Pfeiffer, Simmermacher, and Rippel (1919) with oats, van Itallie (1935) with grassland, Macy (1936) with barley, Crowther (1937) with cotton, Craig (1940) with sugar-cane, Lundegårdh (1941, 1943, 1951) with oats, and Goodall (1948, 1949) with wheat and barley). In most of these investigations, the material for analysis was collected *after*—often many months after—the treatment used to determine the response potentialities of the plants. Most commonly, fertilizer treatments were applied at or before the time of sowing; responses were estimated from the differences in yield between treated and untreated plants; and material from the untreated plants or those receiving a lower level of treatment, collected at or around harvest time, provided the analytical data. In other words, information required for a forecast was available only *after* an operation to whose effects the forecast referred. As a guide to fertilizer practice, such forecasting methods would, of course, be useless. But the rationale of these investigations was the expectation that the nutrient-supplying powers of the soil would remain reasonably similar from year to year,

and that information on the nutritional status of one crop would be valuable in determining the fertilizer regime for the next crop grown in the same field. That is, the analyses were envisaged as indicating the nutritional status of the plants analysed, not with a view to correcting any deficiencies detected in those plants, but in order that the correlations between their nutritional status on the one hand and that of the substrate and the plants subsequently to be grown in it on the other might enable the fertilizer regime of the latter to be adjusted.

In addition to being more immediately applicable in practice, the idea of analysing plants early in their development with a view to forecasting the response to a fertilizer treatment applied subsequently (e.g. by top-dressing or foliar application) seems theoretically more acceptable than the use of analyses of material collected at harvest-time. Some of the reasons for this have been mentioned in an earlier publication (Goodall and Gregory 1947); as they approach maturity, differences between plants in nutrient uptake tend to be apparent more in growth than in percentage composition; and it seems likely that the response of a plant to a nutritional treatment would be much more directly related to its composition at or before the time of application than to the composition of untreated plants several months later.

Partly in response to these considerations, interest has, in recent years, shifted from analysis of plant material at harvest-time as an index of soil conditions to analysis at earlier stages of development, with a view to using the information in improving the growth of those same plants. But very little of this work has been more than qualitative; the rapid "tissue tests" (see Nicholas 1953), for instance, have proved valuable in indicating whether plants are likely to respond to a nutrient addition during their development, but they have not been used to estimate the magnitude of these responses except in the broadest categories. It would appear that the only cases in which plant material has been analysed early enough in development for deficiencies still to be remediable, and in which the analyses could be used for quantitative forecasts of response, are in manganese deficiency of wheat (Goodall 1949) and possibly nitrogen deficiency in cotton (Crowther 1937).

The purpose of the present work was to obtain such data in respect of the major-element nutrition of lettuce. Apart from the first order relationships between the response to an element and the content in the plant of the same element, it was hoped to discover whether these relationships were affected by the status of the plant with respect to the other major nutrients. Lundegårdh (1941) found that the curve showing the relationship between the response of oat plants to potassium fertilizers and their potassium content varied with their phosphorus content—the higher the phosphorus content, the greater the response to potassium shown by plants of a given potassium content. He also found similar interactions between nitrogen response and phosphorus content, and between phosphorus response and nitrogen content. Generally, in work on nutritional diagnosis by plant analysis, it has been assumed that each nutrient can be treated independently. It was hoped to find whether, in the very different conditions of the present investigation, Lundegårdh's conclusions could be substantiated and perhaps extended.

It was first necessary to grow plants which, at an age of a few weeks, differed widely in nutritional status and in potential response to the three major nutrients — nitrogen, phosphorus, and potassium. Such plants could most readily be obtained by sand-culture methods. When they had reached a suitable stage, the responses were to be measured by supplying additional amounts of the same nutrients. Immediately before these treatments were applied, and on several earlier occasions, samples of plant material were to be taken for analysis. The analyses were to cover the major-element content of several different organs, and, in the case of nitrogen and phosphorus, different chemical fractions were to be estimated.

The results of such an experiment clearly have interest considerably beyond the questions which they were designed to answer. Information is obtainable from it on the effects of the nutrients and their interactions on the growth of the plants, on their nutrient uptake, and on their composition. Although the initial treatments were intended simply to provide plants differing widely in nutritional status, they included so many combinations as to provide more extensive material for the study of nutrient interactions than in many experiments designed expressly for that purpose. The first paper of this series will accordingly not deal with those parts of the results bearing directly on deficiency diagnosis, but will concern itself only with the effects of the treatments on plant growth, expressed in terms of dry weight. Subsequent papers will discuss the analytical results and their interpretation in relation to deficiency diagnosis.

It will be appreciated that the complete numerical data of a large and complex experiment cannot be presented in a printed paper. They are, however, recorded in full in the theses on which these papers are based (Grant Lipp 1952; Slater 1952), which may be consulted in the library of the University of Melbourne.

II. MATERIALS AND METHODS

The lettuce plants to be studied were grown in sand culture, under a cage of wire netting to prevent bird damage. The pots used were of glazed earthenware, fitted with a glass level tube, and each contained 6-7 kg of a mixture of white quartz sand and crushed quartz, thoroughly washed with tap and distilled water. They were arranged in double rows under glass shelters, in 125 groups of four. Each of these groups of four pots was allotted at random to one of the 125 initial treatments. By grouping the pots in this way, the effect of local differences in external conditions on the responses to subsequent nutrient additions was reduced, the measurement of these responses being one of the primary purposes of the experiment. These later additions, hereafter referred to as sub-treatments, were distributed randomly within each set of four pots.

The nutrient levels were chosen on the basis of results obtained in a preliminary experiment. The lowest levels (N_1 , P_1 , K_1) were those at which the plants would barely survive, the highest (N_5 , P_5 , K_5) were, judging by these preliminary studies, in excess of the requirements for optimal growth.

The amounts supplied per pot were as follows:

Nutrient level	1	2	3	4	5
N as NaNO_3 (mg)	50	200	500	2000	5000
P as Na_2HPO_4 (mg)	2	5	20	100	500
K as K_2SO_4 (mg)	0	100	300	1000	3000

Each pot also received 1200 mg of calcium as chloride and 600 mg of magnesium as sulphate. All the above were added in solution before the seeds were sown. In addition, each pot received a weekly supply of 2 mg of iron in the form of a solution of ferric citrate, and a trace element solution applied once a fortnight contained the following:

B as H_3BO_3	..	110 μg
Mn as MnSO_4	..	550 μg
Cu as CuSO_4	..	65 μg
Zn as ZnSO_4	..	25 μg
Mo as H_2MoO_4	.	10 μg

The sub-treatments (S_N , S_P , S_K) were applied 46 days after planting, the amount supplied in each case being equal to that in the lowest initial treatment level at which growth was satisfactory at this stage (N_2 , P_3 , and K_2), i.e.

S_N	: 200 mg N per pot,
S_P	: 20 mg P per pot,
S_K	: 100 mg K per pot.

All nutrient solutions were prepared from analytical reagent grade chemicals and distilled water. Distilled water was also used to maintain the solution at a level approximately 15 cm below the surface of the sand.

The seeds of lettuce, variety Great Lakes, were sown on October 31, 1949, each pot receiving five groups of about ten seeds. At 11 days the seedlings were thinned to three in each of the five groups, and at 22 days to one in each group. The seedlings removed during these thinning processes provided the first two harvests; late-germinated seedlings were rejected, but among the others those to be retained on each occasion were selected at random. These first two harvests included only those 27 treatments combining the 1, 3, and 5 levels of each nutrient. On subsequent occasions, plants were harvested from all 125 treatments; at the third (29 days) two plants were taken from each pot, at the fourth (37 days), fifth (44 days), and sixth (98 days) harvests one only. The plants removed for harvesting were brushed clean, and the roots washed. In the first five harvests, before the sub-treatments had been applied, the plants from the four replicate pots were combined into two groups for weighing and analysis, each group representing a pair of pots.

In the first harvest, plants were dried and weighed intact, but in subsequent harvests the roots were cut off at the cotyledonary node, the fresh weight of the shoot was determined, and in some cases certain organs were separated from the shoot before drying. In the 22-day harvest, the cotyledons were separated in this way; in the 37- and 44-day harvests, the tops were divided into younger leaves, laminae of older leaves, and midribs plus stems, in so far as the

size of the plants made it possible. All samples were enclosed in paper bags and dried at 85-95°C.

TABLE 1
ANALYSES OF VARIANCE: DRY WEIGHT

Days from Sowing	Source of Variation	Degrees of Freedom	Mean Square	F
11†	N	2	0.3750	3.87
	P	2	0.1376	1.42
	K	2	1.5448	15.96**
	N × P	4	0.3314	3.42
	N × K	4	0.0987	1.02
	P × K	4	0.1010	1.04
	N × P × K	8	0.0968	
	Error	(23)	(0.0572)	
22†	N	2	82.498	7.76*
	P	2	187.233	17.60**
	K	2	97.889	9.20**
	N × P	4	16.370	1.54
	N × K	4	10.507	—
	P × K	4	16.641	1.56
	N × P × K	8	10.638	
	Error	(27)	(2.988)	
29‡	N	4	0.8318	16.31***
	P	4	3.0737	60.27***
	K	4	1.1867	23.27***
	N × P	16	0.1373	2.69**
	N × K	16	0.0488	—
	P × K	16	0.1382	2.71**
	N × P × K	64	0.0510	
	Error	(125)	(0.0289)	
37‡	N	4	0.7454	8.91***
	P	4	8.3978	100.33***
	K	4	0.7975	9.53***
	N × P	16	0.1629	1.95*
	N × K	16	0.0884	1.06
	P × K	16	0.2111	2.52**
	N × P × K	64	0.0837	
	Error	(125)	(0.0525)	
44‡	N	4	0.8649	9.27***
	P	4	12.1716	130.46***
	K	4	1.3946	14.95***
	N × P	16	0.2066	2.21*
	N × K	16	0.0853	—
	P × K	16	0.1805	1.93*
	N × P × K	64	0.0933	
	Error	(125)	(0.0740)	

TABLE 1 (*continued*)

Days from Sowing	Source of Variation	Degrees of Freedom	Mean Square	F
98§†	N	4	0.931	4.39**
	P	4	15.816	74.60***
	K	4	1.432	6.75***
	N×P	16	0.540	2.55**
	N×K	16	0.147	—
	P×K	16	0.221	1.04
	N×P×K	62	0.212	

* $P = 0.01-0.05$.

† Untransformed values.

** $P = 0.001-0.01$.

‡ Logarithmic transformation.

*** $P < 0.001$.

§ Pots without sub-treatment only.

In view of the considerable differences in size at 44 days between plants subjected to the same treatments, and the importance attached to accurate estimation of the effects of the sub-treatments which were then about to be applied, the length and breadth of the largest leaf of each plant harvested at this time and of the remaining plant in each pot were measured; these measurements enabled analysis of covariance to be applied to the sub-treatment effects.

When the final harvest of all remaining plants was taken at 98 days, hearting was already well in progress in the best grown plants, though commercial maturity had not yet been reached.

III. STATISTICAL TREATMENT OF DATA

(a) Analysis of Variance

Except in the first harvest, the main analysis of the results was conducted on dry weights of aerial parts only, the root weight data being considered less reliable through losses in removing from the pots. These dry weight data were subjected to analysis of variance. In the harvests from 29 days onwards it was found that the differences between duplicate weights were nearly proportional to their means. The logarithmic transformation was accordingly applied, though this tends to over-correct at the highest weights. For the first two harvests with data from only 27 treatments the untransformed weights were used, since here the dependence of variance on mean was slight.

At each harvest the variances due to each of the three initial treatment series and their first and second order interactions were separated. A "within-treatments" error term was calculated, but could not be used in testing the significance of main effects and interactions, because a position effect was confounded with them on account of the non-random distribution of the replicate pots. The $N \times P \times K$ interaction was used instead in testing for significance. The results of analysis of variance at each harvest date are shown in Table 1.

In the analysis of sub-treatment effects at 98 days, the final shoot dry weights have been corrected for differences in size at the time the sub-treatments were applied. In order to do this, an analysis of covariance was performed, the dependent variable being log dry weight at 98 days, and the independent variable log (product of length and breadth of largest leaf at 44 days).

(b) *Missing Values*

In its final stage the experiment comprised 500 different treatments and sub-treatments, without replication. Of these 500 plants 97 died before the final harvest, of which 16 were lost by accident or fungal attack, while the remaining 81 died without overt signs of pathogenic disease, probably as a result of the nutritional treatment to which they had been subjected (most were in the low-phosphorus treatments). It was necessary to replace these missing values before proceeding to the analysis of covariance.

Since the 81 plants dying as a presumed result of the nutritional treatments were very small, and their growth had appeared stationary since the harvest at 44 days, it was assumed that their dry weights had not increased appreciably during this period; accordingly, their weights at 44 days were estimated, and were used in the analysis for 98 days. These estimates were calculated from a regression on the measurements intended for the final analysis of covariance. Computing the regression was made more difficult by the fact that the plants had been paired before weighing. Since in this way only the mean weight of each pair was available for relating to the individual leaf measurements, and a linear regression seemed unsuitable, only pairs in which the two plants were reasonably similar in size could be used in estimating the regression. The standard of similarity arbitrarily chosen was such that the weight of the larger plant was not more than one and a half times that of the smaller. Of the 250 pairs of values, 87 met this requirement. Of various possible regressions of plant dry weight on leaf measurements that were tested, the linear regression of the logarithm of dry weight on that of the product of length and breadth of the largest leaf was found to give the most satisfactory results. This regression was highly significant ($r = 0.995$; $n = 85$) and led to the following expression

$$\log (W_1 + W_2) = 0.593 + 1.100 \log (L_1 B_1 + L_2 B_2),$$

where W_1 , W_2 are the weights of the two plants, and L_1 , L_2 , B_1 , B_2 the lengths and breadths of their largest leaves. If the two plants are assumed to be equal in size and weight, this reduces to

$$\log W = 0.623 + 1.100 \log LB,$$

for one plant. This method of estimating dry weight was used only when the loss of the plant could reasonably be ascribed to nutrient deficiency, but not when the plant had grown to any extent after 44 days or when a sub-treatment had been applied which would have been expected to induce a response. As the estimates were based on the dimensions of the same plants, no degrees of freedom were deducted for them in the analysis of variance.

The method used to replace missing values for the other 16 plants was the one, of several tried, which gave the closest fit when applied to existing

values. The regression of $\log W$, 98 days, on $\log LB$, 44 days, was calculated for the 101 surviving plants which received no sub-treatment. From this regression the calculated dry weight, based on the leaf dimensions, was found for each of the 403 surviving plants. The differences between the actual dry weights and those calculated from the regression were the variates used in fitting the 16 missing values. The mean difference was found for each level of N, P, and K in each of the four sub-treatment groups; interactions among

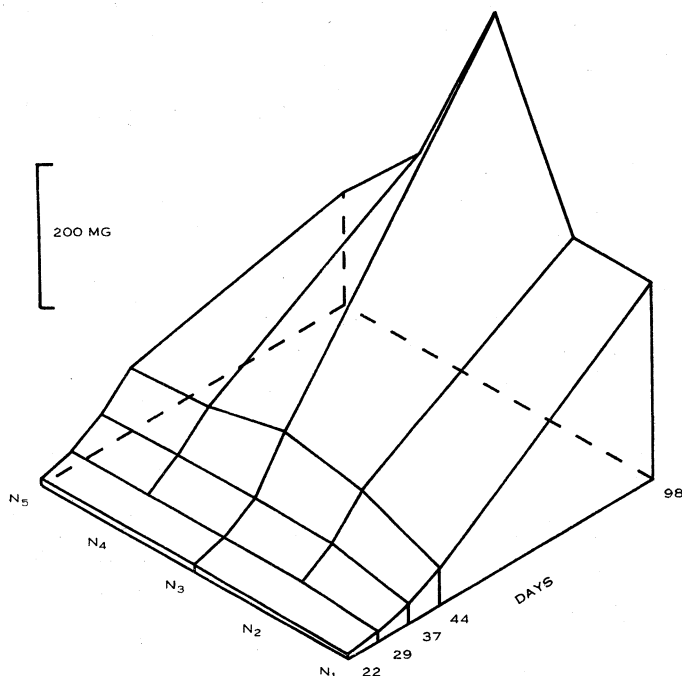


Fig. 1.—Effect of nitrogen supply on dry weight at different stages of development (logarithmic means except at 22 days).

the initial treatments were not taken into account. There were thus 60 means ($3 \times 5 \times 4$) each based on 25 values, less any estimated or missing values. The fitted value was obtained by adding to the calculated dry weight (from the regression equation) a correction term based on the mean of the three relevant mean differences. For example, the correction term for the treatment $N_3P_4K_2$, without sub-treatment, was the mean of the mean differences for N_3 , P_4 , and K_2 , all without sub-treatment. One degree of freedom was subtracted for each of these fitted values in the analysis of variance.

IV. RESULTS

(a) Appearance and Dry Weight as Affected by Initial Treatments

(i) *Nitrogen Initial Treatments.*—Plants receiving the treatments N_1 and N_2 were small and generally pale. Yellowing of the leaves was evident within

a week from emergence, and after a month was most marked at the tips of the older leaves. Hearts were not formed by N_1 plants. The mean dry weight for each nitrogen treatment at each harvest is shown in Table 2; the significant differences* quoted, together with the relevant entries in the analyses of variance (Table 1), enable the significance of the various effects to be assessed. To facilitate consideration of these effects, they are presented in the form of a solid diagram in Figure 1.

The dry weights of the whole plants at 11 days showed a distinct, but non-significant increase over the range N_1 - N_3 - N_5 . At later harvests, however, the treatment N_5 appeared to be supra-optimal. At 29 days there was no significant change in dry weight over the range N_2 - N_5 . At 37 days the increase between N_1 and N_2 was followed by a smaller increase up to N_3 - N_4 , and a decline at N_5 . This trend was continued at 44 days, when the maximum was again reached at N_3 , and the decline to N_5 was more marked. The dry weights at 98 days also showed a maximum at N_3 , and here the decline between N_4 and N_5 was very substantial.

TABLE 2
EFFECT OF NITROGEN SUPPLY ON DRY WEIGHT (MG)

Nitrogen Supply Level	Days from Sowing					
	11	22	29*	37*	44*	98*
1	1.7	4.1	12	27	54	277
2	—	—	23	50	101	278
3	1.8	7.7	23	53	122	538
4	—	—	24	52	97	277
5	2.0	7.9	23	49	90	153
Significant difference	0.34	3.55	—	—	—	—
Significant ratio	—	—	1.34	1.46	1.49	1.82

* Logarithmic means.

(ii) *Phosphorus Initial Treatments.*—The visual effects of phosphorus deficiency were not evident as early as those of nitrogen or potassium, but differences in size were apparent a fortnight after emergence. After 4 weeks, plants from treatments P_1 and P_2 were yellow, but the characteristic red and purple tints usually associated with phosphorus deficiency (e.g. Wallace 1951) were not observed. In many cases, however, the outer leaves were withered. Except for a few P_3 plants, those with phosphorus supply below P_4 did not heart. At later stages, most of the plants with low phosphorus supply showed little or no growth, and many died.

* Based here, as in Tables 3 and 4, on the $N \times P \times K$ interaction, the replication being ignored.

In each harvest from 22 to 44 days, increased phosphorus supply resulted in a marked increase in dry weight over the whole range P_1 - P_5 (Table 3, Fig. 2). In the 98-day harvest, however, the increase from P_4 to P_5 was negligible. In general, increases in growth with increasing phosphorus supply were more substantial than for either of the other nutrients — at 44 days, for instance, the mean dry weight for P_5 was nearly 14 times that for P_1 , while corresponding ratios for nitrogen and potassium were in each case about 3.

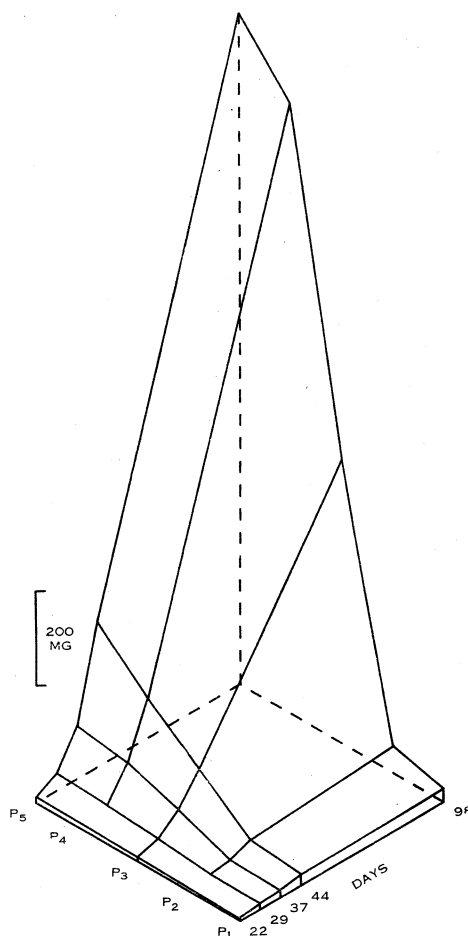


Fig. 2.—Effect of phosphorus supply on dry weight at different stages of development (logarithmic means except at 22 days).

(iii) *Potassium Initial Treatments.*—Within one week from emergence, plants from K_1 , and to a smaller extent from K_2 , showed slower growth than those from higher potassium levels. After 1 month several features which have apparently not been described previously for potassium-deficient plants were evident. The leaves were thick and rounded, in some cases heart-shaped, becoming smoother and rather fleshy when older. The margins were flat and

almost entire while those of normal plants were crinkled and indented. The effect on colour was not very marked, but generally the leaves were a dull, dark green with paler midribs and veins. During later stages of growth the leaves tended to become hook-shaped, as if by increased growth of the midrib. The stem bases were thickened and root systems extremely poor. Plants from K_1 did not heart.

Potassium deficiency affected the rate of growth visibly, before deficiency of either of the other nutrients, and this is again shown in the analysis of variance of nutrient effects on dry weight (Tables 1 and 4, Fig. 3). At 11 days the effect of potassium on the untransformed dry weights of whole plants was highly significant ($P < 0.01$), although none of the other treatment or interaction effects reached the 5 per cent. level. In all subsequent harvests the plants receiving no potassium (K_1) were considerably smaller than the others, but among the latter, differences were less marked and rather irregular. In the final harvest there is a suggestion of an optimum at K_3 .

TABLE 3
EFFECT OF PHOSPHORUS SUPPLY ON DRY WEIGHT (MG)

Phosphorus Supply Level	Days from Sowing					
	11	22	29*	37*	41*	98*
1	1.8	3.5	10	15	24	28
2	—	—	12	18	32	57
3	1.7	6.3	24	61	120	600
4	—	—	30	97	206	1295
5	1.9	9.9	38	114	311	1422
Significant difference	0.34	3.55	—	—	—	—
Significant ratio	—	—	1.34	1.46	1.49	1.82

* Logarithmic means.

(iv) *Interactions of Initial Treatments.*—The principal interest in these data centres in the interaction effects between the three nutrients. From Table 1 it will be seen that there is a significant interaction between phosphorus and nitrogen in all harvests from 29 days onwards and between phosphorus and potassium at 29, 37, and 44 days.

The nitrogen-phosphorus interactions are illustrated in Figure 4, which shows in the form of solid diagrams the mean dry weight for each nitrogen-phosphorus combination at each harvest. A diagram for the 22-day harvest is included, the effect being already in evidence at this stage, though not yet significant. An increase in the nitrogen supply from N_1 to N_2 increases the proportional response to phosphorus at all harvests and from 44 days onwards a further rise in the response to phosphorus can be seen when the nitrogen supply is

increased to N_3 ; the latter rise, however, occurs only at the upper phosphorus levels—the proportional increase in yield due to increase in phosphorus supply up to P_3 is unaffected by nitrogen above N_2 . The increase in dry matter with increase in phosphorus supply occurs throughout the range if the nitrogen supply is adequate, but if not (i.e. at N_1) dry matter increases only up to P_3 .

If one now considers the response to nitrogen at different levels of phosphorus, one notices that at the lowest level (P_1) the dry matter yield varies only slightly with nitrogen supply. At higher phosphorus levels the responses to nitrogen become progressively more marked. This is true both of the positive responses up to N_3 or N_4 and of the negative responses when this optimum is exceeded. The optimum nitrogen level does not seem to depend greatly on the phosphorus supply. The magnitude of the interaction effects, like those of the mean responses, tended to increase as the plants grew older.

TABLE 4
EFFECT OF POTASSIUM SUPPLY ON DRY WEIGHT (MG)

Potassium Supply Level	Days from Sowing					
	11	22	29*	37*	44*	98*
1	1.5	4.0	11	27	46	111
2	—	—	22	51	110	283
3	1.9	7.1	22	52	99	465
4	—	—	28	56	119	380
5	2.0	8.6	23	46	98	316
Significant difference	0.34	3.55	—	—	—	—
Significant ratio	—	—	1.34	1.46	1.49	1.82

* Logarithmic means.

Figure 5 illustrates the interactions between phosphorus and potassium supply. In the absence of added potassium (K_1) dry weight increased with increasing phosphorus supply only up to P_3 . At the potassium levels K_2 and K_3 , the increase continued up to P_4 and in later harvests to P_5 . At the highest potassium levels, increased phosphorus supply caused increase in yield throughout the range even at 29 days.

At any given level of phosphorus supply, the maximum dry weight usually occurred at an optimum intermediate level of potassium supply, this optimum being somewhat displaced according to the phosphorus supply. At the lower levels of phosphorus, K_3 generally represented the optimum; for higher levels it was K_4 .

The nitrogen-potassium interaction is nowhere significant if tested rigorously against the second order interaction; but the variance due to it was significantly greater than that between replicates (which, as already explained, did not

involve any position effect) at 22, 29, and 37 days. Since at each harvest the interaction is in the same direction, it may probably be regarded as meaningful; it is shown in the solid diagrams of Figure 6. The proportional increase in dry weight with increasing potassium supply is greater at higher levels of nitrogen supply. This is particularly true if N_1 and K_1 are compared with the means of N_2-N_5 and K_2-K_5 as is shown by the values in Table 5.

No rigorous test of the significance of the second order interaction is possible, since it is confounded with a position effect, probably small; but the two together are significantly greater than the variance between replicates at 22, 29, and 37 days. This second order interaction takes the form of a reinforcement of first order interactions at favourable levels of the third factor. Consider, for instance, that part of the interaction between phosphorus and potassium, which is represented by

$$(K_3 P_5 - K_1 P_5) - (K_3 P_1 - K_1 P_1).$$

The values for this interaction element at successive harvests for different levels of nitrogen supply are shown in Table 6.

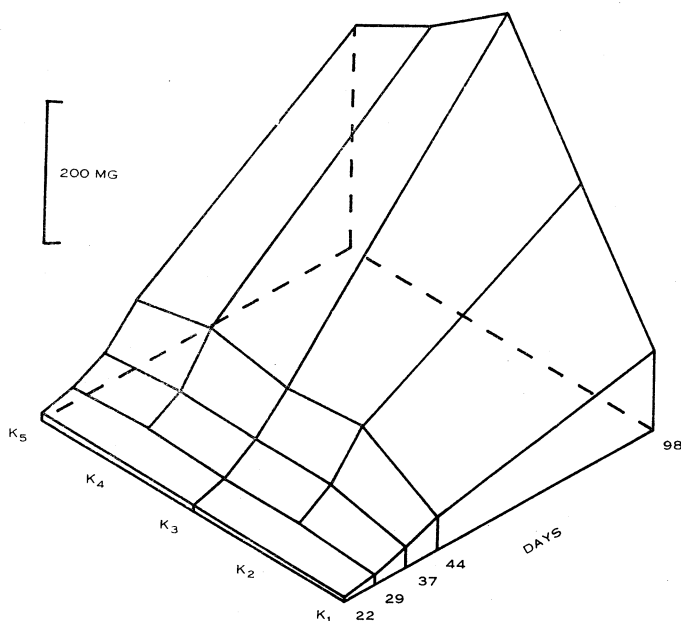


Fig. 3.—Effect of potassium supply on dry weight at different stages of development (logarithmic means except at 22 days).

(b) Relative Growth Rate as Affected by Initial Treatments

Relative growth rates (R.G.R.) were calculated for each of the periods from 11 to 44 days, for the 27 treatments in which data for all these harvests were available. For this purpose, of course, dry weights of the whole plant were required, and it was therefore necessary to include the rather unsatisfactory root dry weight data—the contribution of which was, however, comparatively

small. These growth rates were subjected to analysis of variance, and the results are presented in Tables 7 and 8. It will be noted that the mean R.G.R. was highest in the earliest period, and then fell to a steady level.

TABLE 5
MEAN PERCENTAGE INCREASE IN DRY WEIGHT WITH
POTASSIUM SUPPLY AT DIFFERENT NITROGEN LEVELS

Days from Sowing	N ₁	N ₂ -N ₅
11*	48	32
22*	66	118
29	37	138
37	23	109
44	42	162
98	60	278

* Levels 1, 3, 5 only.

Increase in nitrogen supply from N₁ to N₃ appears to have increased R.G.R. during most periods, although the effect did not at any stage reach significance. Above this level, there was little change.

From 11 to 22 days, potassium was the only nutrient having a significant effect on R.G.R. When its supply was raised from K₁ to K₃, there was a substantial rise in R.G.R., but further increase did not affect it. The effect of

TABLE 6
P × K INTERACTION AT DIFFERENT NITROGEN LEVELS (SEE TEXT)

Nitrogen Level	Days from Sowing					
	11*	22*	29†	37†	44†	98†
N ₁	-0.29	-3.58	-0.44	-0.10	-0.57	-1.55
N ₂	—	—	0.47	0.58	0.11	0.41
N ₃	0.15	8.59	0.78	0.48	0.75	0.53
N ₄	—	—	0.83	0.55	1.14	1.15
N ₅	1.04	8.61	0.59	1.27	0.74	0.89

* Absolute weights.

† Log weights.

potassium persisted during subsequent development, though it did not reach significance.

After 22 days the place of potassium as the important factor controlling R.G.R. was taken by phosphorus. Except in one instance, increase in phos-

TABLE 7
RELATIVE GROWTH RATE (PER CENT. PER DAY)

Nitrogen Levels		N ₁				N ₃				N ₅				Mean N ₁ -N ₅			
Days from Sowing	Phosphorus Level Potassium Level	P ₁	P ₃	P ₅	Mean P ₁ -P ₅	P ₁	P ₃	P ₅	Mean P ₁ -P ₅	P ₁	P ₃	P ₅	Mean P ₁ -P ₅	P ₁	P ₃	P ₅	Mean P ₁ -P ₅
11-22	K ₁	14	10	14	12.7	11	15	12	12.7	12	13	15	13.3	12.3	12.7	13.7	12.9
	K ₃	17	13	12	14.0	17	17	20	18.0	14	18	22	18.0	16.0	16.0	18.0	16.7
	K ₅	15	16	15	15.3	19	19	22	20.0	17	15	20	17.3	17.0	16.7	19.0	17.6
22-29	Mean K ₁ -K ₅	15.3	13.0	13.7	14.0	15.7	17.0	18.0	16.9	14.3	15.3	19.0	16.2	15.1	15.1	16.9	15.7
	K ₁	14	12	15	13.7	11	15	8	11.3	10	15	12	12.3	11.7	14.0	11.7	12.4
	K ₃	14	10	18	14.0	11	18	20	16.3	12	12	21	15.0	12.3	13.3	19.7	15.1
29-37	K ₅	13	12	15	13.3	9	20	18	15.7	11	13	23	15.7	11.0	15.0	18.7	14.9
	Mean K ₁ -K ₅	13.7	11.3	16.0	13.7	10.3	17.7	15.3	14.4	11.0	13.3	18.7	14.3	11.7	14.1	16.7	14.1
	K ₁	6	15	9	10.0	6	15	14	11.7	6	7	6	6.3	6.0	12.3	9.7	9.3
37-44	K ₃	2	18	9	9.7	10	13	14	12.3	-2	18	16	10.7	3.3	16.3	13.0	10.9
	K ₅	-3	6	4	2.3	6	-2	12	5.3	9	12	10	10.3	4.0	5.3	8.7	6.0
	Mean K ₁ -K ₅	1.7	13.0	7.3	7.3	7.3	8.7	13.3	9.8	4.3	12.3	10.7	9.1	4.4	11.3	10.4	8.7
37-44	K ₁	-2	7	14	6.3	3	4	16	7.7	1	14	17	10.7	0.7	8.3	15.7	8.2
	K ₃	7	2	12	7.0	-2	6	19	7.7	13	9	12	11.3	6.0	5.7	14.3	8.7
	K ₅	7	14	13	11.3	16	16	22	18.0	0	18	12	10.0	7.7	16.0	15.7	13.1
37-44	Mean K ₁ -K ₅	4.0	7.7	13.0	8.2	5.7	8.7	19.0	11.1	4.7	13.7	13.7	10.7	4.8	10.0	15.2	10.0

phorus supply resulted in a rise in R.G.R. During the period from 22 to 29 days, two phosphorus interactions also reached significance. Of these, the meaning of the $N \times P$ interaction is far from obvious, and it may well be a chance effect. The $P \times K$ interaction is clearer. At K_1 , increase in the phosphorus

TABLE 8
RELATIVE GROWTH RATE: ANALYSIS OF VARIANCE

Days from Sowing	Source of Variation	Degrees of Freedom	Mean Square	F
11-22	N	2	20.6	4.05
	P	2	9.4	1.97
	K	2	55.8	10.97**
	$N \times P$	4	8.6	1.69
	$N \times K$	4	6.2	1.22
	$P \times K$	4	0.4	—
	$N \times P \times K$	8	5.1	—
22-29	N	2	1.6	—
	P	2	56.3	11.95**
	K	2	19.7	4.18
	$N \times P$	4	24.3	5.16*
	$N \times K$	4	6.0	1.27
	$P \times K$	4	20.4	4.33*
	$N \times P \times K$	8	4.7	—
29-37	N	2	14.4	—
	P	2	126.4	7.02*
	K	2	56.2	3.12
	$N \times P$	4	26.6	1.48
	$N \times K$	4	31.2	1.73
	$P \times K$	4	29.0	1.61
	$N \times P \times K$	8	18.0	—
37-44	N	2	21.8	—
	P	2	245.5	10.98*
	K	2	65.8	2.94
	$N \times P$	4	21.9	—
	$N \times K$	4	32.2	1.44
	$P \times K$	4	31.2	1.40
	$N \times P \times K$	8	22.4	—

* $P = 0.01-0.05$.

** $P = 0.001-0.01$.

supply had no effect on R.G.R., but at K_3 and K_5 plants receiving the highest phosphorus supply (P_5) had a higher R.G.R. than those at an intermediate level (P_3). Increase in potassium supply increased R.G.R. only when phosphorus was abundant.

(c) Sub-Treatment Effects

The effects of the treatments applied at 44 days were studied on the basis of the analysis of covariance, using the logarithm of shoot dry weight at 98 days as the dependent and the logarithm of leaf dimensions at 44 days as the inde-

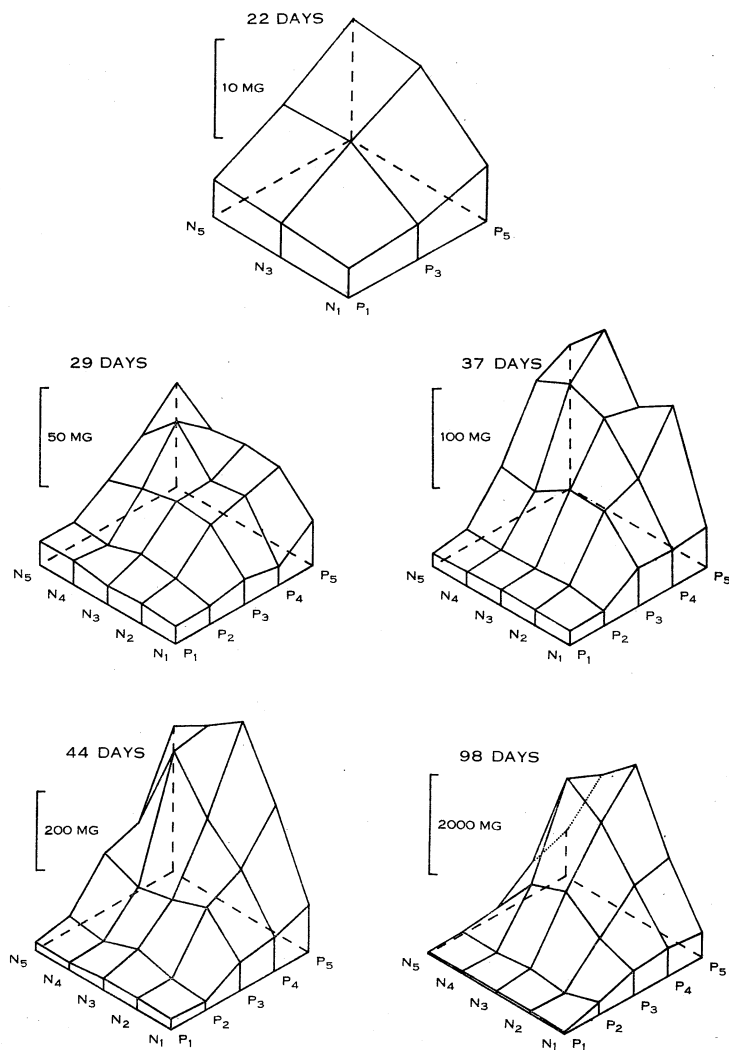


Fig. 4.—Interaction effects of nitrogen and phosphorus supply on dry weight (logarithmic means except at 22 days).

pendent variable. Since analysis of the data as a whole would not have led to a division of the degrees of freedom conveniently displaying the effects of each of the three sub-treatments in comparison with the common control, the data have been analysed in three separate portions, enabling these comparisons to

be made separately. But a common estimate of error was used in all three portions, based on the whole experiment. For this estimate the third order interaction between sub-treatments and main treatments ($S \times N \times P \times K$) was used, with 175 degrees of freedom; consideration of the independent variable reduced this error variance from 0.203 to 0.129.

As the quantities in these analyses are logarithms, the effects tested are the percentage responses. These were evaluated wherever they exceeded the significance level of 0.05, as follows:

$$\text{Percentage response} = \left\{ \text{antilog} \frac{(y_s - y_0) - b(x_s - x_0)}{n} - 1 \right\} \times 100,$$

where y_s is the total of n values of log dry weight at 98 days for sub-treatment S ,
 y_0 is the total of n values of log dry weight at 98 days without sub-treatment,

x_s is the total of n values of log product of leaf length and breadth at 44 days for sub-treatment S , and

x_0 is the total of n values of log product of leaf length and breadth at 44 days without sub-treatment.

The value of the regression coefficient b was obtained from the analysis of covariance for all sub-treatments: $b = 0.928$.

(i) *Nitrogen Sub-Treatment*.—The analysis of covariance is presented in Table 9, and the calculated values of some of the responses are given in Table 10. It may be seen that the average effect of the nitrogen sub-treatment was very small; this average conceals a fairly marked difference between a negative response at N_3 and N_4 and a positive response at N_1 and N_2 . The positive response at N_1 and N_2 was to be expected; the negative response at N_3 and N_4 must have been due to the total nitrogen supply reaching a toxic level, but as the sub-treatment (equal to the initial nitrogen supply in N_2) represented only a 4 per cent. increase in total nitrogen supply at N_5 , no further reduction in growth could be demonstrated here. This interaction between the nitrogen sub-treatment and the initial treatment just fails to reach significance.

A more definite interaction is found between the nitrogen sub-treatment and the initial phosphorus treatment ($S_N \times P$). A positive response of approximately 50 per cent. occurs at P_4 and P_5 ; at P_3 there is no response, and at the lowest phosphorus levels there is a negative response.

The responses to the nitrogen sub-treatment at different levels of initial nitrogen and phosphorus treatments become somewhat clearer when the second order interaction between all three factors ($S_N \times N \times P$) is considered. This interaction, though not reaching significance, indicates that the major positive responses to the nitrogen sub-treatment occurred in those cultures where initial nitrogen supply was low and phosphorus high. With other combinations of initial treatments, the response to nitrogen was usually small or negative—either because the nitrogen supply was already ample, or because the phosphorus status of the plants was too low to permit increased growth.

The only second order interaction of nitrogen sub-treatment with initial treatments which reached significance was that with phosphorus and potassium ($S_N \times P \times K$). Details of this interaction are also given in Table 10. Although there are several anomalous values in this table, the general trend is towards higher positive responses to nitrogen wherever the supply of phosphorus and potassium were both adequate for good growth (P_4 and P_5 , and K_2 - K_5). For example, there is no distinct trend at P_1 , but at P_5 there is a progressive increase in response from K_1 to K_5 .

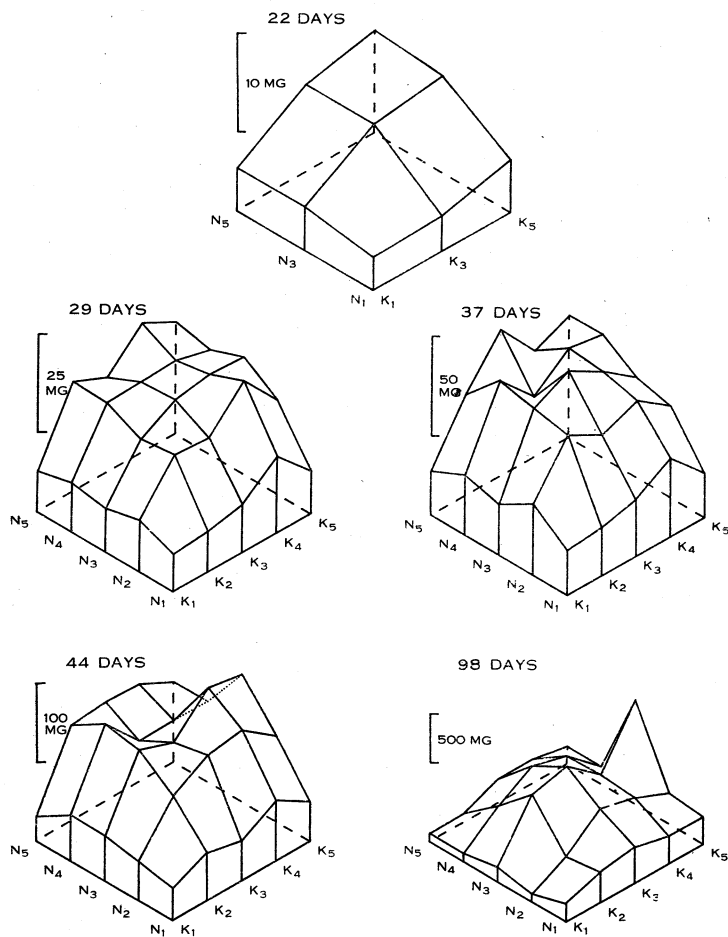


Fig. 5.—Interaction effects of phosphorus and potassium supply on dry weight (logarithmic means except at 22 days).

(ii) *Phosphorus Sub-Treatment*.—The overall effect of phosphorus sub-treatment is highly significant (S_P , Table 9), as also are the interactions with nitrogen initial treatment ($S_P \times N$) and with phosphorus initial treatment ($S_P \times P$). The significance of the second order interaction between phosphorus

sub-treatment and nitrogen and phosphorus initial treatments ($S_P \times N \times P$) almost reaches the 1 per cent. level. The calculated values of these responses are given in Table 10.

The effect of the sub-treatment was strongly dependent on the phosphorus initial treatment, the magnitude of the response declining steadily from P_1 to P_5 .

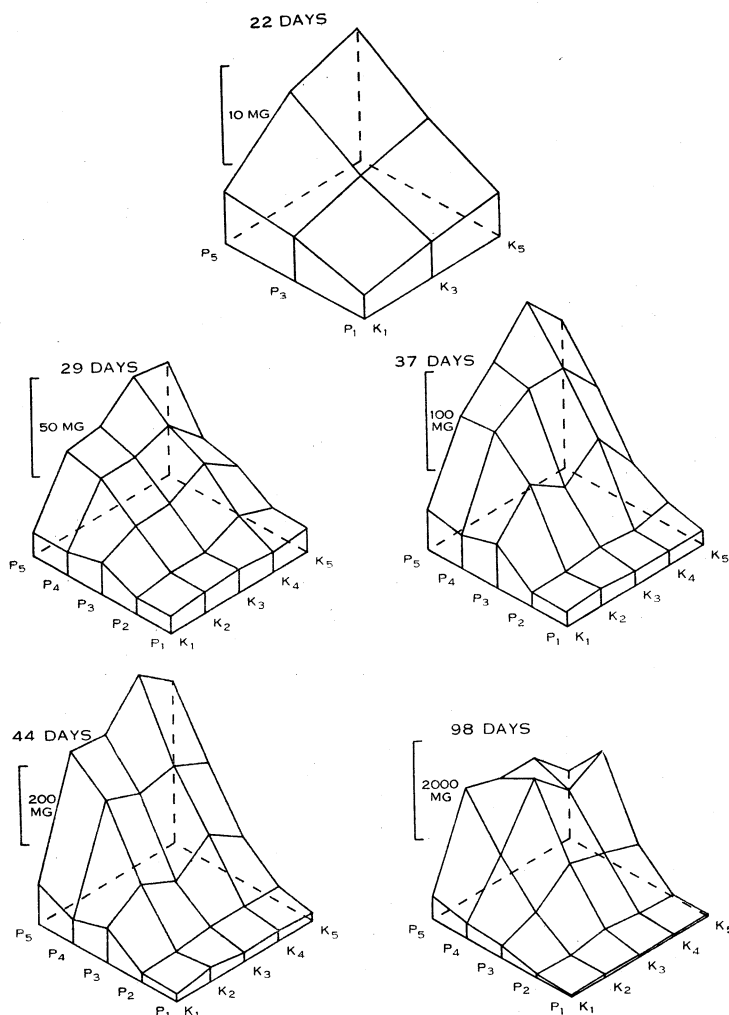


Fig. 6.—Interaction effects of nitrogen and potassium supply on dry weight (logarithmic means except at 22 days).

The significant interaction with nitrogen supply already mentioned depends on the difference between a small response to phosphorus at N_1 , and the much larger, although variable, responses at higher nitrogen levels. The effect may be specified more precisely from the second order interaction; generally the response was largest at the optimum levels of nitrogen supply (N_2 - N_4), although

at P_1 and P_2 there was a positive response at each nitrogen level. The responses at P_3 were small, and at P_4 and P_5 they were negligible. Inspection of the original data shows that over the range of initial treatments where yield was increasing as a result of increases in initial phosphorus supply, positive responses to the phosphorus sub-treatment were found. There is no evidence of adverse effects of excess within the range of phosphorus supply used in this experiment.

TABLE 9

SUB-TREATMENT EFFECTS: ANALYSIS OF COVARIANCE

All mean squares have been compared with the all-subtreatments error term to determine F . Degrees of freedom = 175; mean square = 0.129

Sub-Treatment	Source of Variation	Degrees of Freedom	Reduced Mean Square	F
Nitrogen	S_N	1	0.013	—
	$S_N \times N$	4	0.276	2.16
	$S_N \times P$	4	0.333	2.60*
	$S_N \times K$	4	0.016	—
	$S_N \times N \times P$	16	0.175	1.37
	$S_N \times N \times K$	16	0.082	—
	$S_N \times P \times K$	16	0.234	1.83*
Phosphorus	S_P	1	18.524	143.60***
	$S_P \times N$	4	0.744	5.76***
	$S_P \times P$	4	4.318	33.47***
	$S_P \times K$	4	0.097	—
	$S_P \times N \times P$	16	0.266	2.06*
	$S_P \times N \times K$	16	0.121	—
	$S_P \times P \times K$	16	0.122	—
Potassium	S_K	1	0.091	—
	$S_K \times N$	4	0.200	1.55
	$S_K \times P$	4	0.160	1.24
	$S_K \times K$	4	1.022	7.92***
	$S_K \times N \times P$	16	0.099	—
	$S_K \times N \times K$	16	0.099	—
	$S_K \times P \times K$	16	0.129	1.00

* $P = 0.01-0.05$.

** $P = 0.001-0.01$.

*** $P < 0.001$.

(iii) *Potassium Sub-Treatment*.—In this case only the interaction between sub-treatment and potassium initial treatment ($S_K \times K$) is significant (Table 9). The only definite response is at K_1 ; the others are small, and negative above K_2 . Although the other first order interactions are not significant, Table 10 shows that the response is positive at P_4 and P_5 , and negative at the lower phosphorus levels—a result reminiscent of the effect of phosphorus supply on nitrogen response mentioned above. There is no clear relationship with nitrogen supply.

V. DISCUSSION

The first point to be made in discussion is, perhaps, the physiological dissimilarity in the ranges tested for the three nutrients. In the case of nitrogen, there seems no doubt that the optimum supply, for the conditions of the experiment, was exceeded. The highest potassium supply was also, it would appear, in excess of that needed for maximum growth; and even where potassium was completely omitted from the nutrient solution (K_1) the plants could still make a reasonable amount of growth. But, for phosphorus, the optimum has barely been reached; at the higher nitrogen levels, there is still an increase in growth between P_4 and P_5 , and the effect of the phosphorus sub-treatment, though small, is positive. On the other hand, at the lower levels of phosphorus supply, the plants could barely survive, let alone grow. The phosphorus treatments were thus pitched at a lower physiological level than those of either of the other nutrients.

The marked adverse effects of high nitrogen doses, even where phosphorus supply was adequate, are noteworthy. Adverse effects on yield or quality of seed and fruit crops are commonplace, but in a purely vegetative crop like the lettuce it is more surprising. The optimum region for nitrogen would seem to be much more circumscribed than that for potassium, where the differences in growth over the range K_2 - K_5 were small although the supply varied by a factor of 30.

The data for R.G.R. do not assist greatly in the interpretation of the results. It must be remembered that they are based on less than a quarter of the results, so the lower significance levels than are seen in the analyses of dry weight data are not surprising. In general, they do not suggest that there is one stage of development more than another at which the nutrient effects on growth rate are particularly marked. The range of figures for R.G.R. appears much smaller than for the dry weight data, but since R.G.R. is a "compound interest" rate, and the differences persist in general throughout the period studied, this was to be expected. It is unfortunate that the data collected did not enable net assimilation rates to be calculated.

The responses to the sub-treatments at 44 days, though essential for correlating with the analytical data, do not in themselves add much to our knowledge of the nutrient status of the plants at that time. Where the effects of the initial treatments gave reason to suppose that a particular plant was suffering from deficiency of a nutrient, an additional supply of that nutrient increased its weight over that of the untreated pot; where there was already more than enough, an addition might depress growth.

These data provide interesting material for comparison with certain formal treatments of nutrient effects and interactions which are to be found in the literature. von Liebig's (1840) "Law of the Minimum," based on even less experimental evidence than Blackman's (1919) very similar Law of Limiting Factors in photosynthesis, postulated that the curve connecting yield with nutrient supply had a linear rising portion and an abrupt transition to a horizontal portion; the supply of another nutrient would affect only the point of transition.

This law has been long discredited in the form in which Liebig presented it (though one still often reads of a nutrient as being "in minimum"); and it fails to find support in the present data except perhaps as an extreme limiting

TABLE 10
PER CENT. RESPONSES TO SUB-TREATMENTS

*Nitrogen Sub-Treatment**

Average Effects at Different Levels of Initial Treatments

N ₁	+25	P ₁	-16	K ₁	-3
N ₂	+62	P ₂	-37	K ₂	+8
N ₃	-30	P ₃	-1	K ₃	-7
N ₄	-22	P ₄	+47	K ₄	+11
N ₅	+7	P ₅	+54	K ₅	+10

Interactions between Initial Treatments

	P ₁	P ₂	P ₃	P ₄	P ₅
N ₁	-4	-81	+8	+257	+345
N ₂	-30	+111	+58	+107	+130
N ₃	-45	-44	-17	-9	-32
N ₄	+25	-54	-57	+5	+11
N ₅	-12	-4	+56	-2	+11
K ₁	+24	-48	+115	-34	-9
K ₂	-67	-11	+63	+88	+7
K ₃	+43	-78	+19	+48	+27
K ₄	-49	-5	-27	+103	+132
K ₅	+38	+7	-69	+17	+196

Phosphorus Sub-Treatment†

Average Effects at Different Levels of Initial Treatments

N ₁	+36	P ₁	+1790	K ₁	+200
N ₂	+460	P ₂	+1020	K ₂	+260
N ₃	+290	P ₃	+130	K ₃	+200
N ₄	+410	P ₄	+16	K ₄	+240
N ₅	+250	P ₅	-7	K ₅	+390

Interactions between Initial Treatments

	N ₁	N ₂	N ₃	N ₄	N ₅
P ₁	+1470	+1470	+3830	+5230	+370
P ₂	+11	+3970	+1460	+1300	+1730
P ₃	-46	+370	+41	+280	+370
P ₄	-22	+100	+12	+3	+16
P ₅	-38	-10	-8	+15	+19

TABLE 10 (*continued*)
Potassium Sub-Treatment†
 Average Effects at Different Levels of Initial Treatments

N ₁	-16	P ₁	0	K ₁	+220
N ₂	+62	P ₂	-8	K ₂	+22
N ₃	-7	P ₃	-18	K ₃	-43
N ₄	-11	P ₄	+34	K ₄	-7
N ₅	+37	P ₅	+52	K ₅	-25

* Average overall effect = +3.

† Average overall effect = +250.

‡ Average overall effect = +9.

condition — the effects of increasing phosphorus supply when nitrogen or potassium is extremely low may approximate to the situation it implies.

Mitscherlich's (1909) formulation of the relationship between yield and nutrient supply has had much more consistent support, particularly in Europe and latterly in South America. This, as is well known, relates the gradient of the curve to the shortfall of the yield below an assumed maximum; it is a particular mathematical form of the Law of Diminishing Returns, so familiar in economics. In the present data, the curves representing effect of increase in the supply of a single nutrient, the others being held constant (i.e. the sections of the solid diagrams in Figs. 1-6), are in adequate agreement with Mitscherlich's representation of growth-nutrient relations, provided his later introduction of a "damage factor" is accepted (Mitscherlich 1928). But five points do not enable a satisfactory test of this method of dealing with the data to be made.

The most serious criticisms of Mitscherlich's view (see, for instance, Briggs 1925; Balmukand 1928) have been levelled against his treatment of nutrient interactions. He claimed that the "effect factor" was constant, irrespective of the supply of a second nutrient (and, indeed, of climatic environment, crop variety, or species, and any other factor which might influence the result). This claim has been disproved sufficiently frequently to need no further attention, were it not that, in certain quarters, much attention is still paid to Mitscherlich's work and the criticisms ignored. The present data may accordingly be used to buttress the opposition to his claims and to draw attention again to their inadequacy.

If Mitscherlich's effect factor were constant, the ratio of yields at two particular levels of phosphorus supply, say, would be independent of nitrogen supply. But at 44 days, for instance, the ratios of dry matter yields with P₁ and P₅ were N₁ : 4.0, N₂ : 11.0, N₃ : 17.0, N₄ : 20.8, and N₅ : 17.0.

Mitscherlich sought to answer some of the criticisms by his introduction of the damage factor. If this were assumed constant, like the effect factor, the ratio of yields with two concentrations of one nutrient should still not be affected by other environmental factors. But Mitscherlich did not claim constancy of the damage factor. It was an unspecified function of all the environmental variables, which certainly greatly reduced the attractive simplicity of his

hypothesis. Thus, to fit the data of a 5×5 interaction experiment (25 treatments) would, on his original theory, have needed three parameters only; it would now need six. Furthermore, adequate consideration has not been given to methods for fitting damage factors to observed interaction data.

As an alternative to Mitscherlich's formulation, the hypotheses suggested by Maskell (1925, 1928*a*, 1928*b*, 1929) (see also Brenchley, Maskell, and Warington 1927) — that the reciprocal of the yield could be expressed as the sum of elements each a function of the level of one external factor — may be tested. That is

$$\frac{1}{y} = F(N) + F'(P) + \dots,$$

where y is the yield, and N, P, \dots the amounts of the various nutrients supplied. This, by analogy with electrical theory, was called the "General Resistance Formula." A special form of the hypothesis (the "Special Resistance Formula"), in which the function was proportional to the reciprocal of the supply, was also studied; thus

$$\frac{1}{y} = \frac{a_n}{n + N} + \frac{a_p}{p + P} + \dots,$$

where y, N , and P have the meanings indicated above, n and p are the available amounts of the nutrients initially present in the substrate, and the other terms are constants. Balmukand (1928) developed statistical methods for testing these hypotheses, and found that they gave very satisfactory fits to nutrient interaction data in field trials and pot-culture experiments; so far as we are aware, however, no one has since analysed nutrient interaction data in this way.

Among the various sets of data in the present experiment showing significant first order interactions, one was taken at random for the purpose of testing Maskell's hypotheses; this was the shoot dry weight at 44 days, for the various combinations of nitrogen and phosphorus treatments. Both general and special hypotheses were tested, and the results are shown in the analysis of variance in Table 11.

The expectations from the general formula are in very satisfactory accord with the observations. The values of $F(N)$ and $F'(P)$ for the five levels of nitrogen and phosphorus supply used are:

Level of Supply	$F(N)$	$F'(P)$
1	0.00633	0.04266
2	0.00036	0.03051
3	-0.00057	0.00747
4	-0.00042	0.00405
5	0.00045	0.00265

when y, N , and P are all expressed in mg. Even in the case of the Special Resistance Formula, the deviations from expectation did not quite reach significance, although this hypothesis cannot cover the possibility of yield decreasing when

nutrient supply increases above a certain point — as has occurred in this lettuce experiment with nitrogen supply. In these data, the expression becomes

$$\frac{1}{y} = \frac{0.1470}{-31 + N} + \frac{0.1292}{1 + P} + 0.00194.$$

The negative value of n can hardly have any physical meaning, and may presumably be ascribed to the exigencies of fitting data in which the nutrient supply enters the supra-optimal region.

The degree of agreement attained suggests that this method of analysis, particularly the General Formula, deserves more attention than it has been accorded in the past by students of nutrient interaction.

Serious studies of nutrient interaction have generally led to conclusions similar in the main to those emerging in the present work. The work of Gregory with barley (Goodall and Gregory 1947), and that of Wittwer, Schroeder, and Albrecht (1947) with spinach likewise show the increase in magnitude of proportional responses to one nutrient with increasing supply of another, and up-

TABLE 11

ANALYSIS OF VARIANCE OF DATA FOR LOGARITHMS OF SHOOT DRY WEIGHT AT 44 DAYS, NITROGEN AND PHOSPHORUS COMBINATIONS, AFTER FITTING MASKELL'S FORMULAE

Source of Variation	Degrees of Freedom	Mean Square	<i>F</i>	<i>P</i>
General Formula	8	6.6909	71.714	<0.001
Residual	16	0.1203	1.289	>0.20
Special Formula	4	13.1040	140.450	<0.001
Residual	20	0.1518	1.627	0.05-0.10
Error (see Table 1)	64	0.0933		

ward displacement of the optimum amount of one as the supply of another is increased. These features of nutrient interaction results have often led to emphasis being placed on the concept of "nutrient balance." Various meanings have been ascribed to this phrase. Gregory (1937), for instance, regarded a balanced nutrient solution for barley as one in which the proportions of the nutrients were similar to those in barley grain. He pointed out that, for some of his interaction experiments, in a series of nutrient solutions in which the supply of two nutrients was increased in the same proportion, the growth and yield of the plants were linearly related to nutrient supply. This does not appear to be true for the present lettuce data. Indeed, it would be surprising if it were true in general. Increases in plant size generally carry with them changes in morphology, anatomy, and composition, and it is therefore reasonable to expect that the optimal ratios of nutrients will not be identical at different levels of nutrition.

In the case of most investigators who have referred to nutrient balance, no satisfactory definition is given; indeed, there seems to be an aura of loose

thinking around this concept. In general, the argument would appear to be that different nutrients have mutual optima—that for any given level x of a nutrient X there is a level y of nutrient Y giving maximum yield, and that if plants are supplied with x of X and y of Y then not only does an increase in the supply of Y reduce the yield but the same is also true of X . To put it geometrically, the surface connecting yield with supply of these two nutrients has a ridge, and from any point on the ridge only directions representing increases in the supply of *both* nutrients also represent increases in yield. The advocates of nutrient balance have in no case put forward a collection of data sufficient to enable such a relationship to be demonstrated rigorously, and the present lettuce data do not suggest a surface of that type.

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