Growth changes of plants following the removal of nutritional stresses

D. BOUMA

Dit proefschrift met stellingen van

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is goedgekeurd door de promotor, Dr. A. C. Schuffelen,
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De Rector Magnificus der Landbouwhogeschool,
W. F. EIJSVOOGEL

Wageningen, 20 maart 1965

Growth changes of plants following the removal of nutritional stresses

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS
IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE
BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 14 MEI 1965 TE 16 UUR

DOOR

D. BOUMA

DRUK: „DE VIKING" - OOSTERBEEK

STELLINGEN

I

In tegenstelling tot de gebruikelijke opvatting blijken matige voedingsgebreken, althans van fosfor en zwavel, wel invloed op de assimilatiesnelheid per eenheid bladoppervlakte te hebben.

Dit proefschrift.

D.J. Watson, The physiological basis of variation in yield. Adv.Agron.4:101-45, 1952.

II

Het bepalen van de voedingstoestand van de plant door het meten van verschillen in groei of in metabolische processen veroorzaakt door bepaalde behandelingen, heeft het voordeel dat de gevonden verbanden gewoonlijk causaal zijn. In principe zou dit een meer positieve diagnose mogelijk maken dan bij methoden die berusten op correlatieve betrekkingen.

Dit proefschrift.

III

De stikstofbemesting van Citrus hangt enerzijds af van de behoefte aan dit element voor het handhaven van groei en opbrengst en anderzijds van de nadelige invloed van stikstof op de kwaliteit van de vrucht. Welke van deze twee invloeden doorslaggevend wordt geacht hangt tot op grote hoogte van economische factoren af.

D. Bouma, A factorial experiment with Citrus. J. Hort.Sci.38:175-98, 1963.

IV

Aangezien het nadelige effect van stikstof op de kwaliteit van de vrucht gewoonlijk pas tot stand komt gedurende de laatste fase van haar ontwikkeling is het van belang de stikstoftoestand van de boom zodanig te regelen dat in het begin van het groeiseizoen een beperkte doch voldoende hoeveelheid stikstof aanwezig is, teneinde een overmaat in het laatste stadium te voorkomen.

D. Bouma, The development of the fruit of the Washington Navel Orange. Aust.J.Agr.Res.10:804-17, 1959.

V

De ontwikkeling van de landbouw in Australië wordt voorlopig niet zozeer beperkt door de beschikbare hoevéelheid water, dan wel door een veelal nog ondoelmatig gebruik van het water in gebieden met een gunstige regenval.

VI

Twee belangrijke factoren die het doelmatig gebruik van de regenval beperken zijn:

(a) De veelal grotere beperkingen van de plantengroei door voedingsgebreken.

(b) Het ondoelmatig gebruik van de graslandproduktie door het vee waar voedingsgebreken niet of niet meer bestaan.

VII

Een grotere schapenbezetting in gebieden met winterweiden kan bereikt worden door een gelimiteerde beweiding in de winter, teneinde de vegetatie beter in staat te stellen de door lage temperaturen en geringe lichtenergie beperkte groeimogelijkheden te benutten.

J.C. Cotsell, Sheep manegement
and pasture use.
Agr.Gaz.N.S.W. 69:169-77, 1958.

VIII

Ondanks een hoog zwavelgehalte van de wol zijn de hoeveelheden zwavel die daardoor aan de weide worden onttrokken van betrekkelijk gering belang bij het ontstaan van zwavelgebrek in het weidegewas.

IX

Een bezwaar van zeer veel proeven, waarin de opneembaarheid wordt vastgesteld van aan bladeren toegediende voedingselementen, is dat te weinig aandacht wordt besteed aan de kwantitatieve betekenis van de gevonden opname voor de groei van de plant.

D. BOUMA
WAGENINGEN, MEI 1965.

Aan de herinnering van mijn vader,
aan mijn moeder
Aan mijn vrouw en kinderen

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PREFACE

Most of the experimental work for this investigation was carried out while I was on the staff of the Division of Plant Industry, C.S.I.R.O., Canberra, Australia. It is with pleasure and gratitude that I acknowledge the award by the Organization of a Postgraduate Overseas Studentship, which enabled me to complete this work under the direction of Professor A.C.Schuffelen, Laboratory for Soils and Fertilizers, State Agricultural University, Wageningen, Netherlands.

To you, Professor Schuffelen, I am grateful for your interest in this work, and for the privilege of having been able to complete this work in your Laboratory. It was a great pleasure to return to your Laboratory for a while, so many years after receiving my training in Plant Nutrition and Agricultural Chemistry from you.

During the course of this investigation there have been several persons who have given their help, criticism, and advice. I acknowledge with gratitude the help and stimulating criticism throughout this work given by Dr. A.J. Anderson, C.S.I.R.O., Canberra, whose knowledge of Plant Nutrition, particularly in relation to pastures, has been of considerable help to me.

My sincere thanks are also due to Dr. R.F. Williams, C.S.I.R.O., Canberra. I enjoyed and appreciated the many valuable discussions and the encouragement given, not only during this work, but going back several years before that during my work at the Irrigation Research Laboratory, Griffith, N.S.W.

I also wish to acknowledge the advice and help in statistical matters so freely given by Mr. G.A.McIntyre, C.S.I.R.O., Canberra, during this investigation and during my years with the Irrigation Research Laboratory, Griffith, N.S.W.

I am indebted to Dr. D. Spencer and to Mr. K. Spencer, both of C.S.I.R.O., Canberra, for valuable discussions during this work.

My special and warm gratitude is due to Mr. E.J. Dowling, C.S.I.R.O., Division of Plant Industry, Canberra, for his loyal and enthusiastic assistance and support in all the experimental work of this investigation.

In conclusion, I wish to thank Dr. S.L. Tisdale of The Sulphur Institute, Washington D.C., U.S.A., for his encouragement, and for the financial support by the Institute towards the cost of printing this thesis.

1. INTRODUCTION

Since Liebig developed his theories on the mineral nutrition of plants, the determination of the nutrient requirement of crops has been an important subject of physiological and agricultural research. This subject has been reviewed and discussed by several authors, for example by Goodall and Gregory (1947), and by Wallace (1961). The techniques that have been developed for the assessment of the nutrient status or the nutrient requirements of crop plants are briefly as follows:

(a) The diagnosis of nutrient deficiencies in plants based on the recognition of symptoms.
(b) Pot experiments with soil to determine the effects of addition or omission of nutrients on plant performance.
(c) Chemical analysis of the soil or soil extracts to estimate the nutrient supply in the rootzone.
(d) Chemical analysis of plants or parts of plants to determine the nutrient status.
(e) The observation of plant responses, usually the disappearance of deficiency symptoms, after spraying or injection with mineral nutrients.
(f) The measurement of plant responses after the addition of nutrients in field experiments.

Some techniques have been more useful than others, but all have been applied in the assessment of the nutrient status or the nutrient requirement of plants.

Specific symptoms are usually not apparent under moderate deficiency conditions and this restricts the usefulness of method (a). There are also cases (Wallace 1961) where symptoms produced by pests and diseases, or by weather conditions, may be indistinguishable from mineral deficiency symptoms. Pot experiments with soil (method b) have often been found useful in the identification of likely deficiencies. Although plant responses to nutritional treatments in pots do not necessarily apply to field conditions, they can often provide useful leads for field experimentation. Methods (c) and (d) are in general based on the relationship between the content of certain nutrient elements in the soil or in plant extracts and the yield responses resulting from nutrient applications. These relationships are usually established on the basis of field experimentation. The success of plant or soil analysis techniques depends on the agreement between the forecast and the yield increases obtained after fertilizer additions. The crops for which these methods have been developed to a satisfactory degree are usually grown under intensive conditions. These crops include certain fruits, vegetables and others whose economic importance has often led to a considerable bank of basic information and knowledge of their mineral nutrition essential to the development of a diagnostic technique.

Only methods (e) and (f) are direct in the sense that they are based on the response of the actual crop plant. Injection and spray techniques (e) involve visual observation of the disappearance of deficiency symptoms following the application of solutions containing one or more nutrient elements (Roach 1938). For deficiencies in fruit trees of certain minor elements such as zinc and iron, which often produce fairly well defined symptoms, these methods have been employed with some success. Although the field experiment (f) is the ultimate test to which any diagnostic method must be submitted, there are a number of disadvantages associated with field experiments as a diagnostic technique. Apart from their cost, responses to fertilizers applied to the soil are often slow, particularly when applied as a topdressing to pastures. In addition, only relatively gross differences can be measured.

Fertilizer practices for pastures in southern Australia have largely been based on the results of pot and field experiments, and on local experience gained over the years. Deficiency symptoms are often not apparent or easily recognizable as such. The deficiencies that do occur are usually caused by suboptimal supplies, often in the marginal range, of the elements nitrogen, phosphorus, sulphur, or potassium, and sometimes by the minor elements molybdenum, boron, and others. Soil and leaf analysis techniques have not been used to any extent. This has been due, at least partly, to the lack of basic correlative information applicable to the wide range of soil and other environmental conditions in the pastoral areas of southern Australia.

In attempting to overcome the disadvantages of the field experiment as a diagnostic technique, a research project was initiated in the Division of Plant Industry, C.S.I.R.O., Canberra, Australia, which aimed firstly at obtaining a more rapid removal of nutritional stresses than is normally achieved by the application of mineral nutrients to the surface of the soil, and secondly at a more sensitive index of the induced plant responses than dry weight. Such an approach, if successful, would retain the outstanding virtue of the field experiment, in that it would make use of the response of the living plant to the removal of a nutritional stress.

In preliminary experiments the foliar spray method (e) (Roach 1938) was tried as a means of achieving a more rapid removal of a nutritional stress. Subterranean clover (var. Mt. Barker) was used as a test plant in these and all subsequent experiments because of its importance to the Australian pastoral industry. In these experiments plants were sprayed with different phosphorus compounds, with and without wetting agents, and at several frequencies. Although some treatments were more effective than others, the general response to foliar applications was small compared with that of phosphorus supplied via the roots. In a typical experiment plants were raised in sand cultures at a low level of phosphorus (4 p.p.m.). Daily sprays over a period of 25 days gave the following result:

	Sprayed on leaves			In nutrient solution
Compound	H_3PO_4	$NH_4H_2PO_4$	KH_2PO_4	KH_2PO_4
Concentration (%)	0.1 0.3	0.1 0.3	0.1 0.3	4 20 p.p.m.
Dry wt. of tops(mg)	225 260	167 219	159 198	134 920

($P \leqslant 0.05$ = 85 mg)

Only the dry weights of the plants sprayed with phosphoric acid or with ammoniumphosphate at the 0.3 per cent. concentration were significantly above that of the controls without phosphorus. Although other workers (Wittwer and Teubner 1959) have shown that phosphorus from foliar sprays can move freely through the plant, it appears that the plants in the present experiments were not able to obtain sufficient phosphorus to meet their requirements. In view of the scorching which occurred on some of the leaves sprayed with the 0.3 per cent. concentrations, it seems unlikely that higher concentrations would have given further growth responses. It is possible that different results would have been obtained with deficiencies of trace elements. In view of the comparatively small amounts of these elements needed, the plant may be able to take up sufficient from foliar applications to meet its requirements.

Subsequent experimental work was intended to form the basis for a different approach in the assessment of the nutrient status of plants. In this approach use was made of the differential growth changes induced by transfer of plants to appropriate nutrient solutions. These solutions included complete nutrient solutions and other solutions each lacking a different element. The experiments described in Section 3.1 were designed to provide information on the relationships between the nutrient status of a plant and the changes in leaf area induced in this manner. It will be shown that over short periods these changes may be related, at least qualitatively and for some elements possibly also quantitatively, to the nutrient status of the plant at the time of transfer.

The approach developed in Section 3.1 for the indentification of nutritional stresses in plants rests essentially on the early growth changes, and particularly on those in leaf area, induced by the removal of these stresses. The experiments were usually designed so that the concepts and functions of growth analysis (Watson 1952) could be applied to characterize the early adjustment from stress conditions and the differences in this respect between the nutrient elements under consideration. Because the analysis of growth covered the more theoretical aspects of the experiments, they are presented separately in the second part of this paper in Section 3.2. The emphasis was placed on a comparison between the elements nitrogen, phosphorus, and sulphur. A further series of experiments was carried out to enable a more detailed examination of the time trends in the growth changes during the recovery from stresses caused by these three elements. These changes are considered in relation to the accompanying changes in nutrient composition of the plant and its parts.

2. METHODS

2.1 General.

In the experiments reported here, subterranean clover seeds (Trifolium subterraneum L. var. Mt. Barker) were germinated in freely draining seedling flats filled with riversand. The seedlings were watered when required with excess distilled water, but at least once daily.

Seven days after sowing, when the two cotelydons were nearly fully expanded and the unifoliate leaf just visible, the seedlings were carefully removed from the sand and placed in the appropriate pretreatment nutrient solutions in order to obtain plants of the desired nutrient status. The seedlings usually had a taproot of 8-10 cm and only a few small or no lateral roots. In all experiments only seedlings of uniform size were used.

The nutrient solutions were contained in seedling flats lined inside with clear polythene sheeting and having a volume of 15 l. Hardboard squares, in which 1.25 cm holes were drilled to accomodate the plants, were fitted on the boxes. Squares of green polythene sheeting of the same size were glued on the tops. Slits were cut in the polythene covering to act as support for the plants (Fig. 1). There were 11 rows of 8 holes in each cover, so that there were 88 plants in each box.

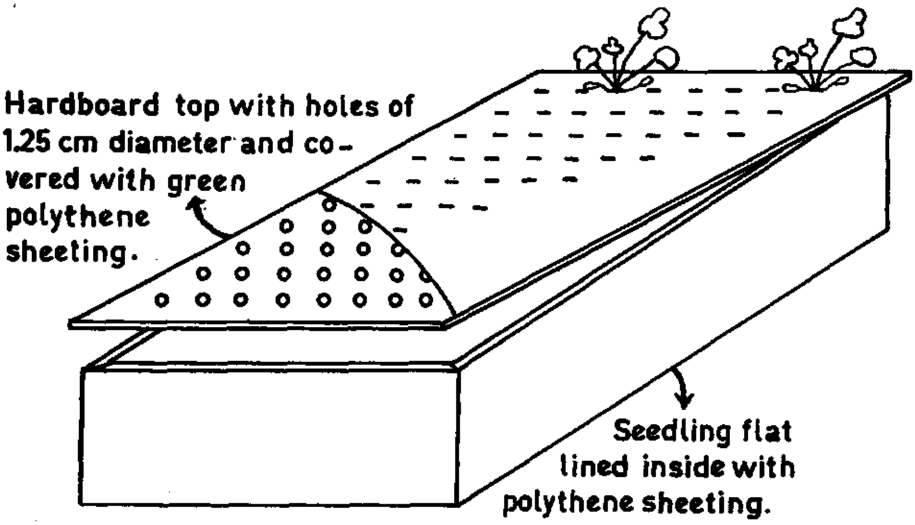

Figure 1.- Container and support used to grow plants in culture solutions during pretreatment.

The growth of the plants was good under these conditions. No aeration was found necessary. This arrangement made it possible to raise uniform plants and also enabled easy culling of plants outside the average for the particular pretreatment. The composition of the basal nutrient solutions for optimum growth was as follows (m-equiv./l): nitrogen 10, phosphorus 0.4, sulphur 2, calcium 8, potassium 5, magnesium 4, and ammonium 2. Minor elements were supplied as chlorides in the following concentrations (p.p.m.): boron 0.1, manganese 0.5, zinc 0.05, copper 0.02, molybdenum 0.01, and iron 1. The pH of the solutions varied between 5.1 and 5.5.

At the end of the pretreatment period the plants were transferred to appropriate test solutions, which, depending on the experiment, included a complete solution and one or more others each lacking one different element. These solutions are referred to as complete, without nitrogen, without phosphorus, etc. The solutions were contained in fruit-cans lined inside with polythene bags and having a volume of 360 ml. There were two plants per container in all experiments. The experiments were carried out in a glasshouse kept at a temperature of approximately 24° C during the day and 17° C at night (6 p.m. - 8 a.m.).

To avoid ambiguity in subsequent Sections, the experimental procedure is summarized here. All glasshouse experiments consisted of three periods. During the first period seedlings were raised in riversand to which only distilled water was applied. This period lasted seven days from sowing. During the second period plants were raised at different nutrient levels in culture solutions to obtain plants of the desired nutrient status. This period lasted, depending on the experiment, 14 - 20 days. The treatments during this period will be referred to as pretreatments and the solutions as pretreatment solutions. The third period commenced on the day of transfer from pretreatment solutions to complete solutions and to one or more other solutions each lacking a different element. These solutions will be referred to as test solutions. The test periods lasted seven days, the day of commencement being referred to as day 0 and the last day as day 7.

2.2 Growth measurement.

Growth changes over a period of seven days after transfer from pretreatments to the appropriate test solutions were described by estimates of total leaf area per plant and by dry weight at the beginning (day 0) and at the end (day 7) of the test period.

Leaf area estimates were obtained by visual comparison of individual leaves with a set of photographic standards prepared for subterranean clover leaves by Williams, Evans, and Ludwig (1964). Drawings of the standards are presented in Fig. 2.

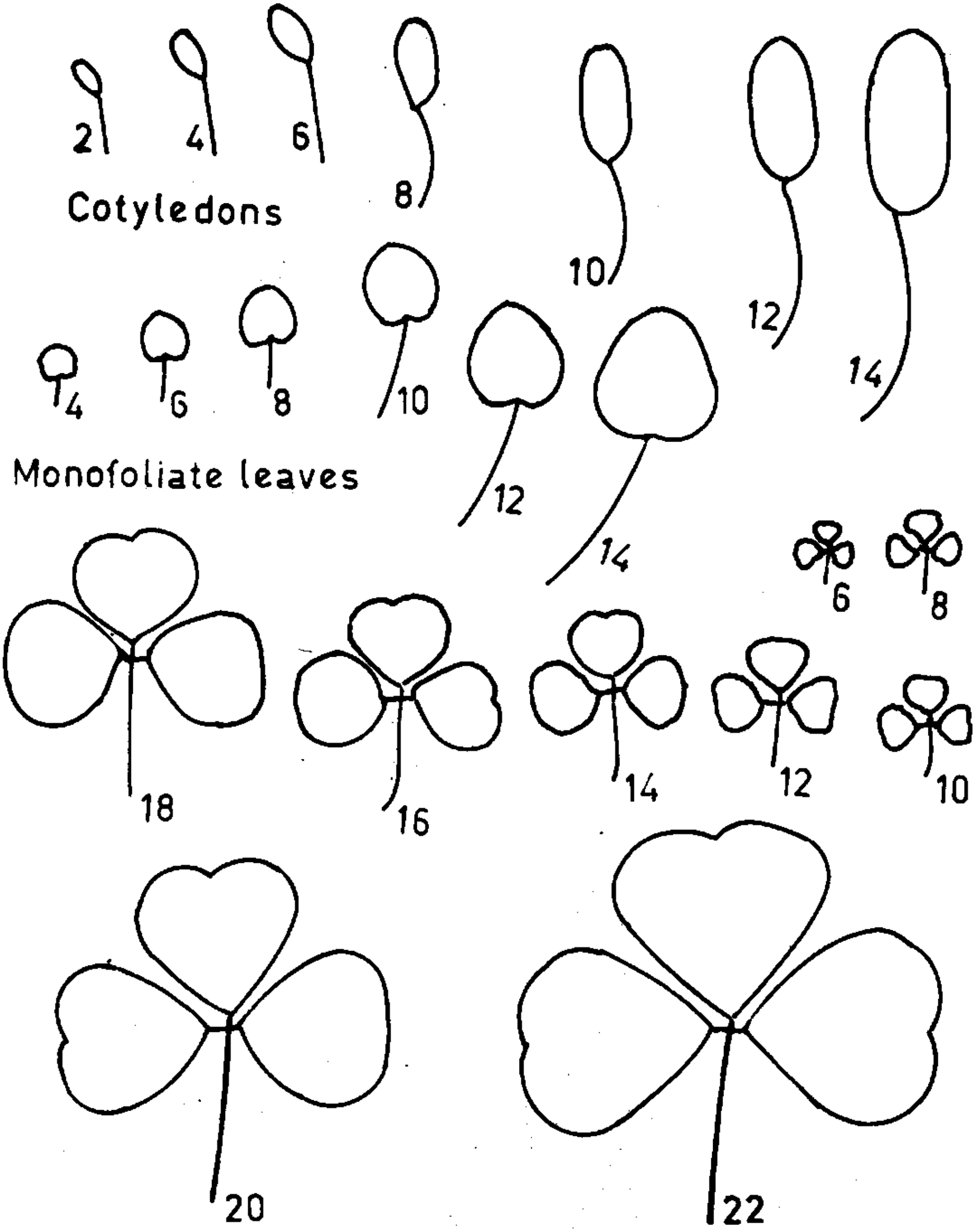

Figure 2.- Leaf area standards. The figures represent the ratings. The relation between rating and leaf area is: rating = 10 × log10 × leaf area (cm^2).

The method is a rating procedure, and the rating scale is based on the formula:

$$\text{rating} = 10 \times \log10A$$

in which A is the leaf area in square centimetres. A full description of the procedure was given by Williams (1954) for tomato leaves.

The first estimate was made on the day of transfer from pretreatments to the various test solutions (day 0). Leaf areas on subsequent occasions were corrected for initial variation on day 0 by regression analysis as outlined by McIntyre and Williams (1949). An example of this procedure is given below. This was taken from the experiment presented in Section 3.1.1.1, in which plants were raised at five phosphorus pretreatment levels and then transferred to complete solutions and to solutions without phosphorus. This particular example applies to the plants raised at the P_3 level.

	Leaf area on day 0		Leaf area on day 7	
Replicates	Complete Soln.	Soln. without P	Complete soln.	Soln. without P
1	12.64	11.61	30.08	20.81
2	10.20	11.11	26.30	16.93
3	11.59	12.06	29.28	21.51
4	11.00	12.78	27.85	23.09
5	9.92	12.57	26.35	20.88
6	10.33	11.51	25.13	18.66
7	14.32	13.19	30.03	22.03
8	11.85	11.30	28.48	18.80
9	10.59	12.50	27.48	20.13
10	12.31	10.90	27.80	19.75
Total	114.75	119.53	278.78	202.59
Mean	11.48	11.95	27.88	20.26
General mean	11.71			
Deviations (dev.)				
	+0.23	-0.24		

Regression of leaf area day 7 (Y) on leaf area day 0 (X)

Pretreatment	Transferred to:	Σx^2	Σxy	Σy^2	b	b × dev day 0	$\hat{y}_7$
P_3	Complete soln.	16.666	16.910	24.350	1.015	+0.23	28.11
	Soln.without P	5.465	9.793	29.449	1.792	-0.43	19.83

The adjusted leaf areas on day 7 ($\hat{y}_7$) were obtained by adding the correction factor (b × deviation of leaf area on day 0 from the general mean on day 0) to the experimentally determined leaf area on day 7. For the P_3 plants transferred to the complete solution $\hat{y}_7$ was therefore $27.88+(+0.23 \times 1.015) = 28.11$ cm^2. Similarly, $\hat{y}_7$ for the plants transferred from P_3 to solutions without phosphorus was $20.26+(-0.24 \times 1.792) = 19.83$ cm^2.

At harvest, plants were usually separated into tops and roots. Dry weights were obtained after drying in a forced draught oven at 75-80° C. Dry weights of plants harvested on day 7 were also adjusted for initial variation on day 0. The procedure was similar to that for leaf area, except that it was based on the regression of the dry weight on day 7 on the leaf area of the same plants on day 0.

In most experiments a harvest was also taken from each pretreatment on the day of transfer to the test solutions (day 0). From the dry weight and leaf area of the harvested plants the regression coefficient (b) within each pretreatment was calculated. With the product of the regression coefficient and the difference between the mean leaf area on day 0 of the plants transferred to the test solutions and that of the plants harvested on day 0 from the same pretreatment, it was possible to obtain an estimate of the dry weight on day 0 of the plants that were transferred to test solutions. In the example quoted above from the experiment presented in Section 3.1.1.1, the leaf area on day 0 of the plants that were transferred to the test solutions was 11.71 cm^2 and that of the plants harvested on day 0 from the same P_3 pretreatment was 11.95 cm^2, a difference of -0.24 cm^2. The mean dry weight of the harvested plants with leaf area 11.95 cm^2 was 83.80 mg. The regression coefficient (b) of dry weight on leaf area for these harvested plants was 7.893 mg/cm^2. As the harvested plants and the plants transferred to the test solutions with and without phosphorous were obtained from the same P_3 pretreatment, the dry weight on day 0 for the plants transferred to the two solutions was estimated as: $83.80+(-0.24 \times 7.893)=$ 81.91 mg.

2.3 Growth analysis.

The concepts and functions of the analysis of plant growth have been applied to the present examination of the early growth changes induced by the removal of nutritional stresses in subterranean clover. The subject of growth analysis, including the derivation of the growth functions has been reviewed by Williams (1946), by Watson (1952), and by Blackman (1960). Only a brief summary is given here of the symbols and formulae used in calculating the various growth indices.

Symbols:

R_W - Relative growth rate (increase in weight per unit weight per unit time)

R_A - Relative leaf growth rate (leaf area increase per unit area per unit time)

E_A - Net assimilation rate (increase in weight per unit leaf area per unit time)

A - Leaf area

W - Dry weight

$\frac{A}{W}$ - Leaf area ratio (ratio of leaf area to the dry weight per plant)

Growth indices:

The value of the relative growth rate R_W at any instant is given by

$$\frac{1}{W} \times \frac{dW}{dt} = \frac{d\log W}{dt}$$

Integration of this function over the interval $t_2 - t_1$ gives an average value of R_W (Fisher 1920):

$$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{d\log W}{dt} dt = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Similarly R_A is given by: $\frac{\log_e A_2 - \log_e A_1}{t_2 - t_1}$

The net assimilation rate at any instant is given by:

$$E_A = \frac{1}{A} \times \frac{dW}{dt}$$

Williams (1946) pointed out that the mean value of E_A over the interval $t_2 - t_1$

$$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{1}{A} \times \frac{dW}{dt} dt$$

can be calculated if the relation between W and A is linear. This condition is usually met over short intervals (7-14 days), particularly for young plants. The mean value for E_A over the interval $t_2 - t_1$ is then calculated as

$$\frac{W_2 - W_1}{A_2 - A_1} \times \frac{\log_e A_2 - \log_e A_1}{t_2 - t_1}$$

For some of the experiments in the present work harvests were available on day 3 and day 5 in addition to those at the beginning (day 0) and the end (day 7) of the test period. The relation between W and A in these experiments was linear with correlation coefficients of 0.79 - 0.94, depending on the experiment.

The relative growth rate R_W is the product of the net assimilation rate E_A and the leaf area ratio $\frac{A}{W}$, as follows:

$$R_W = \frac{1}{W} \times \frac{dW}{dt} = \frac{1}{A} \times \frac{dW}{dt} \times \frac{A}{W} .$$

2.4 Chemical analysis.

Total nitrogen was determined by a modified micro-Kjeldahl method. Total phosphorus was estimated in the nitric-perchloric acid digest by the molybdenum blue method of Truog and Meyer (1929). Total sulphur was determined in an aliquot of the same ash solution according to the method of Johnson and Nishita (1952).

3. EXPERIMENTAL RESULTS

3.1 The effect of the nutrient status of the plant on the growth changes following the transfer to nutrient solutions.

3.1.1 The effect of different nutrient levels on the leaf area changes induced by the subsequent transfer of plants to solutions with and without the varied element.

In six separate experiments, one week old subterranean clover seedlings were placed in pretreatment solutions containing five levels of the elements phosphorus, sulphur, potassium, calcium, boron, or nitrogen. At the end of the pretreatment period plants from each of the five nutrient levels were transferred to complete solutions and to corresponding solutions lacking the element that had previously been varied. Changes in leaf area were followed over a period of seven days after transfer to the test solutions.

The results of the six experiments are presented in Sections 3.1.1.1 -.6. The experiment with five nitrogen levels is presented last (3.1.1.6), because symbiotic nitrogen fixation introduced effects which complicated those of the applied nitrogen levels.

3.1.1.1 The effect of phosphorus levels.

In this and the following five Sections the first graph shows the effects of nutrient levels on leaf areas and dry weights of plant tops at the end of the pretreatment period, which was also the day of transfer (day 0) to the test solutions. The leaf area changes during the 7 day period after transfer to the test solutions are shown in the second graph of each Section. In each of these graphs the leaf areas on day 0 are the logarithms of the areas shown in the preceding response curve. The nutrient levels (P_1, P_2, ...etc.) shown with the log leaf area value on day 0 also refer to the levels of the response curve.

A comparison of the leaf areas and the dry weights of the tops of plants at the conclusion of a period of 14 days at five phosphorus pretreatment levels is shown in Fig. 3. The phosphorus levels during this period were 0.25, 0.5, 1, 2, and 4 p.p.m. phosphorus, and they are referred to as P_1, P_2, P_3, P_4, and P_5 respectively.

Increases in phosphorus supply at the lower levels had a relatively greater effect on leaf area than on dry weight. The increases in leaf area with successive increments in phosphorus supply were 84, 47, 14, and 11 per cent., and for dry weight they were 65, 24, 15, and 10 per cent. respectively.

The changes in leaf area induced by the transfer of plants from each of the five phosphorus levels to complete solutions and to solutions without phosphorus are shown in Figure 4.

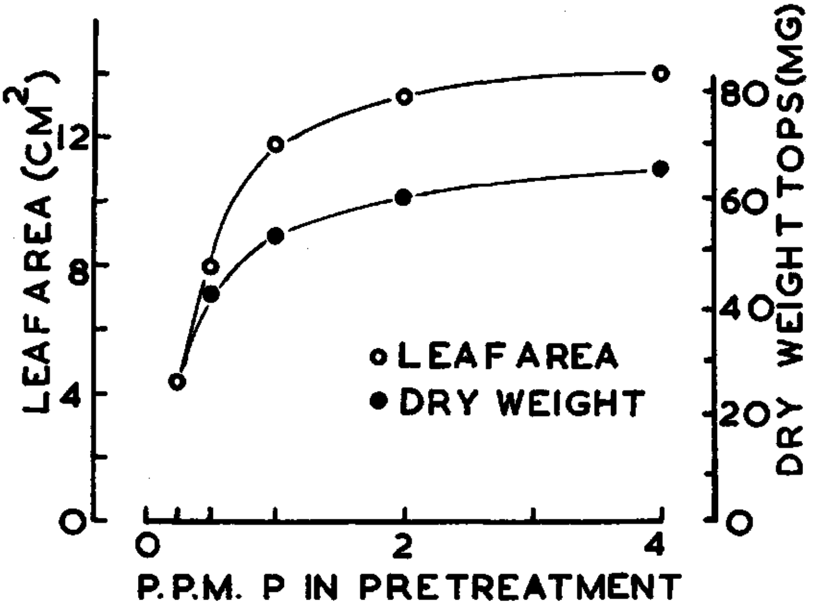

Figure 3.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 14 days at five phosphorus levels. The levels were 0.25, 0.5, 1, 2, and 4 p.p.m. phosphorus and are referred to in the text and in Figure 4 as P_1, P_2, P_3, P_4, P_5, respectively. The scales in this graph, and in the corresponding graphs for the other elements, have been arranged so that the points for dry weight and leaf area at the lowest level coincide.

It will be apparent that the rate of leaf area increase over the 7 day period after transfer to complete solutions was not greatly dependent on the phosphorus status of the plants at the time of transfer. However, leaf area increases after transfer to solutions without phosphorus were greater the higher the phosphorus status of the plants at the time of transfer. For plants raised at the P_5 level there were no significant differences in leaf area between the complete and the no-phosphorus solutions.

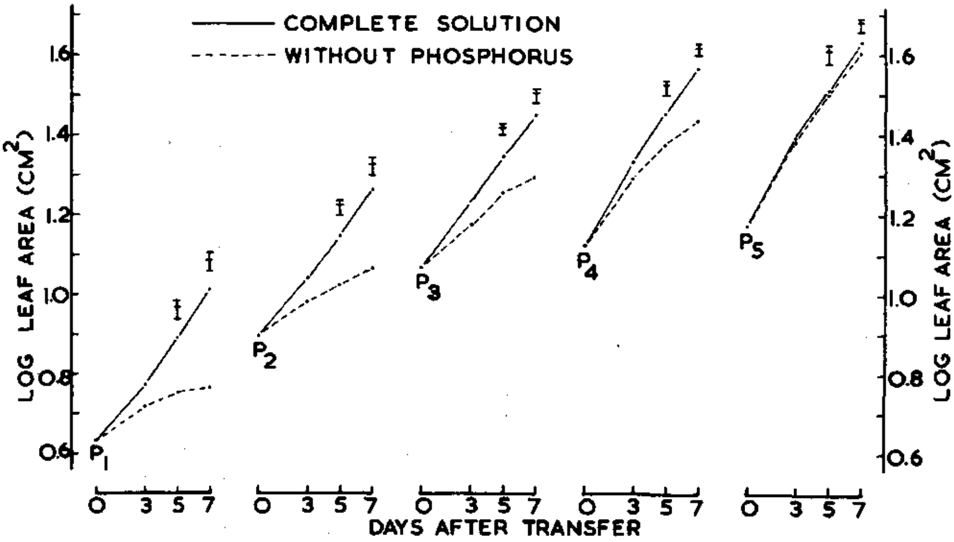

Figure 4.- Changes in leaf area during a period of seven days after transfer to test solutions with and without phosphorus of the plants for which the leaf areas and the estimated top dry weights are shown in Figure 3. The day of transfer to the two test solutions (day 0) was the end of the pretreatment period. The leaf areas shown on day 0 are the common logarithms of the areas in Figure 3, and the phosphorus levels P_1, P_2, P_3, P_4, P_5, refer to the pretreatment levels given in Figure 3. The results of the experiments in which the supply of other elements was varied during pretreatment were plotted in a similar manner (Figures 5 - 15). The vertical lines on day 5 and day 7 represent the minimum differences for significance at 5 and 1 per cent. levels.

3.1.1.2 The effect of sulphur levels.

Figure 5 shows the effect of five sulphur levels on leaf areas and dry weights of tops at the end of a pretreatment period of 19 days. The levels were 0.125, 0.25, 0.5, 1, and 4 p.p.m. sulphur, and are referred to as S_1, S_2, S_3, S_4, and S_5 respectively. Sulphur levels had a relatively greater effect on leaf area than on dry weight, particularly at the intermediate levels. The increases in leaf area between successive sulphur levels were 19, 48, 31, and 8 per cent., and those for dry weight were 14, 34, 12, and 2 per cent. respectively.

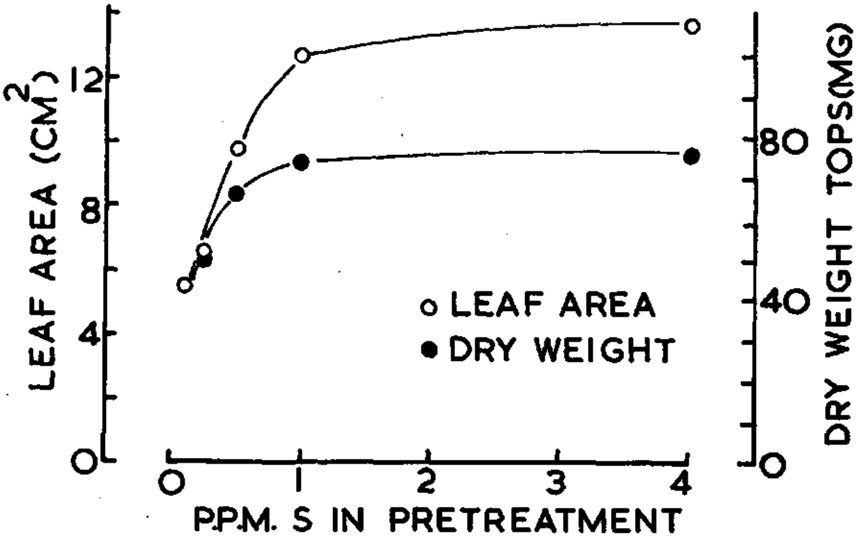

Figure 5.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 19 days at five sulphur levels. The levels were 0.125, 0.25, 0.5, 1, and 4 p.p.m. sulphur, and are referred to in the text and in Figure 6 as S_1, S_2, S_3, S_4, S_5, respectively.

The leaf area changes during the 7 day period after transfer of plants from each of the five sulphur levels to complete solutions and to solutions without sulphur are shown in Figure 6. The relative increases in leaf area induced by the transfer to complete solutions were not greatly affected by pretreatment levels. The leaf area increases of the plants transferred from the S_1, S_2, S_3, and S_4 pretreatments to solutions without sulphur were considerably smaller than for the corresponding plants transferred to the complete solutions. In contrast with the results for phosphorus of the previous Section, there was no evidence that the relative differences in leaf area following the transfer of plants from the sulphur deficient solutions to complete and no-sulphur solutions could be related to the degree of the sulphur stress at the time of transfer. There were no differences in leaf area when plants raised at the S_5 level were transferred to complete and to no-sulphur solutions.

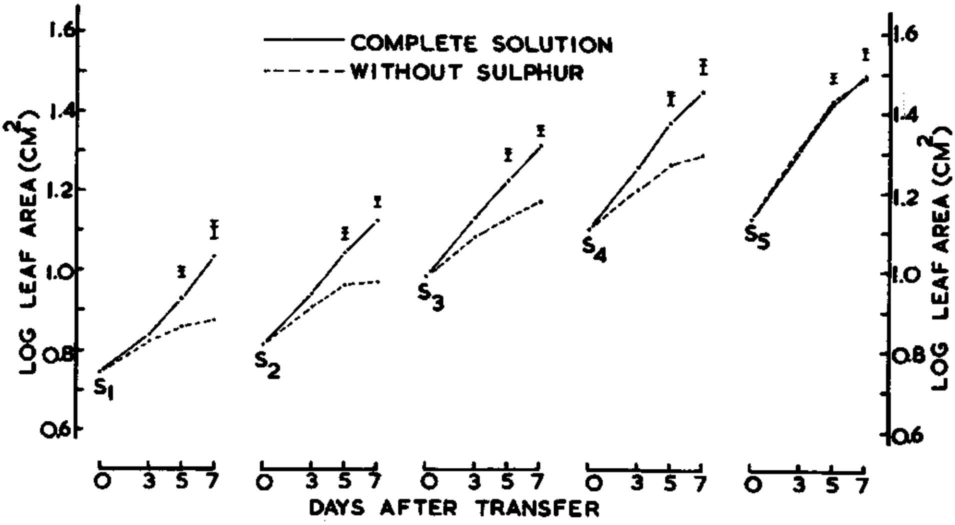

Figure 6.- Changes in leaf area during a period of seven days after transfer to test solutions with and without sulphur of the plants for which the leaf areas and the estimated top dry weights are shown in Figure 5.

3.1.1.3 The effect of potassium levels.

The effects of five potassium levels on leaf areas and dry weights of the plant tops at the end of a pretreatment period of 15 days are shown in Figure 7. The levels were 2.4, 7.2, 21.7, 65, and 195 p.p.m. potassium, and they are referred to as K_1, K_2, K_3, K_4, and K_5 respectively. Increases in leaf area and dry weight were greatest between K_1 and K_2, and the effect on leaf area was relatively greater than on dry weight.

The changes in leaf area over a period of seven days after transfer of plants from each of the five potassium levels to complete solutions and to corresponding solutions without potassium are shown in Figure 8. The relative increases in leaf area after transfer of the five groups of plants to complete solutions were similar. For the plants transferred to solutions without potassium, the increases in leaf area over the 7 day period were greater the higher the potassium level before transfer. These results were similar to those for phosphorus shown in Section 3.1.1.1, where it was also found that the relative differences in leaf area on day 7 between the complete solutions and the corresponding solutions without the varied element were greater the higher the nutrient stress on the day of transfer. In view of the small effect of the K_5 level on

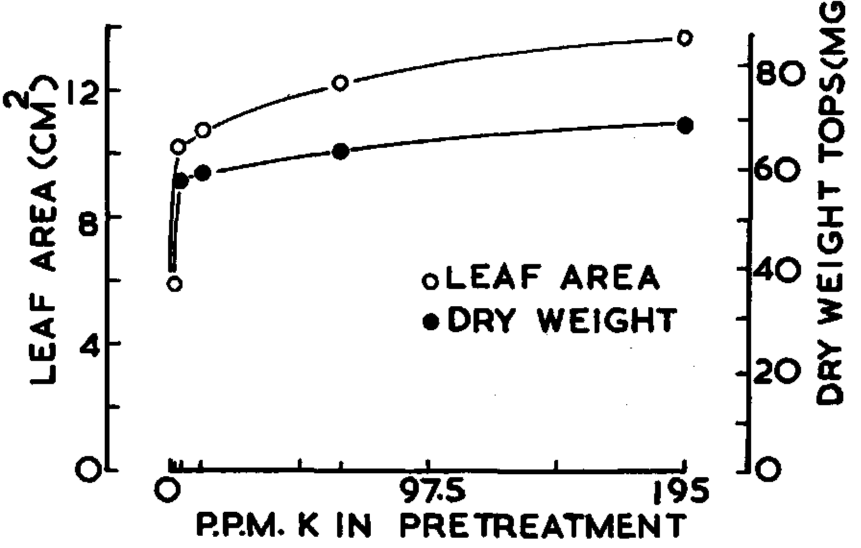

Figure 7.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 15 days at five potassium levels. The levels were 2.4, 7.2, 21.7, 65, and 195 p.p.m. potassium, and are referred to in the text and in Figure 8 as K_1, K_2, K_3, K_4, K_5, respectively.

plant size on day 0 (Fig. 7), and the small differences in leaf area after transfer of the K_4 plants to complete and no-potassium solutions, it appears that the K_4 level, or perhaps a slightly higher level, would have been adequate for satisfactory growth.

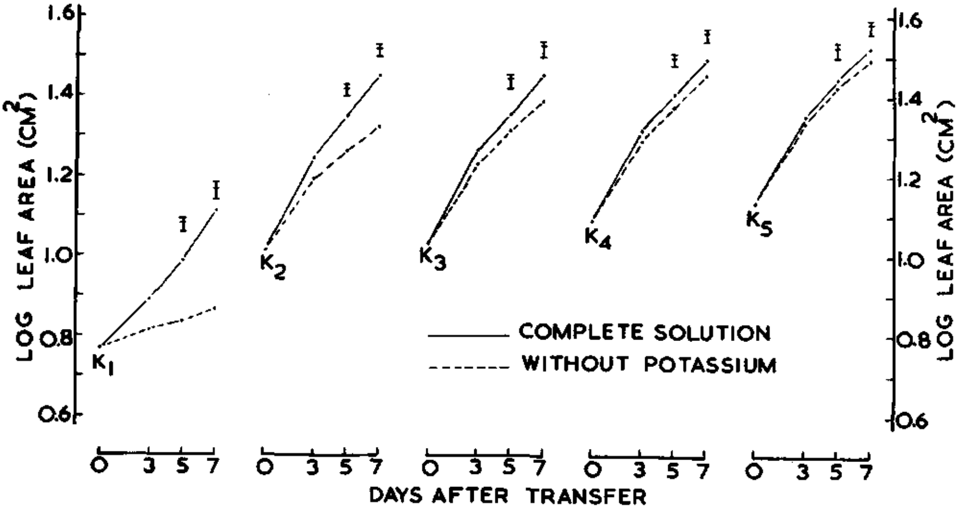

Figure 8.- Changes in leaf area during a period of seven days after transfer to test solutions with and without potassium of the plants for which the leaf areas and the estimated top dry weights are shown in Figure 7.

3.1.1.4 The effect of calcium levels.

It was difficult to obtain a response curve for subterranean clover in water cultures to levels of calcium supply. Initially there were five pretreatment levels, 0.6, 1.25, 5, 40, and 160 p.p.m. calcium respectively. Within six days after the application of these levels, the plants of the lowest two levels showed symptoms typical for calcium deficiency. These consisted at first of malformations of the second trifoliate leaf, and later a shrivelling up of the central growing point of the plants at the lowest level. Most of the latter plants died during the 14 days of pretreatment. Figure 9 shows the effect of the remaining four calcium levels on leaf areas and dry weight of the tops at the end of the pretreatment period (day 0).

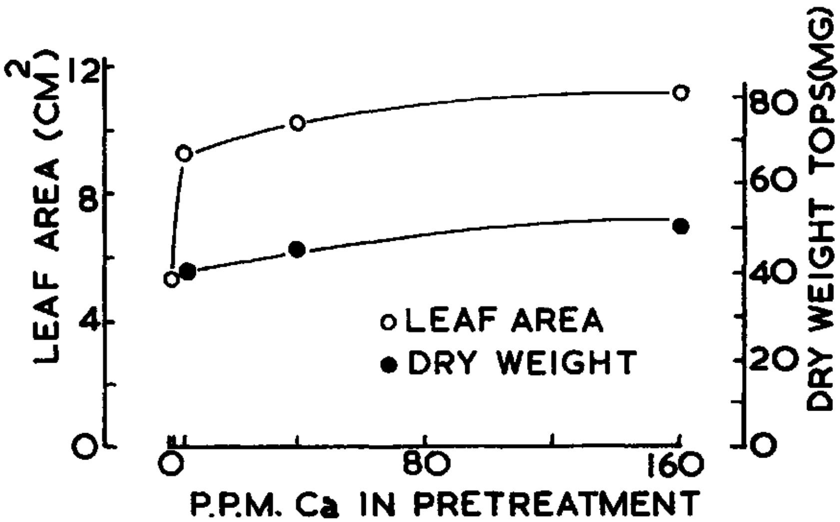

Figure 9.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 14 days at four calcium levels. The levels were 1.25, 5, 40, and 160 p.p.m. calcium, and are referred to in the text and in Figure 10 as Ca_1, Ca_2, Ca_3, Ca_4 respectively.

These levels are referred to as Ca_1, Ca_2, Ca_3, and Ca_4 respectively. Calcium levels had only a small effect on dry weights. The smaller leaf areas of the plants at the lowest calcium level were mainly due to dead leaves, which were excluded from the leaf area ratings, but not from the dry weights. The effects of higher calcium levels on leaf area were also small.

The changes in leaf area after transfer of these four groups of plants to complete solutions and to solutions without calcium are shown in Figure 10. The acuteness of the calcium deficiency at the Ca_1 level was apparent in the fact that in the complete solution the leaf area continued to decline for another three days after transfer, due to the death of more leaves. The response after day 3 was solely due to the appearance of new leaves. The Ca$_1$ plants transferred to the solutions without calcium were virtually dead on day 7.

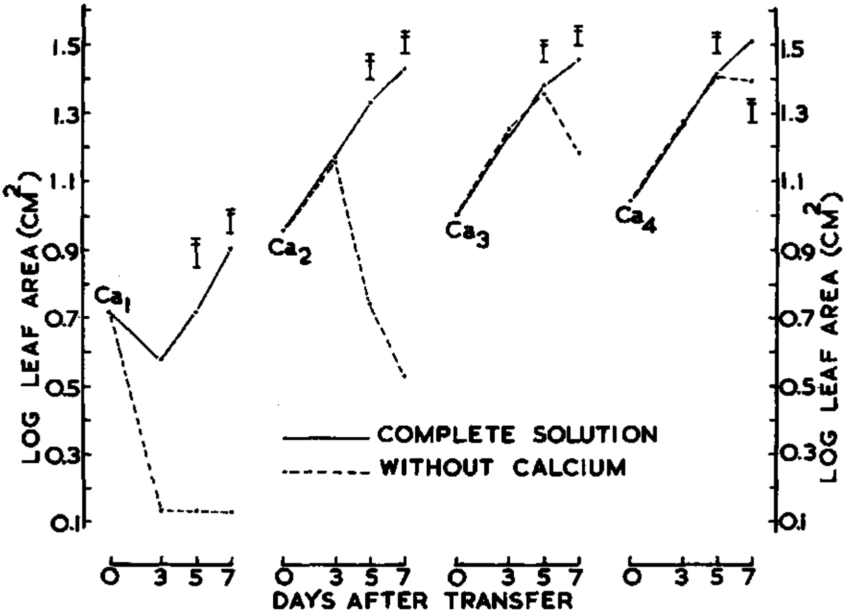

Figure 10.- Changes in leaf area during a period of seven days after transfer to test solutions with and without calcium of the plants for which the leaf areas and the estimated top dry weights are shown in Figure 9.

The plants transferred from the Ca$_2$ pretreatment showed no differences in leaf area between the solutions with and without calcium till day 3. The sudden drop in leaf area of the plants in the no-calcium solution after day 3 was caused by a collapse of petioles and subsequent death of the leaves attached to them. These leaves were not included in the area estimates. The collapse of petioles only occurred on the older leaves, and was always very sudden.

As the calcium status of the plants at the time of transfer increased, leaf area expansion in the two solutions proceeded at nearly the same rate till day 5. Thereafter a collapse of petioles also occurred in the plants of both the Ca$_3$

and the Ca_4 pretreatment levels, but it was less severe for the plants transferred from the highest pretreatment level.

3.1.1.5 The effect of boron levels.

Figure 11 shows the dry weights and leaf areas of plants after a period of 14 days at the following boron levels: 0.002, 0.004, 0.008, 0.016, and 0.064 p.p.m. boron. These levels are referred to as B_1, B_2, B_3, B_4, and B_5 respectively. Increasing the boron level from B_1 to B_5 caused an increase in dry weight of approximately 20 per cent., and an increase in leaf area of over 40 per cent. The greatest increase in leaf area occurred from B_1 to B_3. The anomalous value for the B_4 level was probably caused by variability. Deficiency symptoms, which consisted of a purple blueish discoloration of the leaves, were only apparent at the lowest boron level.

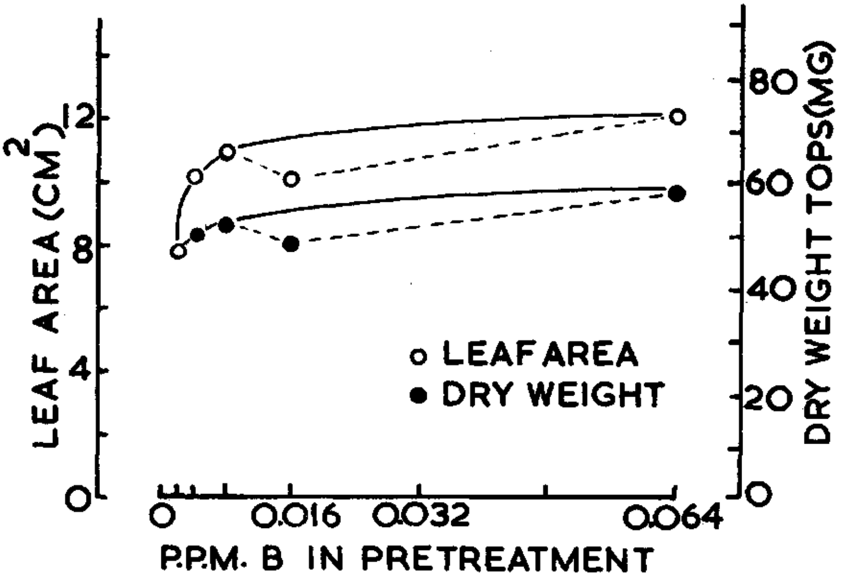

Figure 11.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 14 days at five boron levels. The levels were 0.002, 0.004, 0.008, 0.016, and 0.064 p.p.m. boron, and are referred to in the text and in Figure 12 as B_1, B_2, B_3, B_4, B_5 respectively.

The changes in leaf area over a period of seven days after transfer of these plants to solutions with and without boron are shown in Figure 12.

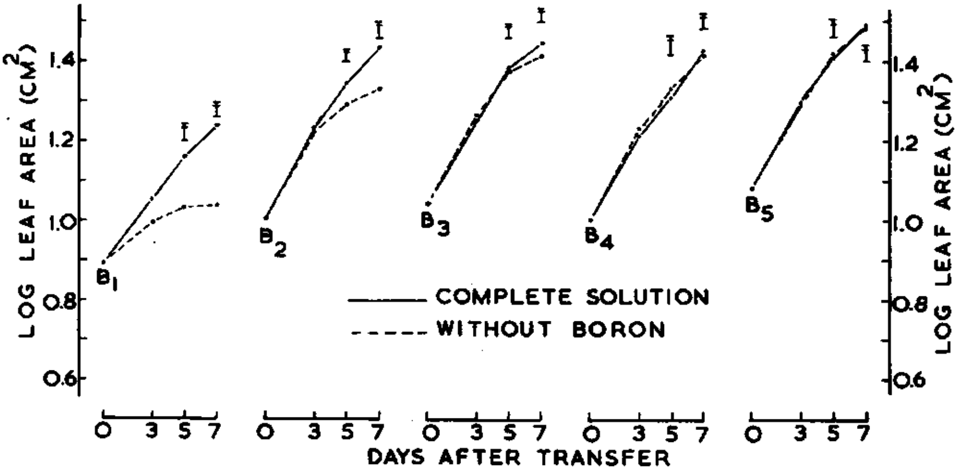

Figure 12.- Changes in leaf area during a period of seven days after transfer to test solutions with and without boron of the plants for which the leaf areas and the estimated top dry weights are shown in Figure 11.

There were no differences between plants transferred to the two solutions from the B$_4$ and B$_5$ levels. This also suggests that the response curves of Figure 11 were those represented by the full lines and that the low value for dry weight and leaf area at B$_4$ was an anomaly caused by plant variability. It appears that the B$_3$ level was nearly adequate.

A small difference in leaf area was apparent on day 7 for the plants transferred from the B$_3$ level. As the pretreatment level decreased the differences became relatively greater. The relative increases in leaf area for plants in the complete solutions were similar at all pretreatment levels, although somewhat lower for the B$_1$ plants. In the solutions without boron the rate of leaf area increase was smaller the lower the boron status of the plant at the time of transfer. At the B_1 level, where the plants showed marked deficiency symptoms at the time of transfer, the leaf area per plant in the no-boron solution increased from 7.8 to 11.0 cm^2 between day 0 and day 7. These results show that the onset of boron deficiency, or the aggravation of a boron deficiency, had a gradual effect on plant growth, in contrast to calcium where the effects were sudden and drastic.

3.1.1.6 The effect of nitrogen levels.

This section presents the results of two experiments with plants raised at different nitrogen levels. The first experiment was primarily intended to evaluate the effect of the nitrogen status of the plant on the leaf area differences induced by transfer to test solutions with and without nitrogen. Nodulation effects had not occurred in the experiments of the preceding Sections because the amounts of combined nitrogen in the culture solutions were adequate for plant growth, and high enough to suppress nodulation almost entirely. In the present experiment, which included much lower nitrogen levels, the pattern of leaf area changes induced by the transfer to the test solutions strongly indicated an effect of symbiotic nitrogen fixation as a result of nodulation during pretreatment. This aspect was further investigated in the second experiment.

The results of the first experiment are shown in Figures 13 and 14. Figure 13 gives the comparison of leaf areas and dry weights of the tops of plants at the end of a period of 17 days at five nitrogen pretreatment levels. The levels were 1.25, 2.5, 5, 10, and 40 p.p.m. nitrogen. They are referred to as N_1, N_2, N_3, N_4, and N_5 respectively.

In the lower range of the response curves the increases in leaf area were relatively greater than those in dry weight. Between N_1 and N_2 leaf area and dry weight increased by 55 and 45 per cent., and between N_2 and N_3 by 21 and 16 per cent. respectively. At the higher nitrogen levels the differences were small. The increase in nitrogen supply from N_4 to N_5 resulted in a leaf area increase of 21 per cent. and in an increase in dry weight of the tops of 24 per cent. There was also a pretreatment in which plants were grown from seed inoculated with the appropriate Rhizobium strain, but without combined nitrogen. This pretreatment is referred to as N_o. For greater clarity in the response curves in Figure 13, leaf area and dry weight of the N_o plants have been omitted. These plants were similar in size to the N_1 plants, which is evident from the following comparison:

	Leaf area (cm^2)	Dry weight tops (mg)
N_1	5.1	24.2
N_o	5.5	26.0

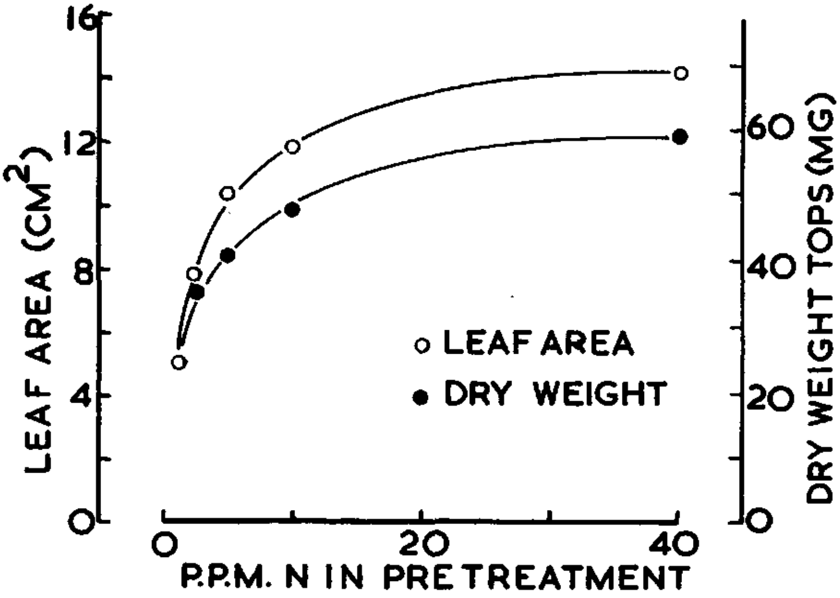

Figure 13.- Leaf areas and estimated dry weights of the tops of plants at the end of a pretreatment period of 17 days at five nitrogen levels. The levels were 1.25, 2.5, 5, 10, and 40 p.p.m. nitrogen, and are referred to in the text and in Figure 14 as N_1, N_2, N_3, N_4, N_5 respectively.

The leaf area changes induced by the transfer of plants from each of the six pretreatments to solutions with and without nitrogen are shown in Figure 14. The differences in leaf area after transfer of plants raised at N_0 or N_1 were small till day 5, but tended to increase thereafter due to a somewhat faster growth rate in the complete solutions. The transfer of N_2, N_3, and N_4 plants resulted in leaf area differences which were greater the higher the nitrogen level had been during pretreatment. Even for the plants transferred from the N_5 level the leaf area differences were considerable, although smaller than for the N_4 plants.

The results of this experiment differed in two respects from those of the preceding Sections for plants raised at several levels of phosphorus, sulphur, potassium, or boron.

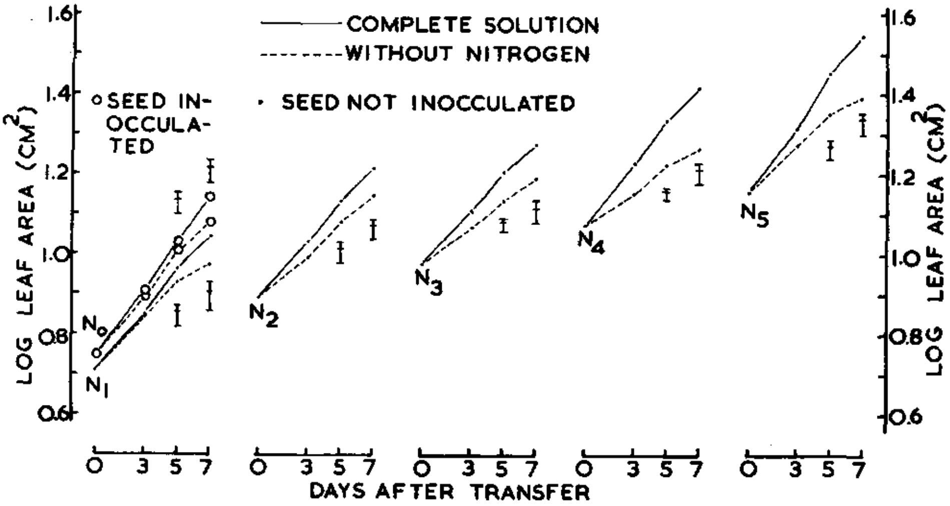

Figure 14.- Changes in leaf area during a period of seven days after transfer to test solutions with and without nitrogen of the plants for which leaf areas and estimated top dry weights are shown in Figure 13. The plants shown as N_o had received no combined nitrogen during the 17 day pretreatment period, but were raised from inoculated seed. Nodules also occurred on the roots of the N_1 plants, probably as a result of spontaneous nodulation, and to some extend on the roots of the N_2 plants (see text).

In these experiments it was shown that the leaf area differences induced by the transfer to test solutions with and without the element concerned, depended on the nutrient status of the plant with respect to these elements at the time of transfer. The leaf area differences during the 7 day period after transfer were non-significant for plants raised at optimim levels of these elements, but quite pronounced for plants deficient in one of these elements. An assessment of the nutrient status of the plant with respect to these elements, at least in a qualitative sense, appeared therefore feasible. The evidence of the present experiment (Figure 14) indicated that leaf area differences induced by the transfer to test solutions with and without nitrogen were not greatly dependent on the nitrogen status of the plant at the time of transfer. This procedure, therefore, does not appear to be a promising approach to the assessment of the nitrogen status of plants.

Another aspect, which could have an important bearing on the marked differences of these results compared with those of the previous experiments, is the possible effect of nodulation during pretreatment on the leaf area differences after the transfer to the two test solutions.

On the day of transfer to the test solutions (day 0) the roots of the plants from the different pretreatments were examined for the occurrence of nodules. Many healthy nodules were apparent not only on the roots of the inoculated N_o plants, but also on those of the N_1 plants. The experiment was not carried out under sterile conditions, and it is likely that spontaneous nodulation had occurred. Nodules on the roots of the plants from the other pretreatments decreased in size and numbers as the nitrogen level that had been applied before transfer increased. There were only a few small nodules on the roots of plants from the N_5 level. Whilst a great deal of significance cannot be attached to these observations, they could nevertheless provide a reasonably satisfactory explanation for the pattern of leaf area changes induced by the transfer of plants from the different pretreatments to the test solutions.

Figure 14 showed that the differences in leaf area induced by the transfer to the two test solutions were small for the plants from N_o and N_1. For the plants transferred from the pretreatments below N_5, the differences in leaf area were greater the higher the nitrogen level had been in the culture solution before transfer. The relative rates of leaf area expansion (R_A) over the 7 day period after transfer of plants from the different pretreatments to the complete solutions were less dependent on pretreatment levels than those for the corresponding plants transferred to the solutions without nitrogen. This is evident from the following comparison of R_A ($cm^2/cm^2/day$) over the 7 day period after transfer to the two test solutions.

Pretreatments

Transferred to:	N_0	N_1	N_2	N_3	N_4	N_5	
Complete solution	0.131	0.112	0.107	0.100	0.114	0.128	
Solution without N	0.107	0.090	0.082	0.070	0.063	0.079	$cm^2/cm^2/day$

Although there was a decrease in R_A for the plants transferred from the lower nitrogen pretreatments to the complete solutions, it appears that the greater leaf area differences between the two test solutions, as a result of the increase in nitrogen pretreatment levels from N_0 or N_1 to N_4, was largely attributable to a decrease in R_A in the solutions without nitrogen. The reason for the increase in R_A for plants transferred from N_5 to solutions without nitrogen, compared with those transferred from N_4, was probably the four times greater nitrogen supply in the N_5 pretreatment.

In view of the suppression of the nodulation observed on the day of transfer, which was mentioned earlier, it appears likely that the supply of symbiotic nitrogen after transfer to the solutions without nitrogen also decreased the higher the nitrogen pretreatment level before transfer. In other words, the difference in the nitrogen supplied to the plant by nitrogen fixation in the solutions without nitrogen, and the nitrogen supplied in combined form to the corresponding plants in the complete solutions, became greater the higher the nitrogen pretreatment level before transfer. It is suggested that this was the main reason for the decrease in R_A in the solutions without nitrogen, and therefore also for the increase in the leaf area differences between the two test solutions shown above.

If this explanation is correct, then the comparison of leaf area differences induced by transfer of plants to complete solutions and to solutions without nitrogen could conceivably be used to assess, at least qualitatively, whether nitrogen fixation before transfer was sufficient for plant growth. To investigate this possibility further a second experiment was carried out in which plants raised with combined nitrogen were compared with well nodulated plants of a similar stage of development.

The pretreatments and nitrogen levels applied were as follows:

Pretreatment number	Description of pretreatment	Pretreatment nitrogen level
1	Plants in solutions for 25 days, nitrogen applied on 11th day	2 p.p.m. N
2	As for no. 1	8 p.p.m. N
3	As for no. 1	64 p.p.m. N
4	Inoculated, plants in solutions for 25 days	0
5	Inoculated, plants in solutions for 25 days, nitrogen applied on 11th day	16 p.p.m. N
6	Sown seven days later than for pretreatments 1 - 5. Plants in solutions for 18 days, nitrogen applied on 4th day	64 p.p.m. N

To prevent spontaneous nodulation as much as possible the sand in which the seeds for pretreatments 1, 2, 3, and 6 were germinated was sterilized. The seeds for pretreatments 4 and 5 were inoculated, and a Rhizobium suspension was added to the pretreatment solutions. Nitrogen applications to pretreatments 1, 2, 3, and 5 were delayed for 11 days in order to allow sufficient time for nitrogen fixation in the inoculated pretreatments to commence. In previous experiments it had been found that 7-10 days were required for nodulated plants to become green and to commence growing at a satisfactory rate. Seeds for pretreatments 1-5 were sown at the same time. Seeds for pretreatment 6 were sown 7 days later in order to make sure that at least one group of plants raised with combined nitrogen would be at similar stage of development to the nodulated plants.

The changes in leaf area over a period of seven days after transfer of plants from each of the six pretreatments to complete solutions and to solutions without nitrogen are shown in Figure 15. The numbers 1-6 on day 0 in the graph refer to the pretreatment numbers given above. The differences in leaf area on day 7 between the two test solutions for the plants of pretreatments 1, 2, 3, and 6 were considerable. By contrast, plants raised from inoculated seed in pretreatments 4 and 5 showed little difference between the two test solutions. Even on day 7 the differences were small and of doubtful significance.

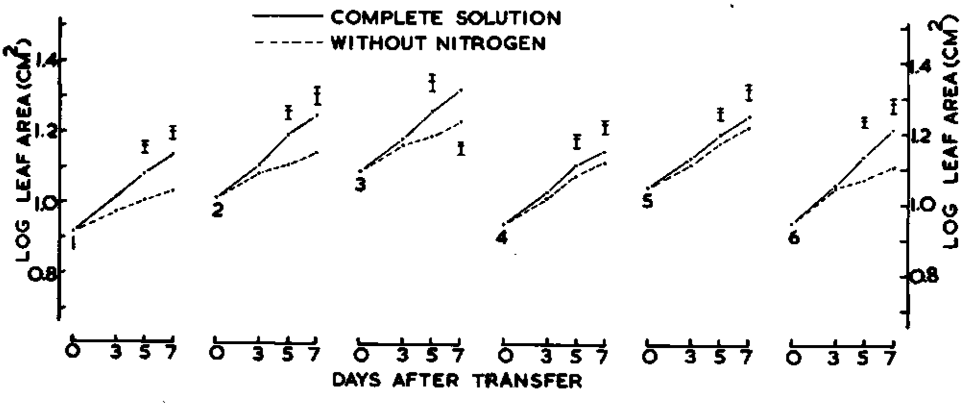

Figure 15.- Changes in leaf area during a period of seven days after transfer of plants raised with different nitrogen pretreatments to solutions with and without nitrogen. The pretreatments are shown as Nos. 1, 2, 3, 4, 5, and 6 on day 0 in the graph. The description of the pretreatments is given in the text.

These results confirm those of the first experiment. Even for plants raised at a high level of combined nitrogen, which in the second experiment was 64 p.p.m. compared with 40 p.p.m. nitrogen in the first experiment, leaf area differences between the two test solutions were considerable. On the other hand, inoculated and nodulated plants were apparently fixing sufficient nitrogen after transfer to solutions without nitrogen to cause a leaf area expansion which approached that of plants transferred to solutions with combined nitrogen.

It can be concluded that the present approach offers no prospects as a means of assessing the nitrogen status of plants. However, the results presented do suggest the feasibility of assessing the adequacy of the symbiotic nitrogen supply for plant growth. This conclusion is justified in view of the small advantage nodulated plants derived from combined nitrogen after transfer to complete solutions, compared with corresponding plants in solutions without nitrogen, at least over the short test period of seven days. That this may also apply to field conditions was shown in an experiment in which plants with healthy nodules sampled in a vigorously growing pasture were transferred to complete solutions and to solutions without nitrogen. Six days after transfer the leaf areas were 17.1 and 15.8 cm^2 for the two solutions respectively (minimum difference for significance 1.5 cm^2, $P \leqslant 0.05$).

It can be argued that this approach would give the wrong answer in cases where nitrogen fixation occurs at a reduced rate because of a suboptimal supply of a nutrient element. Molybdenum, for example, is known to affect nitrogen fixation (Anderson and Thomas 1946). When plants with a symbiotic nitrogen supply limited by molybdenum deficiency are transferred to solutions without nitrogen, the presence of molybdenum in these solutions could conceivably result in a leaf area expansion approaching that of corresponding plants transferred to complete solutions. Further work would have to be done to investigate this point. However, it appears doubtful that an impaired symbiotic system could show such a rapid recovery that the rate at which it is able to provide nitrogen for plant growth would approach that resulting from the uptake of combined nitrogen in complete solutions. The results of Figure 14 for the plants raised at N_2 and N_3, which had nodules of reasonable size on the day of transfer, appear to support this. Leaf area expansion in the solutions without nitrogen was significantly less in than the complete solutions.

3.1.2 The effect of a low nutrient level on the growth changes induced by the subsequent transfer of plants to complete nutrient solutions and to solutions lacking a different element.

In the experiments reported here subterranean clover plants were raised with a suboptimal supply of phosphorus, sulphur, potassium, calcium, or boron. When they were three weeks old the plants of each of these five pretreatments were then transferred to a complete nutrient solution, and to solutions from which phosphorus, sulphur, potassium, calcium, or boron had been omitted. On the day of transfer (day 0) plants were harvested in each pretreatment and their dry matter yield compared with plants that had been raised in full nutrient solutions. To facilitate comparisons the yield differences on day 0 were expressed as the ratio of the dry weight per plant in each of the pretreatments to that in the complete solution. These ratios were: 0.53, 0.84, 0.78, 0.84, and 0.86 for the phosphorus, sulphur, potassium, calcium, and boron pretreatment respectively.

The changes in leaf area over a period of seven days after transfer of plants from each of the five pretreatments to the test solutions mentioned are shown in Figure 16. It will be clear that for each of the pretreatments the rate of leaf area expansion was smallest in the solution lacking the element of which the supply before transfer had been low. In most cases the absence of one of the other elements did not significantly affect the rate of leaf area expansion over the seven day test period, compared with the corresponding plants transferred to the complete solutions.

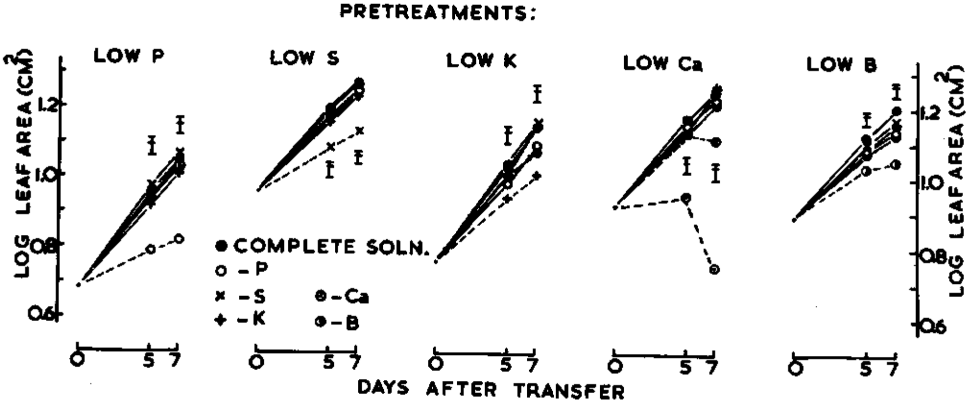

Figure 16.- Changes in leaf area after transfer of plants raised at a low level of phosphorus, sulphur, potassium, calcium, or boron to complete solutions (●), and to solutions without phosphorus (○), sulphur (×), potassium (–), calcium (⊙), or boron (⊙) respectively.

For the low potassium and low boron pretreatments leaf areas on day 7 in the solutions without phosphorus, or without calcium, tended to be lower than in the complete solution. However, the effects of omitting potassium in the case of the low potassium pretreatment, or of omitting boron for the low boron pretreatment were clear cut. The leaf areas on day 7 were considerably below any of the others.

For the low calcium pretreatment leaf areas in the solution without calcium were plotted excluding the leaves of which the petioles had collapsed, and this is the reason for the marked drop of the lower broken line. The upper broken line represents an estimate of the area per plant including the leaves with collapsed petioles. In either case the differences between this treatment and other treatments was highly significant. As the leaves of which the petioles had collapsed usually died and shrivelled up within 24 hours, the exclusion of these leaves is probably more realistic.

It is concluded that the changes in leaf area after transfer to solutions, each without a different nutrient element, were mainly determined by the element in shortest supply at the time of transfer. For the elements studied differences in leaf area between plants transferred to complete nutrient solutions and corresponding plants transferred to solutions without a different element, were small when the supply of the element concerned before transfer had been adequate. When the supply before transfer had been suboptimal, the causal element was identifiable because the expansion in leaf area of plants in the solutions lacking this element was smaller than that of corresponding plants in complete solutions or in solutions from which other elements had been omitted.

The possible application of these results for diagnostic purposes is discussed further in Section 4.1.

3.1.3 The interaction between nutrient elements.

In the experiments reported in the preceding Sections the supply of only one element at a time was varied, while most of the other elements were applied at near optimum levels. In practice it often occurs that more than one element is in short supply and it was therefore essential to determine to what extent this would modify the conclusions of the previous Sections.

This Section reports the results of three factorial experiments. The first experiment involved three levels of nitrogen times three levels of phosphorus, the second experiment three levels of nitrogen times three levels of sulphur, and the third experiment three levels of phosphorus times three levels of sulphur. The nutrient levels applied during pretreatment were based on the previous experiments, and were intended to give low, medium, and optimum supply of the varied elements. The levels are shown in Figures 17, 18, and 19. Other experimental details were the same as for the previous experiments.

3.1.3.1 The interaction between nitrogen and phosphorus.

In the top portion of Figure 17 are shown the leaf areas on the day of transfer to test solutions at the end of 14 days pretreatment with three nitrogen times three phosphorus levels. The levels are referred to from lowest to highest respectively as N_1, N_2, N_3 or P_1, P_2, P_3, or as their nine possible combinations. Pretreatments are referred to in a similar manner in the next two Sections when the other interactions are considered. The trends are those usually found when more than one factor limits plant growth. Only small effects of phosphorus occurred at the N_1 and N_2 levels, and vice versa, only small effects of nitrogen at the P_1 and P_2 levels. Seeds in this experiment had not been inoculated, but spontaneous nodulation occurred at the N_1 level, particularly in the N_1P_3 pretreatment. As in the experiments involving levels of nitrogen reported in Section 3.1.1.6, few nodules were apparent on the roots of N_2 and N_3 plants. Figure 17 also shows the changes in leaf area over a period of seven days after plants of the nine pretreatments had been transferred to complete solutions, to solutions without nitrogen, and to solutions without phosphorus.

Considering the effect of the three phosphorus pretreatments first, it is apparent that the plants transferred from P_1 and P_2 showed a considerably smaller leaf area increase in the solutions without phosphorus than in the complete solutions. The relative differences were greater for the P_1 plants than for those transferred from P_2. This occurred for all nitrogen pretreatment levels. Differences for plants raised at P_3 were small. However, the evidence indicates that the relative differences in leaf area for plants transferred from P_1 or P_2 to solutions with and without phosphorus became greater the higher the nitrogen status of the plants at the time of transfer. This will be clear from the following comparison, which gives the ratios of leaf areas on day 7 in the solutions without phosphorus to that in the complete solutions for the following pretreatment combinations:

	N_1	N_2	N_3
P_1	0.72	0.71	0.60
P_2	0.86	0.84	0.75

For each phosphorus pretreatment the decrease in the ratio was greatest between N_2 and N_3. The relative rates of leaf area increase (R_A) over the 7 day period after transfer to complete solutions varied comparatively little with nitrogen or phosphorus pretreatment levels (range 0.090 - 0.110 $cm^2/cm^2/day$). After transfer of plants from P_1 or P_2 to test solutions with-

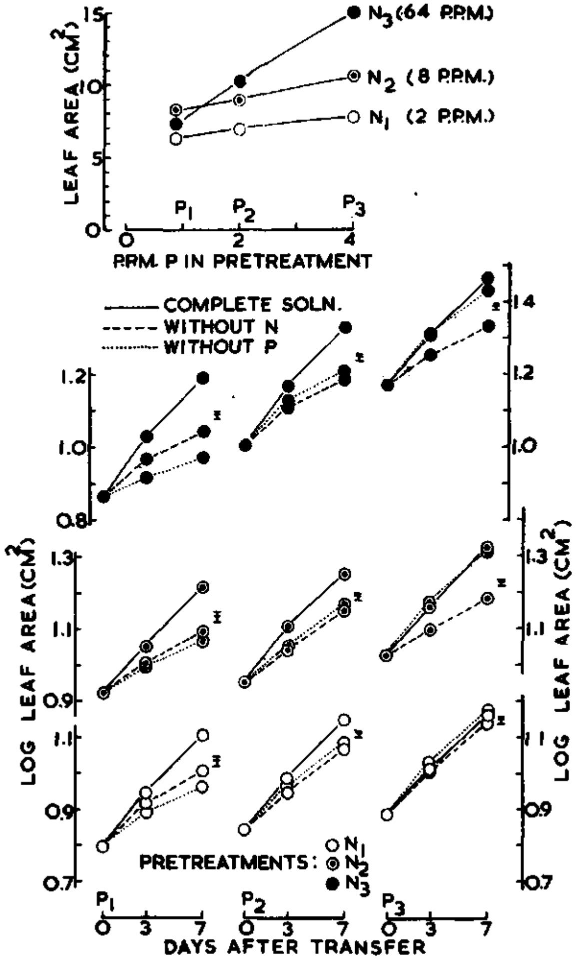

Figure 17.- Leaf areas at the end of a pretreatment period of 14 days at three nitrogen times three phosphorus levels (top). The nitrogen levels were 2, 8, and 64 p.p.m., and the phosphorus levels 1, 2, and 4 p.p.m. Below the leaf areas at the end of the pretreatment period are shown the subsequent changes in leaf area during a period of seven days after transfer of plants from each of the nine pretreatments to complete solutions (———), to solutions without nitrogen (-----), and to solutions without phosphorus (.....). Nitrogen pretreatments are shown thus: ○ N_1, ◉ N_2, ● N_3. The leaf area changes for the plants transferred from P_1 are shown on the left, for those from P_2 in the centre, and for the plants transferred from P_3 on the right of the graph.

out phosphorus, R_A was lower the higher the nitrogen level before transfer. For example, R_A for P_1 plants transferred to solutions without phosphorus from N_1, N_2, or N_3 pretreatments was 0.053, 0.048, and 0.036 cm^2/cm^2/day respectively. These results suggest, as would be expected, that the P_1 or P_2 plants raised at N_3 were under a relatively greater phosphorus stress at the time of transfer than the plants raised at N_1. The total phosphorus concentrations (per cent. dry weight) in the tops of the P_1 and P_2 plants on day 0 were as follows:

	N_1	N_2	N_3
P_1	0.25	0.26	0.23
P_2	0.58	0.43	0.43 % P.

Although there is evidence for a dilution of the phosphorus content at P_2 between N_1 and N_2, this did not occur at P_1. It is suggested that the comparison of relative changes in leaf area after transfer to appropriate test solutions probably showed a physiologically more accurate picture of the phosphorus status of the plant at the time of transfer than the concentrations of phosphorus in the plant tissue.

The changes in leaf area after transfer to solutions without nitrogen are in line with the findings of Section 3.1.1.6, in which it was found that the differences in leaf area after transfer to solutions with and without nitrogen were small only when plants were well nodulated at the time of transfer. In the present experiment this was only the case in the N_1P_3 pretreatment, and to some extent in N_1P_2. This is apparent in the following rating of nodulation carried out on day 0. A rating of 1 represented no or a few small nodules, 2 some healthy nodules, and 3 well nodulated. Only the results for N_1 and N_2 are given. No, or only a few small nodules, were visible on the roots of the N_3 plants.

	P_1	P_2	P_3
N_1	1.6	2.1	2.6
N_2	1.2	1.1	1.3

The better nodulation at N_1 than at N_2, irrespective of phosphorus pretreatment, was probably the reason why the N_1 plants showed smaller relative differences in leaf area after transfer to solutions with and without nitrogen than the N_2 plants. The improvement in nodulation at N_1 with increasing phosphorus levels could be related to a subnormal level of nitrogen fixation under conditions of phosphorus deficiency (Anderson and Thomas 1946). This is also indicated by the nitrogen content of the tops on day 0, which increased from 2.72 per cent. at N_1P_1 to 3.33 per cent. of the dry matter of the tops at

N_1P_3. At the higher nitrogen levels, increases in leaf area after transfer to solutions without nitrogen were always considerably smaller than in the complete solutions. This happened in spite of much higher nitrogen concentrations in the tops on the day of transfer. The comparison on day 0 of the nitrogen concentrations in the tops of the N_1 and N_3 plants at the three phosphorus pretreatments was as follows:

	P_1	P_2	P_3
N_1	2.72	3.35	3.33
N_3	5.26	5.54	5.16 % N.

It will be noticed that, in spite of a high nitrogen content of 5.16 per cent. at N_3P_3, leaf area increases after transfer to solutions without nitrogen were relatively small. Even for the smaller N_3P_1 plants with a nitrogen content of 5.26 per cent., the relative increases in leaf area in the solutions without nitrogen were less than for the N_1P_3 plants with a nitrogen content of only 3.33 per cent. on the day of transfer to the no-nitrogen solutions. It would seem that the N_1P_3 plants after transfer to solutions without nitrogen received sufficient symbiotic nitrogen to maintain a satisfactory growth rate.

3.1.3.2 The interaction between nitrogen and sulphur.

The effects of three nitrogen times three sulphur levels on leaf areas at the end of a pretreatment period of 16 days are shown in the top of Figure 18. The effects were essentially similar to those found for the nitrogen phosphorus interaction of the previous Section. There was no effect of sulphur at the lowest nitrogen pretreatment level (N_1), except perhaps a small depression. Nitrogen pretreatment levels had no effect at the lowest sulphur pretreatment level (S_1). At N_3 sulphur levels showed the greatest effect, and vice versa, nitrogen levels the greatest effect at S_3.

The changes in leaf area over a period of seven days after transfer of plants from each of the nine pretreatments to complete solutions, and to solutions without nitrogen or without sulphur, are shown in Figure 18 below the response curves for day 0.

The effect of pretreatments on the differences in leaf area after transfer of plants to solutions without nitrogen compared with those in complete solutions were less easy to interpret than before. For the N_1 pretreatment with sulphur levels S_2 and S_3 the differences were small, and less than for the N_1 plants raised at S_1. This result was somewhat similar to that of the previous Section for plants raised at a low nitrogen level with increasing phosphorus supply (Figure 17). It may have been due to an effect of sulphur on nodulation during pretreatment, similar to the effect of phosphorus on

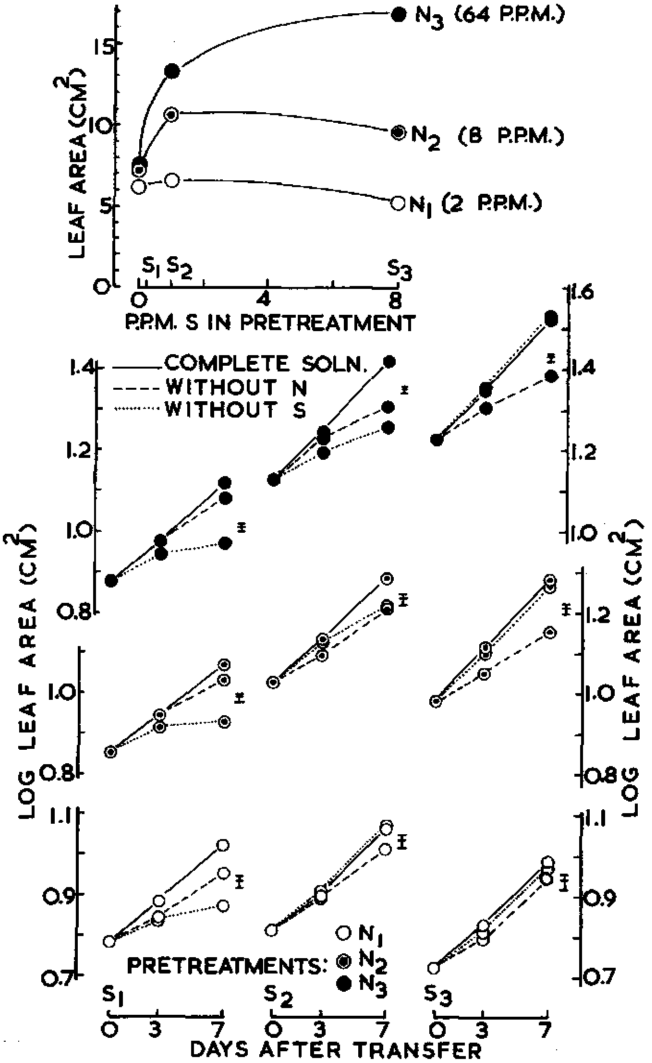

Figure 18.- Leaf areas at the end of a pretreatment period of 16 days at three nitrogen times three sulphur levels (top). The nitrogen levels were 2, 8, and 64 p.p.m., and the sulphur levels 0.125, 1, and 8 p.p.m. Below the leaf areas at the end of the pretreatment period are shown the subsequent changes in leaf area during a period of seven days after transfer of plants from each of the nine pretreatments to complete solutions (———), to solutions without nitrogen (-----), and to solutions without sulphur (·········). Nitrogen pretreatments are shown thus:○N_1,⊙N_2,●N_3. The leaf area changes for the plants transferred from S_1 are shown on the left, for those from S_2 in the centre,and for the plants transferred from S_3 on the right of the graph.

nodulation found in the previous experiment. However, this can be no more than a suggestion, because there were no ratings available of the nodulation in the different pretreatments on the day of transfer.

The main difference in the comparison of leaf area changes after transfer to the test solutions with and without nitrogen with those of the previous experiment occurred for the N_2 and N_3 plants. It is clear from Figure 18 that the leaf area differences between complete and no-nitrogen solutions increased, the higher the sulphur level had been before transfer. This occurred for the N_2 plants as well as for the N_3 plants. To emphasize this point, the following comparison gives the ratios of leaf areas on day 7 in the solutions without nitrogen to that in the complete solutions for the pretreatments shown.

	S_1	S_2	S_3
N_2	0.86	0.83	0.75
N_3	0.91	0.76	0.73

The nitrogen concentration in the tops of the plants on the day of transfer from these pretreatments was as follows:

	S_1	S_2	S_3
N_2	2.75	2.55	2.79
N_3	4.34	4.67	4.79 % N.

It is clear that the nitrogen contents were virtually independent of the sulphur level that had been applied during pretreatment. This would exclude differences in the nitrogen status of the plant on the day of transfer as a likely explanation for the increase in the relative leaf area differences between the two test solutions. No satisfactory explanation can be offered at this stage. However, the present results compared with those of the nitrogen times phosphorus interaction of the previous Section suggest a lower demand in the plant for nitrogen during the initial stages of recovery from a sulphur stress than during recovery from a phosphorus stress.

The trends in the relative leaf areas differences between plants transferred to complete solutions and to solutions without sulphur are less difficult to interpret. It is clear that there were no differences in leaf area between the two solutions for the plants transferred from S_3, irrespective of the nitrogen level during pretreatment.

At the lowest sulphur level during pretreatment (S_1) no effects were found of nitrogen pretreatment levels on the leaf areas obtained on the day of transfer (top, Figure 18), and it is reasonable to conclude that during pretreatment plant growth at S_1 had been determined by the limits imposed by the sulphur stress. As a result, rates of leaf area expansion after transfer to solutions without sulphur were very low and virtually independent of the nitrogen supply before transfer. The values of R_A for the S_1 plants over the 7 day period after transfer from N_1, N_2, and N_3 to solutions without sulphur were 0.029, 0.025, and 0.032 $cm^2/cm^2/day$ respectively.

At the intermediate sulphur level (S_2) the severity of the sulphur stress which developed during pretreatment depended on the interacting effect of nitrogen levels. As the S_2 level did not have any significant effect on leaf areas of the N_1 plants (top, Figure 18), the growth at the N_1S_2 pretreatment level was probably still limited by a low nitrogen supply. When the plants raised in the N_1S_2 culture solutions were transferred to solutions without sulphur, leaf area expansion was the same as for the corresponding plants in the complete solutions. At the intermediate nitrogen level (N_2) the S_2 plants possibly developed a slight sulphur stress during pretreatment, which became apparent as a small but significant reduction in leaf area seven days after transfer to solutions without sulphur compared with the corresponding plants in the complete solutions. The increase in leaf area between S_2 and S_3 at the N_3 level established on day 0 (top, Figure 18) indicates that the sulphur stress of the S_2 plants during pretreatment had increased as a result of the increase in nitrogen supply from N_2 to N_3. Subsequent transfer of the N_3S_2 plants to solutions with and without sulphur resulted in considerably greater leaf area differences than for the N_2S_2 plants.

3.1.3.3 The interaction between phosphorus and sulphur.

The leaf areas after a pretreatment period of 17 days at three phosphorus times three sulphur levels are shown in the top portion of Figure 19. The trends are similar to those discussed in the previous Sections for the nitrogen times phosphorus, and for the nitrogen times sulphur interaction.

The effect of the sulphur pretreatment levels on the relative leaf area differences after transfer to complete solutions and to solutions without sulphur generally followed the same pattern as that discussed for sulphur in relation to the interaction with nitrogen of the previous Section. At the lowest sulphur pretreatment level the differences after transfer to complete and no-sulphur solutions were considerable, and were not related to phosphorus pretreatment levels. The relatively greater difference between the two solutions at P_2S_1 compared with P_1S_1 and P_3S_1 was probably not real, and at least partly caused by an anomalous replicate in P_2S_1. It is likely that at S_1 the sulphur stress was the major determinant of the relative differences in leaf area between complete and no-sulphur solutions. This is also suggested by the small effect of phosphorus levels on the leaf areas of the S_1 plants on day 0 (top, Figure 19).

At the highest sulphur level (S_3) there were no differences in leaf area between plants in the complete solutions and in the solutions without sulphur. This applied irrespective of

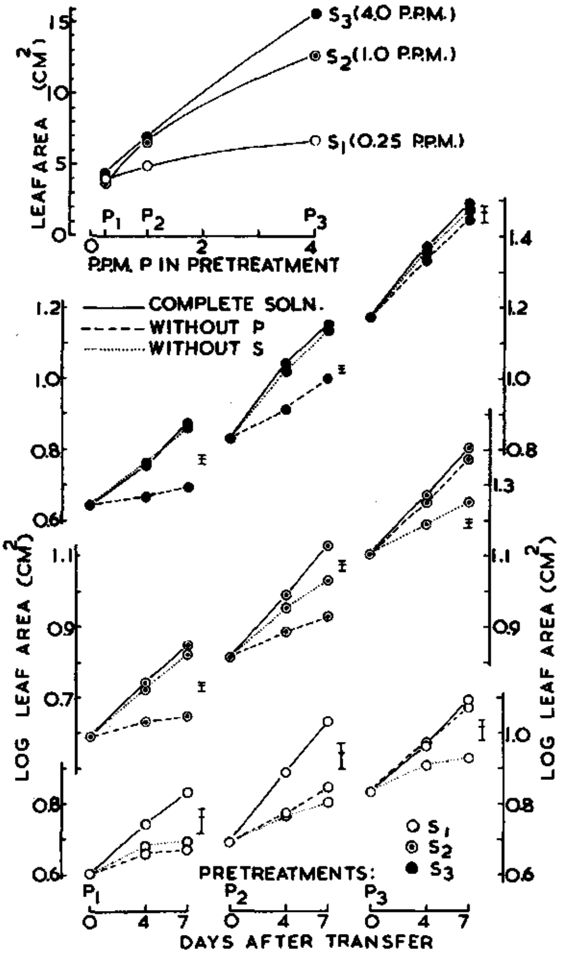

Figure 19.- Leaf areas at the end of a pretreatment period of 17 days at three phosphorus times three sulphur levels (top). The phosphorus levels were 0.25, 1, and 4 p.p.m.,and the sulphur levels 0.25, 1, and 4 p.p.m. Below the leaf areas at the end of the pretreatment period are shown the subsequent changes in leaf area during a period of seven days after transfer of plants from each of the nine pretreatments to complete solutions (———); to solutions without phosphorus (----), and to solutions without sulphur (·········). Sulphur pretreatments are shown thus:○S_1, ⊚S_2, ●S_3. The leaf area changes for the plants transferred from P_1 are shown on the left, for those from P_2 in the centre, and for the plants transferred from P_3 on the right of the graph.

phosphorus pretreatment levels.

Plants raised at the S_2 pretreatment level showed a considerable response to phosphorus, and the relative changes in leaf area after transfer to complete solutions and to solutions without sulphur strongly suggest that the severity of the sulphur stress during pretreatment depended on the accompanying phosphorus level. At P_1 phosphorus was the more important factor. As the phosphorus level increased, the stress caused by sulphur became more severe, and as a consequence the relative differences in leaf area induced by transfer to complete and no-sulphur solutions increased. This is apparent from the following comparison of the ratios of leaf areas in the two solutions for S_2 plants raised at P_1, P_2, and P_3 respectively: 0.97, 0.80, and 0.71.

The results for the three phosphorus pretreatments also fall into a reasonably simple pattern. No or only small differences between the complete and the no-phosphorus solutions were recorded for plants raised at the P_3 level. This applied irrespective of the sulphur pretreatment levels.

At the lowest phosphorus pretreatment level (P_1) the phosphorus stress limited plant growth, and the relative differences in leaf area after transfer to complete solutions and to solutions without phosphorus were independent of sulphur pretreatment levels. This is also indicated by the low phosphorus contents in the dry matter at P_1 for each of the three sulphur pretreatments on day 0. The values were: 0.107, 0.108, and 0.102 per cent. at P_1S_1, P_1S_2, and P_1S_3 respectively.

The results for the plants raised at P_2 were essentially similar to those for the P_1 plants. The response curves on day 0 (top, Figure 19) showed that the difference in leaf area between P_1 and P_2 at both S_2 or S_3 were small compared with those between P_2 and P_3. In other words, plant size at P_2 was considerably closer to P_1 than to P_3, suggesting that phosphorus was still the major factor determining growth at P_2. This is also indicated by the phosphorus contents in the dry matter of the tops of the plants on day 0. At S_2 for example, the contents were 0.108, 0.133, and 0.414 per cent. for P_1, P_2, and P_3 respectively. The increase between P_1 and P_2 was small compared with the one from P_2 to P_3. If a phosphorus stress at P_2 was the major factor limiting growth, the absence of any marked sulphur effect on the relative differences in leaf area between complete and no-phosphorus solutions becomes understandable. It would follow from this that, if the P_2 level were increased, a point would be reached where sulphur pretreatments would affect the relative stress caused by phosphorus. It may be recalled that this kind of pattern was found in the preceding Sections for sulphur pretreatments and the phosphorus or nitrogen interactions therewith, with respect to the relative differences in leaf area after transfer to complete solutions and to solutions without sulphur. A similar pattern was also found in Figure 17 for the nitrogen-phosphorus pretreatment interaction in relation to the leaf area comparison after transfer to complete solutions and to solutions without phosphorus.

The results of the three experiments just presented are essentially in agreement with those of the earlier experiments in which only the supply of one element was varied during pretreatment. In the present experiments too, differences in leaf area between plants in complete solutions and in solutions lacking phosphorus or sulphur were small if the level before transfer had been adequate. With a suboptimal supply during pretreatment the differences were considerable, provided the supply of the other element had been adequate.

When the supply of two elements had been deficient during pretreatment, both to approximately the same extent, the leaf area increases after transfer of plants to the two test solutions without one of the elements were smaller than in the complete solutions. This was demonstrated best in the experiment with three phosphorus times three sulphur levels (Figure 19). It, therefore, would appear feasible to identify stresses caused by these two elements, provided they are of approximately equal severity. When phosphorus and sulphur had been supplied at greatly differing suboptimal levels during pretreatment, the evidence indicated that the leaf area differences at the end of the test period were greatest between plants in the complete solutions and the corresponding plants in the solutions without the element which had been in shortest supply before transfer. Leaf area changes after transfer to the solutions lacking the other element were closer to those in the complete solutions the greater the difference in the supply of the two elements before transfer. Figure 19 showed that there may be situations in which the leaf area differences induced by the transfer to test solutions would justify the conclusion that only one element was deficient at the time of transfer, simply because the supply of this element had been low enough to mask the deficiency of the other element. The trends in the leaf area differences after transfer, at least those resulting from the interaction between different phosphorus and sulphur levels applied during pretreatment, followed the Law of Minimum factors rather closely.

The effects of nitrogen pretreatments on the comparison of leaf area changes induced by transfer to complete solutions and to solutions without nitrogen, particularly in the experiment with nitrogen and phosphorus levels (Figure 17), were also in agreement with the results of the earlier experiments (Section 3.1.1.6). It was again found that plants raised at an adequate nitrogen level, when transferred to solutions without nitrogen, showed a considerably smaller leaf area expansion than corresponding plants in complete solutions. However, differences in leaf area between solutions with and without nitrogen were small for plants that were well nodulated at the time of transfer, which was also found in Section 3.1.1.6.

3.1.4 Factors affecting the changes in leaf area induced by the transfer of plants to nutrient solutions.

3.1.4.1 The effect of the stage of development.

In the previous experiments plants remained in the pretreatment solutions for the same length of time. The differences in nutrient levels applied during that period resulted in marked differences in plant size on the day of transfer. The plants raised at the higher pretreatment levels generally had not only larger, but also more, leaves. These plants were therefore more advanced in their development than those of the lower pretreatment levels. The question may be asked, to what extent the results would be modified when plants of differing and suboptimal nutrient status are transferred to test solutions if plant sizes are equal. To answer this question , plants were raised at two suboptimal levels of phosphorus and at two suboptimal levels of sulphur. The phosphorus levels were 0.5 and 1 p.p.m., and the sulphur levels were 0.25 and 0.5 p.p.m. These four pretreatments are referred to as P_1, P_2, S_1, and S_2 respectively. The plants remained in these solutions for a period of 14 days. A comparison was made between these plants and another group of plants raised at the second phosphorus level (1 p.p.m.) and at the second sulphur level (0.5 p.p.m.), but from seed which had been sown four days later. These plants are referred to as P_2' and S_2'. On the day of transfer from pretreatment solutions the P_2' and S_2' plants were therefore four days younger than those of the other four pretreatments. The leaf area on day 0 of the P_2' plants was close to that of the P_1 plants, and that of the S_2'plants nearly the same as that of the S_1 plants. This is shown in the following comparison:

P_1	P_2	P_2' (Sown four days later)	S_1	S_2	S_2' (Sown four days later)
8.25	9.92	8.70	9.16	10.40	8.85 cm^2 .

The changes in leaf area induced by transfer of these six groups of plants to solutions with and without the varied element are shown in Figure 20. The results for the comparison between P_1 and P_2, and between S_1 and S_2 confirm those already presented.

It will also be apparent that the delay in sowing of the P_2' and the S_2' plants caused somewhat smaller relative differences in leaf area than those found for the older plants raised at the same level (P_2 and S_2 respectively). However, the differences in leaf area on day 7 for the P_2' and S_2' plants were still highly significant.

Table 1 gives a statistical evaluation of the differences between pretreatments with respect to the relative leaf area differences in the solutions without and with the varied ele-

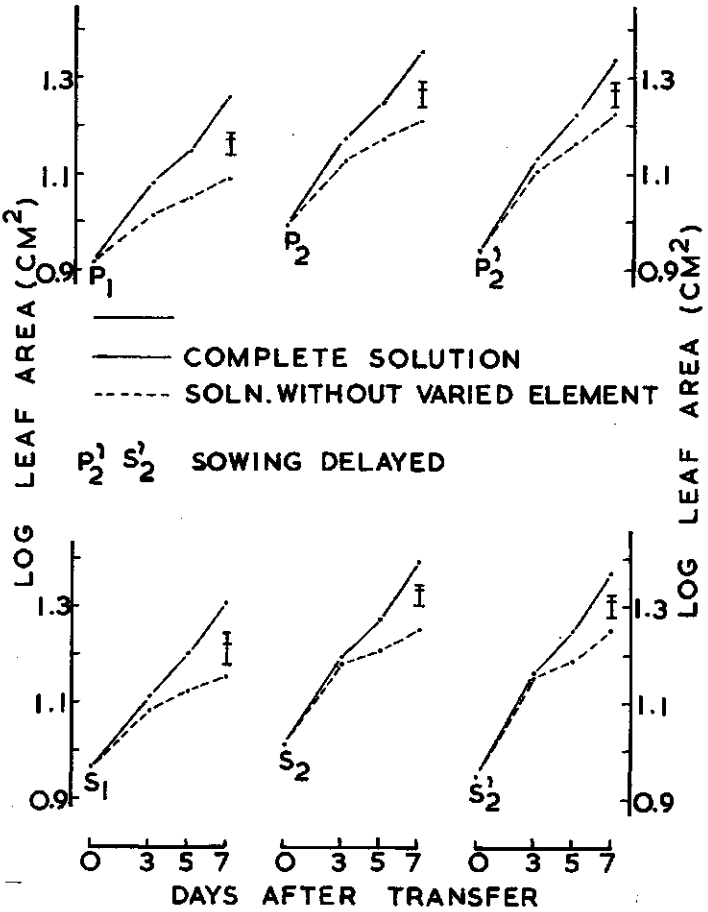

Figure 20.- Changes in leaf area during a period of seven days after transfer of plants raised at two deficient phosphorus levels (P_1 and P_2) and at two deficient sulphur levels (S_1 and S_2) to complete solutions and to solutions without phosphorus or sulphur respectively. P_2' and S_2' plants were also raised at the second phosphorus or the second sulphur level respectively. They had been sown four days later to obtain plants of similar size as the plants raised at P_1 or S_1 respectively.

ment on day 7. The delay in the sowing of the P_2' and S_2' plants had caused somewhat smaller relative differences in leaf area on day 7 compared with P_2 and S_2 respectively. Although this effect was small and did not reach significance, it probably represented a real treatment effect. Suboptimal levels of phosphorus or sulphur were present in the solutions, and it seems likely that plants grown in it for a shorter period of time (P_2' or S_2'), would be under a less severe stress at the time of transfer (day 0) than the plants that had grown at the same levels for a longer period (P_2 or S_2).

TABLE 1

COMPARISON OF RELATIVE LEAF AREA DIFFERENCES ON DAY 7 BETWEEN PRETREATMENTS.

Pretreatment	Rel. differences in leaf area between solutions without and with varied element*	Differences between pretreatments in rel. leaf area differences**	Min. differences for significance	
			$P < 0.05$	$P < 0.01$
P_1	-0.170	$P_2 - P_1$: 0.034	0.037	0.053
P_2	-0.136	$P_2' - P_1$: 0.060	0.033	0.048
P_2'***	-0.110	$P_2' - P_2$: 0.026	0.031	0.045
S_1	-0.154	$S_2 - S_1$: 0.016	0.028	0.040
S_2	-0.138	$S_2' - S_1$: 0.043	0.032	0.046
S_2 ***	-0.111	$S_2' - S_2$: 0.027	0.036	0.052

* These values are the differences of the logs of the leaf areas in the solutions without and with the varied element.

** The values in this column were obtained by subtracting the relative leaf area differences for the pretreatment comparisons as shown.

*** P_2' and S_2' plants were raised at the same level as P_2 and S_2 resp. but were four days younger.

The smaller relative leaf area differences for the P_2' and S_2' plants on day 7 caused a marked increase in the comparison $P_2'-P_1$ and $S_2'-S_1$ compared with P_2-P_1 and S_2-S_1 respectively.

From a practical point of view, it would seem that under conditions where a stress is caused by the exhaustion of the pool of supply, as for example in pot cultures with soil, results similar to those above may be obtained. As the plant develops, the supply becomes smaller and the stress severer. A situation can also be visualized in which a nutrient stress is caused by a suboptimal rate of supply to the root system, rather than by exhaustion of a suboptimal, but available, pool. Under field conditions the former situation is probably the more usual one. When seed reserves are exhausted the plant would have a suboptimal, but more or less continuous supply of nutrient available, for at least part of its life cycle. In this case the stage of development would probably not be as important as in the kind of situation of which the experiment above is an example. This suggestion is supported by recent field experience. In a number of field experiments, involving phosphorus and sulphur treatments, plants were sampled at a young stage of development in late autumn and early winter, and also at a much later stage of development in late winter and early spring. After washing the roots thoroughly from adhering soil, the plants from the control plots were transferred to complete solutions and to solutions without phosphorus (no-P) or without sulphur (no-S). Plants from the phosphorus treated plots were transferred to complete solutions and to solutions without sulphur. Plants sampled in the sulphur plots were placed in complete solutions and in solutions without phosphorus. A comparison between the two samplings of the relative differences in leaf area seven days after transfer to the above solutions is given in Table 2.

There is no evidence of any consistent trends between samplings that can be related to differences in the stage of development. In most experiments the relative leaf area differences were almost identical on the two sampling occasions. Although greatly differing stages of development existed in these experiments, arising from seeds that had germinated at different times, it was not found difficult to select plants of a reasonably uniform stage of development, even between different treatments.

TABLE 2

COMPARISON BETWEEN TWO SAMPLINGS OF RELATIVE LEAF AREA DIFFERENCES, SEVEN DAYS AFTER TRANSFER OF PLANTS SAMPLED IN FIELD PLOTS TO NUTRIENT SOLUTIONS AS SHOWN. (The differences in leaf area induced by transfer are expressed as the ratios of the leaf area in the solution without phosphorus or without sulphur to that of the corresponding plants transferred to complete solutions, no-P/C and no-S/C respectively).

Field treatment	Control (nil)				Phosphorus		Sulphur	
Transferred to	no-P/C		no-S/C		no-S/C		no-P/C	
Experiment number	1[*]	2	1	2	1	2	1	2
1	0.94	0.90	0.90	0.95	0.96	0.96	0.89	0.81
2	0.83	0.84	0.91	0.92	0.89	0.91	0.88	0.83
4	0.89	0.91	0.80	0.86	0.83	0.84	0.79	0.81
13	0.86	0.87	1.03	1.04	0.97	0.97	0.89	0.88
18	0.92	0.99	0.83	0.82	0.89	0.87	0.93	0.87

[*] Column 1 refers to the late autumn, early winter sampling, and column 2 to the sampling in late winter, early spring

3.1.4.2 The effects of different temperatures during pretreatment and after transfer to solutions.

The experiments reported so far were all carried out in a glasshouse in which the temperature was maintained at approximately 24° C during the day and at 17° C during the night. When plants are raised at different nutrient levels, differences in temperature may affect, not only the expression of the nutrient stress during pretreatment, but also the relative differences in leaf area after transfer to complete nutrient solutions and to solutions without the varied element.

The experiments reported here were carried out in the Controlled Environment Laboratory "Ceres", of the Division of Plant Industry, C.S.I.R.O., Canberra, Australia. In the first experiment plants were raised at phosphorus levels of 1 and 5 p.p.m., both treatments at three different temperatures. The temperature treatments were 15/10, 21/16, and 27/22° C. These are referred to as T_1, T_2, and T_3 respectively. The first figure of each temperature treatment refers to the day temperature (6 a.m.-6 p.m.) and the second figure to the night temperature. In the second experiment, plants were raised at 1 and 5 p.p.m. sulphur, and both sulphur treatments occurred at the same three temperature treatments as for phosphorus. After a pretreatment period of 18 days the plants from each temperature and nutrient level were assigned at random to solutions with and without the varied element, and to each of the three temperatures. They were grown at these treatments for a further period of seven days after transfer.

Table 3 shows the interactions of phosphorus or sulphur pretreatment levels with temperatures during the pretreatment period. Leaf areas and dry weights were those obtained on the day of transfer (day 0). The effects of nutrient levels and temperatures on leaf areas were relatively greater than those on dry weight. The effect of phosphorus at T_1 was small, and there was no effect of sulphur levels at T_1. At higher pretreatment temperatures the stress imposed by phosphorus deficiency appeared to be greater than that imposed by sulphur.

In Table 4 are shown the ratios of leaf areas after seven days in solutions without the varied element to that of the corresponding plants in complete solutions as affected by nutrient level and temperature during pretreatment, and by the temperature during the 7 day period after transfer.

The ratios for plants raised at the higher phosphorus or sulphur level varied little with temperature during pretreatment or after transfer. The fact that the ratio was always near unity suggests an adequate nutrient status at the time of transfer.

Within each temperature the ratio for the P_1 plants was always lower than for the S_1 plants. This was a reflection of the relatively greater stress imposed by the P_1 pretreatment in the first experiment, than by S_1 in the second experiment. If the dry weights for P_1 and S_1 on day 0 for the T_2 pretreatment in Table 3 are expressed as fractions of the yields at P_2 and S_2 respectively, a value of 0.74 is obtained for P_1/P_2, and 0.83 for S_1/S_2. These values are nearly identical to the ratios of leaf areas seven days after transfer of the P_1 and S_1 plants to T_2 or T_3 (see Table 4).

TABLE 3

EFFECTS OF PHOSPHORUS OR SULPHUR LEVELS ON LEAF AREA AND ON DRY WEIGHT OF PLANTS GROWN AT DIFFERENT TEMPERATURES AFTER A PRETREATMENT PERIOD OF 18 DAYS.

	T_1(15/10° C)	T_2(21/16° C)	T_3(27/22° C)
Leaf area (cm^2)			
P_1	4.50	7.52	9.94
P_2	6.25	12.61	15.63
Dry weight (mg)			
P_1	41.1	59.2	78.9
P_2	48.3	80.5	96.4
Leaf area (cm^2)			
S_1	6.92	9.51	12.17
S_2	6.86	14.80	18.16
Dry weight (mg)			
S_1	65.1	95.1	105.8
S_2	61.3	114.5	139.0

It is clear from Table 4 that P_1 or S_1 plants kept at T_1 during the 7 day period after transfer showed a higher ratio of leaf areas than the corresponding plants kept at T_2 or T_3. This was a result of the fact that the lower temperature of T_1 limited the response of P_1 and S_1 plants after transfer to complete solutions, and also reduced the stress on these plants after transfer to solutions without the varied element. This is apparent, for example, from the changes in leaf area of the S_1 plants raised at T_2 and subsequently transferred to T_1 and T_2. On day 0 the leaf area of these plants was 9.15 cm^2. Seven days after transfer to T_1 this value had changed to 12.26 cm^2 in the complete solutions and to 11.14 cm^2 in the solutions without sulphur. The corresponding plants transferred to T_2 however, had increased their leaf area after seven days from 9.51 cm^2 on day 0, to 14.66 cm^2 and to 12.09 cm^2 in the complete and no-sulphur solutions respectively.

Within each temperature regime during the 7 day period after transfer, the ratios for P_1 or S_1 plants raised at the T_1 pretreatment were always lower than for plants raised at the higher pretreatment temperatures. Judging by the small dry weight differences on day 0 as a result of phosphorus or sulphur levels at the T_1 pretreatment, compared with those of plants raised at T_2 or T_3 (Table 3), the plants would be less deficient than those raised at higher temperatures. One would therefore expect the ratios on day 7 for the plants raised at T_1 to be higher than for T_2 or T_3 plants.

TABLE 4

THE EFFECT OF DIFFERENT TEMPERATURES DURING AND AFTER PRETREATMENT ON THE RATIOS OF THE LEAF AREA IN THE SOLUTIONS WITHOUT THE VARIED ELEMENT TO THAT IN THE COMPLETE SOLUTIONS.

		Temperature during 7 day period after transfer					
Pretreatment:		$T_1(15/10^{\circ}$ C)		$T_2(21/16^{\circ}$ C)		$T_3(27/22^{\circ}$ C)	
Experiment 1							
P_1	T_1	0.74		0.64		0.69	
	T_2	0.80		0.74		0.73	
	T_3	0.80		0.75		0.74	
P_2	T_1		0.92		0.98		0.96
	T_2		0.98		0.94		0.96
	T_3		1.02		0.96		0.95
Experiment 2							
S_1	T_1	0.86		0.72		0.70	
	T_2	0.91		0.82		0.82	
	T_3	0.90		0.82		0.82	
S_2	T_1		1.05		1.01		0.98
	T_2		0.96		1.01		1.01
	T_3		1.01		0.96		0.94

The lower ratios for the P_1 and S_1 plants raised at T_1, than for the P_1 and S_1 plants raised at T_2 or T_3, could have been due to an effect of the low pretreatment temperature on the relative rate of leaf area expansion (R_A) of the plants transferred to complete solutions, or on R_A of the plants transferred to the solutions without the varied element, or on both. The comparison of R_A for the S_1 and S_2 plants raised at T_1, over the 7 day period after transfer to complete and no-sulphur solutions placed at T_2, was as follows:

	Transferred to T_2 in:	
Pretreatments	Complete solutions	no-sulphur solutions
S_1T_1	0.109	0.062
S_2T_1	0.115	0.112 cm^2/cm^2/day

In view of the results obtained earlier (e.g.Figures 4 and 6), in which it was shown that the rates of leaf area expansion after transfer of plants from different phosphorus or sulphur pretreatment levels to complete solutions did not differ greatly, the value of 0.109 cm^2/cm^2/day for R_A of the S_1T_1 plants in the complete solutions appears reasonable. The low value of 0.062 cm^2/cm^2/day suggests that a low rate of leaf area increase after transfer of the S_1 plants to solutions without sulphur was the main factor in the lower ratio of leaf areas on day 7 for the plants raised at T_1 shown above (Table 4). This value is low in comparison with the value of 0.109 cm^2/cm^2/day for the corresponding plants in the complete solutions. Table 3 showed that there were no differences in plant size between the S_1 and S_2 plants raised at T_1 at the end of the pretreatment period (day 0). The results for the S_1T_1 plants transferred to T_3 were the same. The trends in the phosphorus experiment were similar to those above, but differed in magnitude, because the P_1 level had imposed a relatively greater stress than the S_1 level in the sulphur experiment.

No satisfactory explanation can be given for the relatively low rates of leaf area increase when plants raised at suboptimal nutrient levels at low temperatures were transferred to solutions without the varied element. Although the resulting reduction in the ratio of leaf areas spoils the quantitative relationship somewhat, the evidence presented nevertheless suggests that the comparison of leaf area changes induced by transfer to test solutions can reveal a suboptimal nutrient supply before transfer, even if its effect is masked by a low temperature. The evidence also suggests that above a temperature somewhere between T_1 and T_2, differences in pretreatment temperature do not have any marked effect on the leaf area comparisons after transfer to test solutions. For practical purposes it is suggested that the temperature during the 0-7 day test period is not very critical above a certain level, which appears to be around that of the T_2 treatment (21/16°C). With lower temperatures during the test period, the relative leaf area differences induced by the transfer would decrease. It may be possible to obtain the same differences as at higher temperatures by prolonging the test period beyond seven days.

3.2 An examination of the growth changes and the nutrient element distribution induced by the removal of nutritional stresses.

In Section 3.1 it was shown that the leaf area changes induced by the transfer of plants raised at different levels of phosphorus, sulphur, potassium, or boron, to test solutions with and without the previously varied element,depended on the supply before transfer. The relationship between the plant's nutrient status and the relative differences in leaf area after transfer to solutions with and without the varied element appeared to be quantitative in the experiments with plants raised at different levels of phosphorus, potassium, or boron, but not in the experiment in which plants were raised at different sulphur levels. In the experiment with plants grown at five nitrogen pretreatment levels, there was no relationship at all between the nutrient status and the subsequent leaf area differences after transfer to complete and no-nitrogen solutions. The differences in the trends between the nitrogen, phosphorus, and sulphur experiments suggested significant differences in the growth changes induced by the transfer. The results were therefore further examined with the aid of the concepts and functions of growth analysis. Harvests had been carried out on the day of transfer (day 0) and on day 7, so that only an overall description of the changes over the 7 day period after transfer was possible. This is presented in Section 3.2.1. A further series of experiments with different nitrogen, phosphorus, and sulphur levels, in which harvests were carried out at intermediate stages, made it possible to examine the time trends in the growth changes during the early adjustment from stress conditions, and the differences in this respect between phosphorus and sulphur. The results of these experiments are presented in Sections 3.2.2 and 3.2.3. The changes in nutrient composition associated with the growth changes of the experiments in Section 3.2.3 are considered in Section 3.2.4.

3.2.1 A comparison of the changes in growth after the removal of stresses caused by nitrogen, phosphorus, or sulphur.

Figure 21 shows a comparison of the relative rates of increase in leaf area (R_A) and in plant dry weight (R_W) when plants raised at five nitrogen, phosphorus, or sulphur levels were transferred to solutions with and without the previously varied element. The growth functions were calculated over the period of seven days after transfer, and apply to the experiments for which the details and the changes in leaf area were given in Sections 3.1.1.2,.3, and .6.

It is clear that in each of the three experiments the differences between R_A and R_W after transfer to solutions without the varied element were small, and that they followed the same trends in relation to the nutrient levels before transfer. In

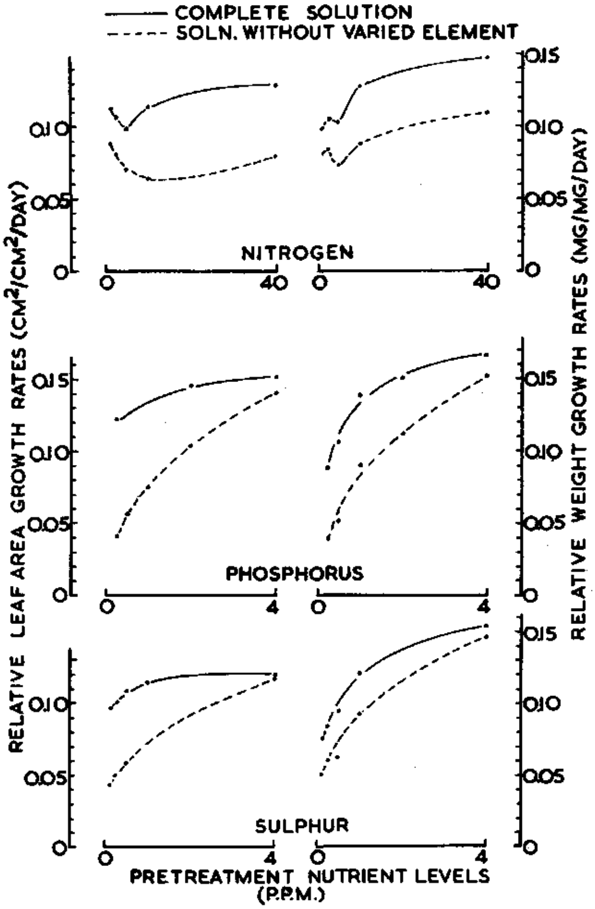

Figure 21.- Relative rates of increase in leaf area (R_A, left) and in plant dry weight (R_W, right) over a period of seven days after transfer of plants raised at five nitrogen, phosphorus, or sulphur levels respectively to solutions with and without the previously varied element. The growth functions were calculated from the data in the experiments for which changes in leaf area are shown in Figure 14, 4, and 6 respectively.

the phosphorus and sulphur experiments R_A and R_W for plants in the solutions without the varied element were smaller the lower the nutrient status of the plant at the time of transfer. The comparison of R_A and R_W after transfer of plants raised at five nitrogen levels was complicated by the presence of nodules on the roots of the plants raised at the lower nitrogen levels. The trough in R_A and R_W after transfer of the plants raised at the intermediate nitrogen levels to solutions without nitrogen, was probably related to the differences in nodulation observed on day 0 in response to pretreatment nitrogen levels. This was discussed in Section 3.1.1.6.

When plants were transferred to complete solutions, R_A over the 7 day period was less dependent on pretreatment levels than R_W. In the experiment with five sulphur pretreatment levels for example, R_A was 0.097 cm^2/cm^2/day for the S_1 plants and 0.120 cm^2/cm^2/day for the plants transferred from S_5 to complete solutions. By contrast, R_W was 0.076 and 0.153 mg/mg/day for the same plants respectively. Although it is possible that differences in R_A between pretreatments were small before transfer, it is more likely that R_A showed a more rapid increase as a result of the removal of stresses than R_W. Results similar to these were found in the experiments with five potassium and five boron levels. They suggest that an early response in the rate of leaf area expansion was an essential part of the adjustment during recovery from stresses, which, in the present experiments at least, appeared to precede marked changes in relative increases in dry matter.

The relatively small effect of phosphorus and sulphur pretreatment levels on R_A after transfer to complete solutions, compared with that on R_W, is clearly the reason why comparisons of leaf area differences induced by transfer to test solutions offer a better chance of success in the present approach in assessing the nutrient status of plants, than comparisons of dry weight differences. The possibility of achieving a quantitative assessment of the phosphorus status of plants was already suggested in Section 3.1.1.1, and this is also evident in Figure 21. The comparison of R_A between the complete and no-sulphur solutions in the sulphur experiment suggests that a quantitative relationship between the sulphur status and the leaf area differences induced by the transfer might have been found had the sulphur pretreatment levels been somewhat higher than the lower levels of the present experiment. The comparisons of R_A in the experiment with five potassium and five boron levels of Section 3.1.1.3 and 3.1.1.5 were similar to that of the phosphorus experiment.

The rate of leaf area expansion of the plants transferred from the highest nitrogen level to complete solutions was nearly twice as high as R_A for the corresponding plants in the solutions without nitrogen. This was in marked contrast to the small differences found after transfer of plants raised at optimum levels of the other elements to solutions with and without the varied element.

As R_W is the product of the net assimilation rate (E_A) and the leaf area ratio (A/W), the contribution made by these two components to the differences in R_W between pretreatments, and between test solutions, was further examined. A comparison of E_A and A/W for the sulphur experiment is given in Table 5. Results for the experiments with levels of phosphorus, potassium, or boron were generally similar to those for sulphur, and are therefore not shown.

TABLE 5

COMPARISON OF E_A ($mg/cm^2/day$) AND A/W (cm^2/mg) DURING A PERIOD OF SEVEN DAYS AFTER TRANSFER OF PLANTS GROWN AT FIVE SULPHUR LEVELS TO COMPLETE (C) AND NO-SULPHUR (no-S) SOLUTIONS.

Pretreatments	S_1	S_2	S_3	S_4	S_5
E_A, C	0.80	0.86	0.90	1.04	1.19
E_A, no-S	0.58	0.68	0.63	0.86	1.29
A/W, day 0	0.088	0.091	0.100	0.120	0.124
A/W, day 7, C	0.102	0.103	0.110	0.114	0.114
A/W, day 7, no-S	0.084	0.085	0.098	0.098	0.107

The net assimilation rate (E_A) over the 7 day period after transfer to complete solutions was lower for plants raised at S_1 than for plants raised at S_5. A more than twofold difference in E_A was found between S_1 and S_5 plants transferred to solutions without sulphur. The net assimilation rate is obviously an estimate of the rate of gain in dry matter by photosynthesis over respiratory losses. Recent experiments (Section 4. 1.5) showed that respiration rates of sulphur or phosphorus deficient leaf tissue were considerably lower than that of non-deficient tissue. It appears, therefore, that the differences in E_A represented real differences in photosynthetic efficiency of the leaf tissue.

The differences in leaf area ratio (A/W) between pretreatments on day 0 had largely disappeared 7 days after transfer to complete solutions. This was due to a small increase at the lower pretreatment levels and a decrease, probably ontogenetic at the higher levels. It is clear that a further seven days of sulphur stress after transfer of plants, which were already deficient, to solutions without sulphur did not change A/W greatly compared with day 0.

The present results indicate, therefore, that during recovery from phosphorus or sulphur stresses smaller relative growth rates partly resulted from reduced rates of carbohydrate assimilation. The relatively greater increases in leaf area than in dry weight (Figure 21) during the recovery period were reflected in a small increase in the leaf area ratio between day 0 and day 7 (Table 5).

To examine whether the more rapid recovery of R_A than of R_W after the removal of stresses could be related to a preferential distribution of dry weight in the plant, the relative increases in dry weight of tops and roots between day 0 and day 7 were plotted in Figure 22. It is clear that at the lower pretreatment levels in all experiments, there was a somewhat

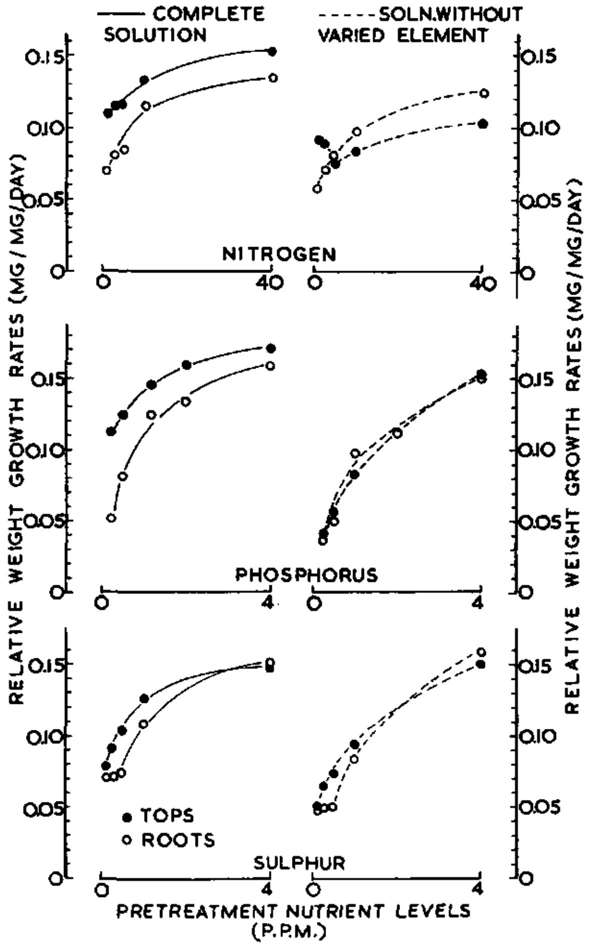

Figure 22.- Relative rates of increase in dry weight (R_W) of tops and roots over the seven day period after transfer to complete solutions (left) and to solutions without the previously varied element (right). The experiments are those for which R_A and R_W per plant are shown in Figure 21.

greater relative increase in the weight of the tops than of the roots. This effect was greatest in the phosphorus experiment, and it may have been a reflection of a preferential distribution of dry matter to the top during recovery from stresses. In each experiment R_W of roots and tops were reduced to approximately the same extent by decreasing pretreatment levels. It can also be seen that, except in the nitrogen experiment, intensification of the stress by transfer to solutions without the varied element reduced R_W of tops and roots to the same extent.

3.2.2 The time trends in dry weight and leaf area changes.

The results of the preceding Section suggested that during the recovery from stress conditions the photosynthetic efficiency of the plant was reduced. The reduction was severer the lower the previous nutrient supply. The nature of the experiments did not permit more than a description of the overall growth changes between the day of transfer and seven days afterwards, and no distinction could be made between leaves existing at the time of transfer and those formed afterwards. This precluded an evaluation of possible differences in the redistribution of dry matter and nutrient elements as a result of the removal of stresses caused by different elements.

To examine these aspects in more detail, three experiments were carried out in which the leaves existing at the time of transfer were identified by placing a small white paper collar around the petioles.

The first experiment involved three pretreatment levels of nitrogen (2.5, 5, and 60 p.p.m.), the second experiment three levels of phosphorus (0.3, 0.7, and 4 p.p.m.), and the third experiment three sulphur pretreatments (0.2, 0.5, and 4 p.p.m). The experiments were carried out as closely as possible in time, and in the same glasshouse. The seeds for the three experiments were sown at intervals of one day, commencing with the nitrogen experiment, and followed by the phosphorus and sulphur experiments respectively. Transfer to solutions with and without the varied element, when the plants had been in pretreatment solutions for 16 days, were also carried out at intervals of one day, and in the same order as above. There were eight replicates of two plants in all three experiments. Harvests were carried out on day 0, day 3, day 5, and day 7. Leaf areas were obtained for all plants on each occasion, and the plants separated into roots, petioles, and leaves. Leaves and petioles were further separated into old and new, referring to parts existing at the time of transfer, and to those formed afterwards.

The names leaves and petioles as used here may need further definition. Subterranean clover in its vegetative phase is a rosette. The first leaf formed after the cotelydons is a unifoliate leaf. Subsequent leaves also arise from the central growing point, and are trifoliate. The trifoliate leaf is connected to the central growing point by a petiole, which increases in length during the unfolding and part of the expansion of the leaflets. The leaflets of each trifoliate leaf are connected to the main petiole by short stalks (1-2 mm). The fraction called "leaves" was obtained by separating the whole trifoliate leaf at the joint of the petiole and the three short stalks to which the leaflets were attached. The three short stalks were included in the leaf fraction. The petioles are at their base surrounded by a small stipule. Immediately below the stipule the main root begins, and lateral roots arise from the main root at a certain distance below this point (0.5 cm or more). The fraction "petioles" was obtained by separating the main root just below the stipule. It includes, therefore, all plant parts enclosed by the stipule. With very young leaves before the leaflets had unfolded, it was not always possible to separate leaf and petiole. In all these cases the new part was separated at the base and included in the new leaf fraction.

The results for the sulphur experiment were plotted in Figure 23 to illustrate the pattern of dry weight changes in the different plant parts during the 7 day period after transfer to complete solutions. The weights for old and new parts were plotted additively, and refer to leaves or petioles present at the time of transfer and to those formed afterwards. Although there were differences in the derived growth indices for the three experiments, which will be discussed later, the general changes in dry weight for the nitrogen and phosphorus experiment were similar to those for sulphur.

The dry weight increases for leaves, petioles, and roots were smaller, the greater the stress at the time of transfer to complete solutions. Increases in dry weight for the S_1 plants were relatively small till five days after transfer, but increased markedly between day 5 and day 7. This is clear from the relative increases in dry weight per plant, which were 0.053, 0.066, and 0.166 mg/mg/day for the intervals 0-3, 3-5, and 5-7 days after transfer respectively. For S_3 the relative increases in dry weight changed little with time. The values of R_W for the same three intervals were 0.153, 0.138, and 0.153 mg/mg/day respectively. R_W for S_2 plants was between the values for S_1 and S_3 plants.

A small decrease was apparent in the dry weight of the old leaves, and particularly of the old petioles, of the S_1 plants between day 0 and day 5. The dry weight of the old petioles of the S_2 plants remained virtually the same, but the dry weight of the old leaves increased by nearly 25 per cent. The old leaves and the old petioles of the S_3 plants increased markedly throughout the 7 day period after transfer.

It is evident that the increases in dry weight of new leaves and new petioles became of greater significance in relation to the total increase of plant dry weight as the stress at the time of transfer increased. This is further illustrated by the following comparison of distribution indices of dry

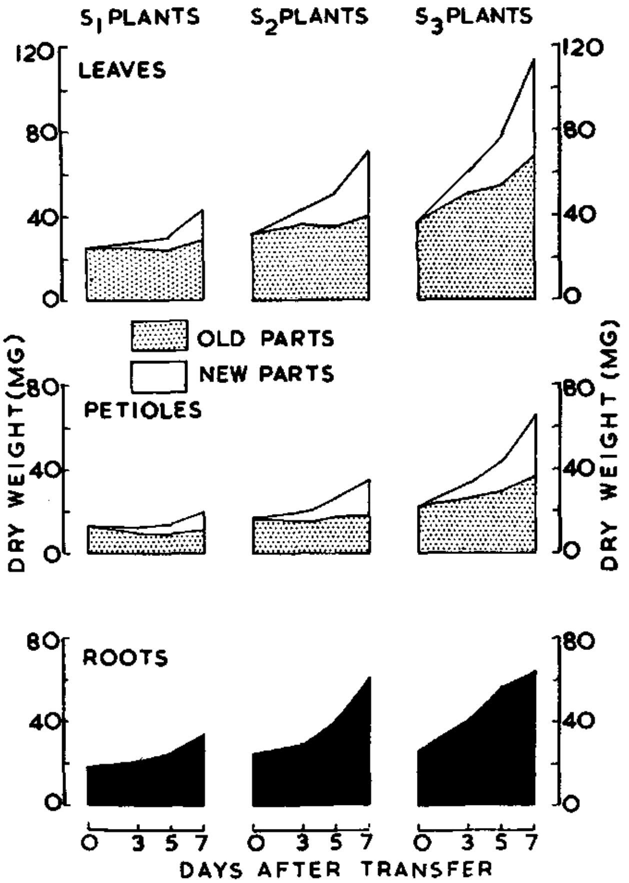

Figure 23.-Changes in dry weight (mg) after transfer of plants raised at three sulphur levels (0.2, 0.5, and 4 p.p.m. sulphur, S_1, S_2, and S_3 respectively) to complete solutions. Leaves, petioles, and roots are shown in the top, centre, and bottom respectively. The old and new parts refer to leaves and petioles existing at the time of transfer, and to leaves and petioles formed afterwards respectively.

weight between day 0 and day 7:

	Pretreatment level		
	S_1	S_2	S_3
New tops	57	52	47
Old tops	4	10	30
Roots	39	38	23

The values are the increases in dry weight of the plant parts shown, expressed as a percentage of the total increase per plant. The tops represent the sum of leaves and petioles. Very little of the dry matter moved to the old tops of the S_1 plants, and most of it was found in the new leaves. It is also evident that the roots exercised a more powerful demand for assimilates in plants that had been under stress than in S_3 plants. The results for phosphorus and nitrogen also showed a preferential distribution of dry weight to new leaf tissue during recovery from stresses. The following comparison gives the distribution indices for dry weight in the new leaves for the three experiments.

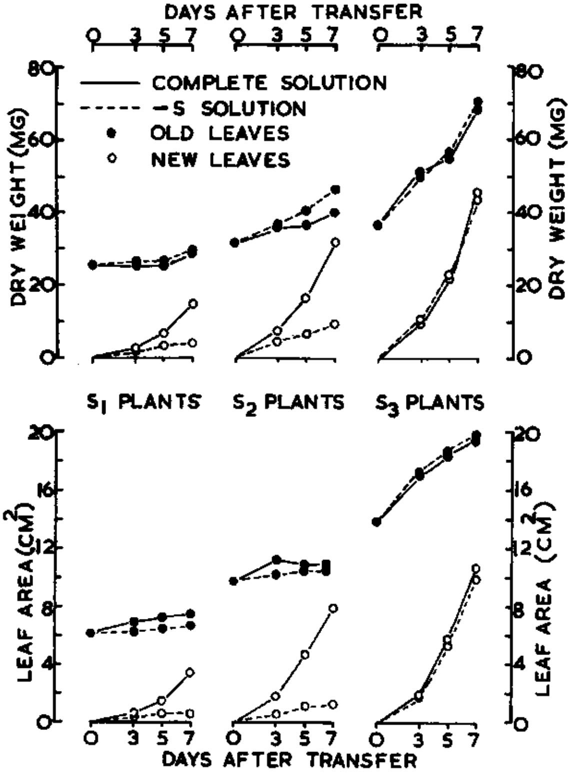

Figure 24.-Comparison of changes in leaf dry weight (mg, top of graph) and in leaf area (cm^2, bottom) after transfer of plants from three sulphur pretreatments to complete solutions and to solutions without sulphur. The results apply to the experiment for which sulphur pretreatment levels are given in Figure 23.

	Pretreatment level		
Experiment	1	2	3
Nitrogen	43	36	34
Phosphorus	35	29	28
Sulphur	36	33	29

Although there were small differences in magnitude, the trends were similar in all experiments.

Figure 24 shows a comparison of dry weights and areas of old and new leaves over the 7 day period after transfer to complete solutions and to solutions without sulphur, obtained in the same experiment. The increases in dry weight of the old leaves of plants transferred to solutions without sulphur were virtually the same as for the plants transferred to complete solutions. The increases were small for S_1 plants, but were greater the higher the pretreatment level. The main differences between complete and no-sulphur solutions were reflected in the dry weight of new leaves. The results for leaf area followed trends similar to those for dry weight. The contribution by the old leaves to the total leaf area increase per plant was small for the plants transferred from S_1 and S_2, but considerable for plants transferred from S_3. The differences between complete solutions and solutions without sulphur were largely determined by the area of the new leaves.

3.2.3 The changes with time of the growth indices.

Table 6 shows the derived growth functions R_A, R_W, E_A, and the leaf area ratio (A/W) over the intervals 0-3, and 3-7 days after transfer, calculated from the data of the sulphur experiment of the preceding Section.

The comparison between R_A and R_W confirms the finding of Section 3.2.1 that R_A during recovery from stress conditions was less dependent on the pretreatment nutrient supply than R_W.R_A over the first interval after transfer to complete solutions was smaller the lower the sulphur level before transfer. Over the second interval the differences had nearly disappeared, due to a further rise for the S_1 plants. The differences in R_A between complete and no-sulphur solutions for the S_1 and S_2 plants was already quite considerable over the first interval. R_A for S_1 and S_2 plants transferred to the solutions without sulphur did not show a decrease during the second interval. The differences in R_W between complete and no-sulphur solutions over the first interval after transfer were relatively small. R_W for the S_1 and S_2 plants increased considerably from the first to the second interval after transfer to complete solutions. R_W for the S_3 plants transferred to complete solutions, or to solutions without sulphur, changed little with time. The relative increases in dry weight of plants in the solutions without sulphur did not change greatly, even when the plants were already under stress at the time of transfer.

TABLE 6

COMPARISON OF DERIVED GROWTH FUNCTIONS OVER THE INTERVALS 0-3, AND 3-7 DAYS AFTER TRANSFER OF PLANTS RAISED AT THREE SULPHUR LEVELS TO COMPLETE SOLUTIONS AND TO SOLUTIONS WITHOUT SULPHUR.

	Transferred to:					
	complete solutions			solutions without sulphur		
Pretreatments	S_1	S_2	S_3	S_1	S_2	S_3
R_A (cm^2/cm^2/day)						
Day 0-3	0.067	0.092	0.108	0.022	0.028	0.106
Day 3-7	0.099	0.094	0.113	0.022	0.025	0.111
R_W (mg/mg/day)						
Day 0-3	0.053	0.087	0.155	0.048	0.071	0.155
Day 3-7	0.116	0.143	0.146	0.044	0.072	0.135
A/W plant (cm^2/mg)						
Day 0	0.115	0.134	0.163	0.115	0.134	0.163
Day 3	0.120	0.136	0.141	0.106	0.118	0.140
Day 7	0.111	0.111	0.124	0.100	0.097	0.127
E_A (mg/cm^2/day)						
Day 0-3	0.45	0.64	1.04	0.43	0.56	1.04
Day 3-7	1.02	1.18	1.11	0.42	0.68	1.02

Min. diff. for significance 0-3 days after transfer to complete solns. ($P \leqslant 0.01$): R_A 0.016; R_W 0.026; E_A 0.12.

Table 6 also shows the two components of the relative growth rate, the leaf area ratio (A/W) and the net assimilation rate (E_A). The leaf area ratios on day 0 increased with increasing pretreatment sulphur levels. No change with time occurred in the ratio for the S_1 plants, and there were no differences between the complete and the no-sulphur solutions, indicating that in both cases dry matter production and leaf area expansion proceeded at the same rate. The decrease in the leaf area ratio between day 0 and day 7 for the S_3 plants probably represented an ontogenetic drift,and was largely attributable to an accumulation of dry matter in the old leaves of these plants (2.62 and 3.54 mg/cm^2 on day 0 and day 7 respectively). The changes in A/W for the S_2 plants were between those for the S_1 and S_3 plants.

An examination of the trends in net assimilation rates shows that they follow those for the relative growth rates rather closely, thus confirming the suggestion of Section 3.2.1, that the smaller growth rates of plants during the recovery from nutrient stresses were at least partly attributable to a reduced assimilatory capacity of the leaf tissue. A point to be noted in Table 6 is that E_A for S_1 and S_2 plants over the first interval was nearly the same in the complete solutions and in the solutions without sulphur. As for R_W, the main increase in E_A did not occur till the second interval.

The growth functions for the experiment with three phosphorus pretreatment levels for which details were also given in Section 3.2.1, showed trends which suggested marked differences between the two experiments. To make the comparison between the two experiments more meaningful, the dry weight and leaf areas per plant on day 0 are given below:

	Pretreatment levels			
	1	2	3	
Dry weight, P experiment	46	65	96	mg
Dry weight, S experiment	57	73	86	mg
Leaf area, P experiment	4.5	6.9	14.5	cm^2
Leaf area, S experiment	6.1	10.0	13.9	cm^2

The nutrient levels applied to P_3 and S_3 during pretreatment were the same. As a result, the differences in dry weight and leaf area between these two pretreatments were small and not significant (11 mg and 1.2 cm^2 for $P \leqslant 0.05$). If the dry weights on day 0 of the lower pretreatment levels are expressed as fractions of that of the highest level, the ratios of 0.48 and 0.68 are obtained for P_1/P_3 and P_2/P_3 respectively, and 0.66 and 0.85 for S_1/S_3 and S_2/S_3. It is clear from these comparisons that the phosphorus pretreatments had imposed relatively greater stresses than the sulphur pretreatments.

Figure 25 shows a comparison of R_W and E_A for the S_1 and S_2 pretreatments in the sulphur experiment, and the corresponding growth functions for P_1 and P_2 in the phosphorus experiment over the intervals 0-3 and 3-7 days after transfer to complete solutions and to solutions without the varied element. The intervals are referred to in the graph as 1 and 2 on the X-axis. Attention is confined to the comparisons of R_W and E_A between the two experiments, because in the phosphorus experiment it was also found that the leaf area ratios for the lower pretreatments (P_1 and P_2) changed little during the 7 day period after transfer to complete solutions or to solutions without phosphorus. As a result, the changes in R_W after transfer of P_1 and P_2 plants were mainly due to corresponding changes in E_A.

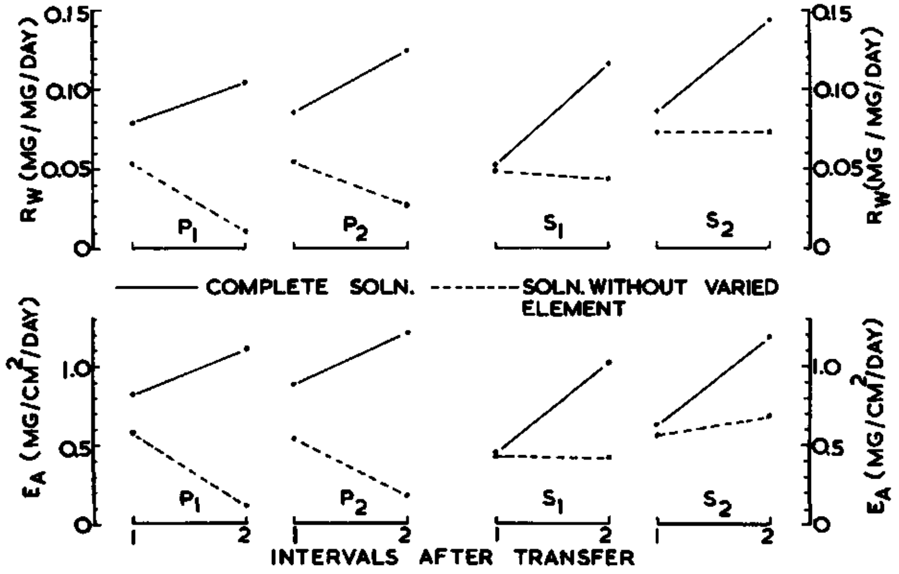

Figure 25.-Comparison of the changes in the relative growth rate (R_W) and in the net assimilation rate (E_A) of plants raised at two deficient phosphorus levels (P_1 and P_2), and of plants raised at two deficient sulphur levels (S_1 and S_2, Figure 23), after transfer to complete solutions and to solutions without the previously varied element. Intervals 1 and 2 are the periods 0-3 and 3-7 days after transfer.

There were marked differences in the changes of R_W and E_A after transfer of the phosphorus deficient plants, compared with those that occurred after the transfer of the sulphur deficient plants. These differences were apparent in the complete solutions, as well as in the solutions without the varied element. Over the first interval, the differences between the test solutions were already considerable for the P_1 and P_2 plants. By contrast, no or only small differences were found for the S_1 and S_2 plants. Over the second interval after the transfer of P_1 and P_2 plants, the differences in R_W and E_A between the complete and no-phosphorus solutions became greater due to a further rise in the complete solutions, and also as a result of a considerable decrease in the solutions without phosphorus. For the S_1 and the S_2 plants no change occurred over the second interval after transfer to the solutions without sulphur, but in the complete solutions the rise in R_W and E_A was steeper than for the P_1 and P_2 plants.

The results just presented suggest differences in the immediate responses after the removal of phosphorus and sulphur stresses. It was pointed out earlier that at the time of transfer the P_1 and P_2 plants were probably under a severer stress than the S_1 and S_2 plants. In spite of this, the P_1 and P_2 plants appeared to show a more rapid initial recovery in R_W and E_A than the S_1 and S_2 plants. In addition, intensification of the stress by omitting the previously varied element caused a marked decrease in E_A for P_1 and P_2, but did not affect E_A for the S_1 or S_2 plants. These differences suggest a more plastic response of E_A in the phosphorus than in the sulphur experiment. Even with the comparatively mild stress of the S_2 pretreatment level, virtually no difference was observed in the net assimilation rate over the first interval after the addition or omission of sulphur.

Three days after transfer of the P_1 and S_1 plants to complete solutions, the area of the new leaves formed only a small portion of the total area per plant. For the P_1 plants the area of new leaves increased from 11 per cent. on day 3 to 35 per cent. of the total area on day 7, and for the S_1 plants from 9 to 33 per cent. It appears likely, therefore, that the contribution of new leaves to the changes in E_A over the first interval after transfer to complete solutions was relatively small, in which case these changes were largely a reflection of the adjustments occurring in the old leaves. If this suggestion is correct, the differences in the results of the two experiments suggest a more rapid initial recovery in the assimilatory capacity of the old leaves of plants after the removal of a phosphorus stress than after the removal of a sulphur stress. The relatively steep rise in E_A over the second interval after transfer of sulphur deficient plants to complete solutions may well have been causally related to the marked expansion of new leaf tissue during this period, with perhaps only a small contribution by the old leaves. It would be of interest to establish the relative contribution of old and new leaves to the response in the net assimilation rate, and how long possible differences in this respect between previously phosphorus and sulphur deficient plants persist.

The results of the nitrogen experiment showed trends similar to those of the phosphorus experiment. They are not presented, because possible effects of spontaneous nodulation at the lower nitrogen levels could not be separated from the effects of applied combined nitrogen.

3.2.4 The uptake and distribution of nutrient elements after the removal of stresses caused by nitrogen, phosphorus, or sulphur.

The concentration of total nitrogen, phosphorus, and sulphur in the dry matter of the different plant parts over the seven day period after transfer, for the three experiments discussed in the preceding Section, are shown in Figure 26.

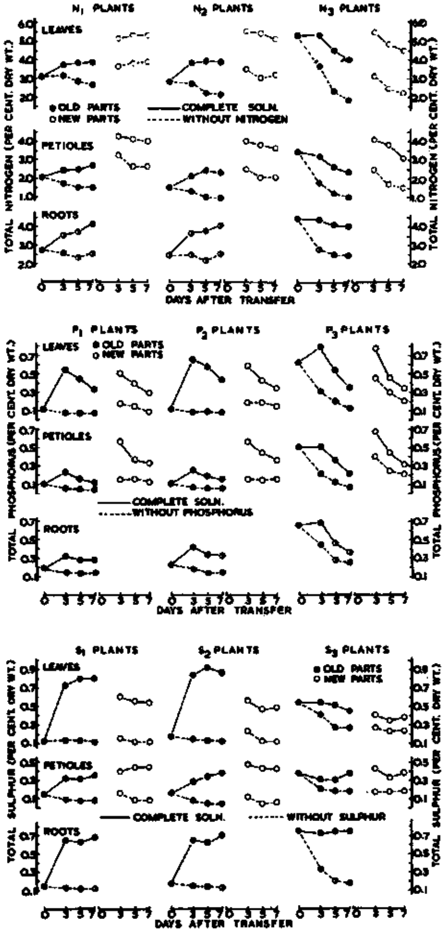

Figure 26.- Changes in nutrient concentration (per cent. dry weight) after transfer of plants raised at three nitrogen levels (top), at three phosphorus levels (centre), or at three sulphur levels (bottom) to complete solutions and to solutions without the previously varied element. The nitrogen levels N_1, N_2, and N_3 during pretreatment were 2.5, 5, and 60 p.p.m. nitrogen. The phosphorus pretreatment levels P_1, P_2, and P_3 were 0.3, 0.7, and 4 p.p.m. phosphorus. The sulphur pretreatment levels are given in Figure 23.

There were some marked differences between the changes in the nitrogen concentration on the one hand, and those for phosphorus and sulphur on the other. In the experiment with plants raised at three nitrogen levels, transfer of N_1 and N_2 plants to complete solutions caused a steady, but not very large, increase in the nitrogen content of the old leaves and old petioles. Increases in the nitrogen concentrations of the roots continued throughout the 7 day period after transfer. It is also apparent that the nitrogen concentration in the new parts of the N_1 and N_2 plants was considerably greater than in the old parts. Transfer to solutions without nitrogen caused a decrease in the concentrations of the old leaves and petioles. A considerable decrease was also apparent in the roots of the N_3 plants after transfer to solutions without nitrogen but not in those of the N_1 and N_2 plants.

The changes in concentration after transfer of the plants raised at three phosphorus or three sulphur levels followed trends different from those for nitrogen in the nitrogen experiment. The removal of the phosphorus stress in the P_1 and P_2 plants resulted in phosphorus concentrations in the old leaves which were at least as high as in the new leaves. The increase in the phosphorus concentration in the old leaves occurred over the period immediately after transfer to complete solutions, and was followed by a steady decrease between day 3 and day 7. In the sulphur experiment, the transfer of the S_1 and the S_2 plants to complete solutions resulted in even greater increases in the sulphur concentration of the old leaves. These increases occurred, as for phosphorus in the phosphorus experiment, immediately after the removal of the stress. In contrast to the results for phosphorus, the sulphur concentrations in the old leaves did not decrease after day 3. If anything, they rose even further. Sulphur concentrations in the old leaves of the S_1 and S_2 plants were always higher than in the new leaves.

Phosphorus concentrations in the roots of the P_1 and P_2 plants increased by nearly 80 per cent. over the first interval after transfer to complete solutions, but decreased somewhat after that. A far greater increase occurred in root sulphur contents over the same interval after the transfer of the S_1 and the S_2 plants to complete solutions. In view of the relatively low phosphorus and sulphur levels in the solutions to which the plants in the phosphorus and sulphur experiments were transferred, which was 4 p.p.m. for both elements, the results just presented probably represent real treatment effects. In all experiments care was also taken to wash the roots thoroughly before drying.

The concentrations of phosphorus and sulphur after transfer to solutions without the varied element changed in a fairly predictable manner. For the plants low in sulphur or phosphorus at the time of transfer, neither the concentrations in old or new parts, nor that in the roots changed greatly. For the P_3 and S_3 plants, the old leaves, the old petioles, and the roots showed an approximately similar fall in the concentrations of phosphorus or sulphur respectively between day 0 and day 7.

The changes in nutrient concentrations in the tissues were related to marked differences in the distribution patterns during the recovery from stress conditions caused by nitrogen, phosphorus, or sulphur. The differences in the uptake and distribution were examined with the aid of Table 7, which shows the absolute increases for the two lower levels over the intervals 0-3, and 3-7 days after the transfer to complete solutions. The distribution indices over the same intervals are shown in Table 8. The figures in Table 8 are the changes in composition of the plant part, expressed as a percentage of the total uptake per plant of the element concerned.

It is clear from Table 7 that the absolute increases per plant over the 7 day period after transfer to complete solutions in all three experiments were greatest at the second pretreatment levels. The increases in nitrogen were greater over the second than over the first period after transfer. In the phosphorus experiment the increases over the 0-3 day period were considerably greater than those over the second interval.

Table 8 shows that even over the first interval most of the nitrogen taken up by the plant moved to the new plant parts, particularly at N_1, where half of it was accounted for in new leaves and petioles. Relatively more nitrogen moved to the new aerial parts over the second interval, and less to the old parts. The roots received nearly the same proportion as before.

By contrast, more than half of the phosphorus or sulphur taken up by the plant over the first interval after the removal of a phosphorus or sulphur stress respectively, moved to the old leaves and petioles. In the phosphorus experiment, this was followed by a loss of phosphorus from these parts over the 3-7 day interval. This is evident from the difference between the small quantities taken up by the plant from the solution, and the much greater increases in the new aerial parts. It was shown in Table 7 that the uptake per plant over the second interval was only 0.02 mg phosphorus at P_1 and 0.06 mg at P_2. The increases in the new leaves and petioles over the same period were 0.04 and 0.07 mg phosphorus for P_1 and P_2 respectively. During the second interval the old tops lost nearly 25 per cent. of their phosphorus, which amounted to 0.04 and 0.06 mg at P_1 and P_2 respectively. It is therefore evident that phosphorus was retranslocated from old to new aerial parts during the second interval. The new leaves and petioles of the P_1 plants obtained as much as 50 per cent. of their phosphorus from the old parts, and those of the P_2 plants much less. In the sulphur experiment, the old tops did not show any net losses of sulphur. Instead, sulphur in the old tops showed a further, though much smaller increase over the second period. The importance of newly formed plant parts over the 3-7 day period is apparent from the increase in the

TABLE 7

INCREASES PER PLANT (mg) IN TOTAL NITROGEN, PHOSPHORUS, AND SULPHUR RESPECTIVELY, AFTER TRANSFER OF PLANTS GROWN AT SUBOPTIMAL LEVELS OF EACH OF THESE ELEMENTS TO COMPLETE NUTRIENT SOLUTIONS.

		Pretreatment level	
Experiment	Interval	1	2
Nitrogen	0-3	0.70*	1.67
	3-7	1.71	2.42
	0-7	2.41	4.09
Phosphorus	0-3	0.17**	0.32
	3-7	0.02	0.06
	0-7	0.19	0.38
Sulphur	0-3	0.30***	0.49
	3-7	0.25	0.44
	0-7	0.55	0.93

* mg nitrogen

** mg phosphorus

*** mg sulphur

TABLE 8

CHANGES IN THE DISTRIBUTION INDICES OF NITROGEN, PHOSPHORUS, AND SULPHUR RESPECTIVELY, DURING THE INTERVALS 0-3, AND 3-7 DAYS AFTER TRANSFER OF PLANTS RAISED AT SUBOPTIMAL LEVELS TO COMPLETE SOLUTIONS.

		Plant part		
Pretreatment	Intervals	Old tops	New tops	Roots
N_1	0-3	19*	50	31
	3-7	14	57	29
N_2	0-3	27	35	38
	3-7	14	53	33
P_1	0-3	58	18	24
	3-7	-299	262	136
P_2	0-3	63	13	23
	3-7	- 88	116	72
S_1	0-3	55	9	36
	3-7	24	36	40
S_2	0-3	54	13	33
	3-7	11	38	51

* The figures are the changes in the amounts of the element in the plant parts shown, expressed as a percentage of the total uptake of the element per plant.

distribution indices.

The results just presented provided an indication of the uptake and distribution of nitrogen, phosphorus, and sulphur that accompanied the growth changes during the adjustment period after the removal of stresses caused by these elements, which were described in Section 3.2.3. It was also shown in Section 3.2.3, and in the earlier Section 3.2.1, that plants raised at optimum phosphorus or sulphur levels were able to expand their leaf area at virtually the same rate after transfer to solutions without one of these elements as in complete solutions, at least over a period of seven days.

Although the expansion of leaf surface present at the time of transfer accounted for nearly 40 per cent. of the twofold or greater increase in the total area per plant, the remaining 60 per cent. was due to the expansion of new leaves. Plants raised at an optimum nitrogen level, on the other hand, showed a much smaller increase in leaf area in solutions without nitrogen than in complete solutions. It was therefore of interest to examine whether the differences in this respect between plants raised at optimum nitrogen levels, and plants raised at optimum phosphorus or sulphur levels, could be related to differences in the mobility and redistribution of the elements concerned after the transfer to solutions without nitrogen, phosphorus, or sulphur respectively.

Table 9 shows the absolute nutrient contents (mg) on day 0, day 3, and day 7 after transfer of the N_3, P_3, and S_3 plants to solutions without nitrogen, phosphorus, or sulphur respectively.

The N_3 plants transferred to solutions without nitrogen lost nearly half of the nitrogen present in the old tops, most of which was translocated to the new leaves and petioles. The corresponding N_3 plants transferred to complete solutions took up 6.17 mg nitrogen over the 0-7 day period. Of this amount 3.74 mg went to the new tops, and even when some luxury uptake is assumed for these plants, this amount was still far greater than that available from the old parts for new growth of the plants transferred to the no-nitrogen solutions. This would explain, at least partly, why the plants in the no-nitrogen solutions grew so much less than those in the complete solutions. The redistribution of phosphorus followed a similar trend as that for nitrogen, except that the old parts lost relatively more of their phosphorus. The increase in phosphorus after transfer to complete solutions over the same period was 0.42 mg, of which 0.29 mg was present in the new parts. The discrepancy between phosphorus in the new parts after transfer to solutions without phosphorus and to complete solutions was far smaller than in the case of nitrogen.

When plants were transferred to solutions without sulphur, relatively little sulphur was retranslocated from the old tops and most of the sulphur present in the new parts was obtained from the roots. As for phosphorus, the difference in the sulphur accounted for in the new tops of plants in complete solu-

TABLE 9

ABSOLUTE NUTRIENT CONTENT (mg) OF PLANT PARTS 0, 3, AND 7 DAYS AFTER TRANSFER OF PLANTS RAISED AT OPTIMUM LEVELS OF NITROGEN, PHOSPHORUS, OR SULPHUR TO SOLUTIONS WITHOUT ONE OF THESE ELEMENTS RESPECTIVELY.

		Old Tops	New Tops	Roots
N_3 plants transferred to solutions without nitrogen	Day 0	2.71	–	1.20
	Day 3	1.90	0.52	1.07
	Day 7	1.31	0.87	1.39
Total loss or gain (mg N)	0–7	–1.40	+0.87	+0.19
P_3 plants transferred to solutions without phosphorus	Day 0	0.38	–	0.20
	Day 3	0.23	0.09	0.22
	Day 7	0.14	0.16	0.26
Total loss or gain (mg P)	0–7	–0.24	+0.16	+0.06
S_3 plants transferred to solutions without sulphur	Day 0	0.28	–	0.20
	Day 3	0.27	0.04	0.14
	Day 7	0.25	0.15	0.10
Total loss or gain (mg S)	0–7	–0.03	+0.15	–0.10

tions compared with plants in solutions without sulphur was smaller than in the case of nitrogen. The plants in the complete solutions increased in total sulphur by 0.74 mg, of which 0.29 mg was recovered in the new tops. It can therefore be concluded that plants raised at optimum phosphorus and sulphur levels, when transferred to solutions without one of these elements, were able to meet the demand for phosphorus and sulphur associated with new growth over the 7 day period reasonable well by retranslocation from other plant parts. In the solutions without phosphorus, the demand for phosphorus was met by retranslocation from old aerial parts. In the no-sulphur solutions the new aerial parts obtained a reasonable supply of sulphur from the roots. In the solutions without nitrogen no nitrogen was exported from the roots to the tops, and the nitrogen available from top parts was insufficient to meet the demand for new leaf growth.

Although it is realized that further work would have to confirm the results of the present experiments, the trends suggest marked differences in the distribution patterns of nitrogen, phosphorus, and sulphur during the growth changes induced by the removal of stresses caused by these elements, and also in the retranslocation when stresses were imposed on plants grown at optimum levels. The results for the nitrogen experiment indicate that, as early as the first three days after the removal of nitrogen stresses, the newly formed leaves and petioles took up most of the nitrogen. By contrast, most of the sulphur taken up over the first interval after the removal of sulphur stresses was recovered in the existing leaves and petioles without any apparent retranslocation from these parts afterwards. The initial movement of phosphorus after transfer of phosphorus deficient plants to complete solutions was also largely directed towards the existing top parts, but this was followed by a significant retranslocation to new leaves and petioles. This was more marked at the lower than at the higher pretreatment levels.

When non-deficient plants were transferred to solutions without nitrogen or phosphorus, the results confirmed the known mobility of these elements. However, this was only apparent for the aerial parts, and no losses occurred from the roots. This was in contrast to sulphur, where most of the sulphur in the newly formed leaves and petioles was derived from the roots.

4. DISCUSSION AND CONCLUSIONS

4.1. The assessment of the nutrient status of plants.

4.1.1. General.

Much of the experimental work was carried out to provide the basis for an approach in the assessment of the nutrient status of plants that would at least overcome some of the disadvantages of the field experiment as a diagnostic technique. The principle underlying the approach rests in a sense on the Law of Minimum factors. It can be stated in general terms that within the limits of its genetic properties, growth of a plant is a function of external conditions, which include climatic and soil conditions. If all external conditions, except the supply of one nutrient element, are present at near optimum levels, plant growth is a function of the limiting element. There is evidence that under conditions where a nutrient element limits growth, uptake of other elements often exceeds their rate of utilization (Goodall and Gregory 1946). It was therefore argued that if in a given situation plant growth is limited by the supply of one element, transfer of these plants to nutrient solutions each without a different element, but containing the limiting element, should at least initially induce a response. Plants transferred to solutions without the limiting element should, of course, respond less or not at all. In exploratory work preceding the experiments presented here, plants were dug up in a phosphorus deficient pasture, and transferred to complete nutrient solutions and to a number of solutions without a different element. Within a few days the plants in all solutions, except in the solutions without phosphorus, had commenced to grow and their appearance had visibly improved.

To use the terminology of this paper, the plants in this case were under a fairly severe phosphorus stress. The initial response after the transfer was induced by the removal of the phosphorus stress, and during the brief experimental period the omission of other elements had no apparent effect. The term "nutritional stress" has been used on purpose. In the context of the present work it conveys better than "nutrient deficiency" that plant growth is being checked by a suboptimal supply of an element. The expression is also more in keeping with the dynamic nature of plant growth, and with the capacity of the living organism to respond to the removal of the check to its growth potential.

The example quoted above was perhaps an extreme case, but it indicated that the approach might work, even under conditions of more moderate nutritional stresses, if a sufficiently sensitive index of the induced responses could be found.

In preliminary experiments plants grown at different levels of nutrition, both in glasshouse work and in the field, were transferred to complete solutions, and to solutions lacking a different element. In this work it was hoped that dry weight differences would be large enough to establish treatment effects 6-8 days after transfer. To increase the precision of the experimentally determined dry weights at the conclusion of the experiments, they were adjusted for initial variation by regression analysis on leaf area ratings carried out on the day of transfer. This technique and its statistical basis was developed and described by McIntyre and Williams (1949). In spite of the improvement in the dry weight estimates, the differences between treatments were rather small. Leaf area estimates were also available for the harvests at the conclusion of the 6-8 day experimental periods and it was found that the differences in leaf area showed the same trends as those in dry weight, but were, relatively speaking, considerably greater.

In a typical experiment, for example, in which plants were raised at different phosphorus levels in sand cultures, the plants transferred from the two deficient levels (P_1 and P_2) to complete and no-phosphorus solutions, had the following dry weights (mg) six days after transfer (leaf areas shown in brackets):

	Complete solution	no-P solution
P_1	34 (7.9)	28 (5.8)
P_2	36 (8.0)	31 (6.2 cm^2)

The reduction in leaf area of the P_1 plants in the solution without phosphorus compared with the control was 26 per cent., but that for dry weight was only 18 per cent. The differences for the P_2 plants were 23 and 13 per cent. for leaf area and dry weight respectively. Correlations between leaf areas and dry weights in this and other experiments were always highly significant and greater than 0.80, a fact which is well documented in the literature (McIntyre and Williams 1949, Watson 1952). In all subsequent work changes in leaf area after transfer have been used as a suitable and reasonably sensitive index of plant responses. An added advantage is, of course, that the use of ratings enables the description of response patterns without destruction of experimental material.

4.1.2 The experiments.

The experiments reported in Section 3.1 comprised six related stages in the development of this approach. The experiments in Section 3.1.1 were carried out to establish whether the changes in leaf area after transfer of plants to complete solutions and to solutions without the element of which the supply before transfer had been varied, could be related to the nutrient status of the plant with respect to that element at the time of transfer. It was shown conclusively that leaf area changes after transfer of plants raised at different levels of phosphorus, sulphur, potassium, or boron, to solutions with and without the varied element, depended on the nutrient status of the plant at the time of transfer with respect to the varied element. Plants raised at an adequate (but not luxury) level of these elements showed little difference in leaf area between the two solutions over a period of seven days after transfer. When they had been grown at suboptimal supplies, the differences in leaf area between the complete and without solutions were detectable as early as 3-5 days after transfer. The results of these experiments are summarized in Table 10, which gives the ratios of leaf areas in the solution without the varied element to that in the complete solution against the level of supply before transfer. It can be seen that for phosphorus, potassium, and boron there was a reasonably quantitative relationship, which was better on day 7 than on day 5

TABLE 10

RATIOS OF LEAF AREA PER PLANT IN SOLUTIONS WITHOUT THE VARIED ELEMENT TO THAT IN COMPLETE SOLUTIONS, FIVE AND SEVEN DAYS AFTER TRANSFER FROM PRETREATMENTS AS SHOWN.

Pretreatment levels	1		2		3		4		5	
Days after transfer	5	7	5	7	5	7	5	7	5	7
P-pretreatment	0.72	0.57	0.76	0.64	0.81	0.71	0.84	0.74	0.98	0.93
S-pretreatment	0.85	0.69	0.83	0.70	0.80	0.71	0.79	0.70	1.01	0.99
K-pretreatment	0.71	0.57	0.82	0.75	0.92	0.86	0.94	0.92	0.96	0.94
B-pretreatment*	0.75	0.63	0.88	0.79	1.01	0.94	1.02	0.97	-	-

* The fourth B-level of Figure 12 has been omitted.

It is possible that for sulphur a quantitative relationship would have been found if the nutrient levels had been spaced somewhat higher along the response curve. Field results (see below) seem to support this.

The results for nitrogen were entirely different. It was shown (Figure 14) that at optimum levels of nitrogen supply, and in a later experiment (Figure 15) at even higher levels, differences in leaf area after transfer to solutions with and without nitrogen were quite considerable. This is no doubt due to the importance of nitrogen in all phases of leaf growth, possibly more so than any other single nutrient element (Watson 1952). Nitrogen can affect leaf growth by its effects on leaf numbers and leaf size. Leaf size may be altered by cell numbers and cell size. Although the incorporation of nitrogen in the plant into organic compounds is to a large extent reversible, Williams (1948) suggested that the cell is able to accumulate relatively larger quantities of inorganic and soluble organic phosphorus, than nitrogen compounds. This would seem to have some bearing on the present results in explaining the differences between nitrogen and phosphorus.

Because the crop under investigation is grown for its ability to fix atmospheric nitrogen, the feasibility of assessing the adequacy of the nitrogen supply to the plant by symbiosis is, from a practical point of view, perhaps of even greater importance. The results of the two experiments reported in Section 3.1.1.6 showed that for plants with healthy nodules, leaf area differences over a period of seven days after transfer to solutions with and without combined nitrogen were small. Results were also quoted from an experiment in which plants were sampled from a healthy vigorously growing pasture. Even as late as eight days after transfer the differences in leaf area were hardly significant. It was therefore suggested that the comparison of leaf area differences after transfer to solutions with and without nitrogen offers a relatively simple way of ascertaining whether symbiotic nitrogen fixation at the time of transfer was sufficient for plant growth.

The results for calcium were in keeping with the known immobility of this element in the plant. The omission of calcium in the solutions to which the plants were transferred brought about a sudden collapse of the petioles, which was severer the lower the calcium status of the plant at the time of transfer. How useful this would be for practical application is difficult to say without further evaluation.

The second series of experiments (Section 3.1.2) were carried out to establish whether a low nutrient status with respect to phosphorus, sulphur, potassium, calcium, or boron, could be indentified by comparing the leaf area changes after transfer to complete solutions, and to solutions without one of the elements mentioned. In all five experiments, the increases in leaf area after transfer to the solution without the element that had been deficient before transfer, were considerably smaller than those in the other solutions. The differences between the plants in the complete solutions and those in the solutions lacking elements other than the deficient one, were generally small. In other words, the patterns of leaf area changes during the brief periods after transfer, were almost entirely determined by the element that had caused the stress, and were not affected to any significant extent by the absence of other elements (Figure 16).

The third phase was concerned with the possibility of detecting stresses caused by two elements (Section 3.1.3). There were three experiments, the first one dealing with nitrogen and phosphorus, the second one with nitrogen and sulphur, and the third one with phosphorus and sulphur. The results showed that stresses caused by two elements can be identified, at least qualitatively, when they are of approximately equal severity. When the supply of one element had been much lower than that of the other element the stress was mainly determined by the element in shortest supply. Leaf area changes after transfer generally followed a pattern in keeping with the Law of Limiting factors, and with the concept of a nutrient stress as discussed earlier. This was best demonstrated in the phosphorus-sulphur experiment (Figure 19). When phosphorus and sulphur were both supplied at a low level, the leaf area changes after transfer to solutions without sulphur or without phosphorus were both smaller than in the complete solutions. An increase in the pretreatment supply of one element increased the relative difference in leaf area between the solutions lacking the other element and the complete solutions. In the other two experiments the interactions of nitrogen with phosphorus or sulphur followed similar trends. The results for nitrogen agreed with what has been said before for the nitrogen experiments (Section 3.1.1.6). They confirmed that the present approach for the assessment of the nitrogen status of plants raised with combined nitrogen does not appear feasible.

In the fourth group of experiments (Section 3.1.4) an attempt was made to evaluate the effect of the stage of development of the plant, and the effect of temperatures before and after transfer on the leaf area differences between complete solutions and solutions without the varied element. The evidence presented suggested that the manner in which a nutrient stress developed may be of greater importance than the stage of development. In the experimental technique employed, suboptimal amounts of sulphur or phosphorus were added to the culture solutions in one or two applications. As a result the plants that had grown at these levels to a further advanced stage than others had also been under a greater stress. It was found that leaf area differences after transfer to solutions with and without the varied element were greatest for the plants that had been in the deficient solutions for the longest period of time. Under field conditions stresses are more likely to be caused by a suboptimal, but more or less continuous supply, rather than by an exhaustion of a soluble and available pool. Field evidence for plants of different phosphorus and sulphur status and sampled early and late in the growing season, appeared to support this. There were only small and no consistent changes in the relative leaf area differences for the two samplings (Table 2, Section 3.1.4.1).

Temperatures during the 7 day test period did not appear to be very critical above a certain level, which was suggested to be around 21/16° C. Pretreatment temperatures affected the results somewhat when they had been low. The effect was small in comparison with that of differences in the level of pretreatment nutrient supply. The results also suggested that although a low temperature can mask the effect of a low nutrient supply, transfer to solutions with and without the element concerned may still induce differences in leaf area, and may thus enable at least a qualitative assessment of the nutrient status of the plant at the time of transfer (Section 3.1.4.2).

4.1.3 Field evaluation.

The results presented have shown that the contrasting of growth responses immediately after transfer of deficient plants to complete solutions and to solutions without a different element, can identify qualitatively which nutrient element, if any, is limiting plant growth at the time of transfer. They have also shown that when other nutrients were available in non-limiting supply, a quantitative assessment, at least for certain elements, may be feasible. To test the latter possibility under field conditions, 21 field trials were initiated in 1963 on the Southern Tablelands and Western Slopes of New South Wales, Australia. Pastures in this area are winter pastures with a growing season which extends, depending on opening rains, from April to November. The mean annual rainfall between different sites varied between 500-900 mm. The greatest direct distance between the sites in a N-S direction was approximately 370 km, and in an E-W direction 230 km. The sites were chosen to cover as wide a range of climatic, soil, and other environmental conditions as possible.

The following treatments were applied to each of the trials: control (nil), phosphorus (as $NaH_2PO_4.H_2O$) at a rate equivalent to the phosphorus in 204 kg/ha of superphosphate, sulphur (as $Na_2SO_4.10H_2O$) at a rate equivalent to the sulphur in 204 kg/ha of superphosphate, and phosphorus and sulphur together at the same rate as the two separate quantities applied to the other plots. A basal dressing of 300 g molybdenum (as molybdenumtrioxyde), and 9 kg boron (as borax) per ha were applied. The size of the plots was 3 × 4 m^2. All treatments were replicated six times. Plants were sampled from the control, phosphorus, and sulphur plots in late autumn, early winter. They were brought to the glasshouse, and on the day after sampling placed in appropriate test solutions after visual grading for uniformity. The procedure adopted in the field was that the plants were dug up, adhering soil was shaken off carefully, the roots were rinsed in water, and the plants were transported in wet paper towelling wrapped around the roots. Although a certain amount of root damage could not be avoided, it was generally small and, in view of the satisfactory growth rates after transfer, of no great concern.

Estimates of clover yield in the field were based on the sum of a winter and a spring cut. Leaf areas were used as before to describe the relative differences in response to the transfer to solutions. Correlation coefficients were calculated between the following ratios.

Estimate of field response (clover yield)	Glasshouse response 7 days after transfer to solutions
1. $\frac{\text{Dry weight (0) plots}}{\text{Dry weight (P) plots}}$	a. $\frac{\text{L.Area (no-P) solution}}{\text{L.A. complete solution}}$ For plants sampled in (0) plots
2. $\frac{\text{Dry weight (S) plots}}{\text{Dry weight (SP) plots}}$	b. $\frac{\text{L.A. (no-P) solution}}{\text{L.A. complete solution}}$ For plants sampled in (S) plots
3. $\frac{\text{Dry weight (0) plots}}{\text{Dry weitht (S) plots}}$	c. $\frac{\text{L.A. (no-S) solution}}{\text{L.A. complete solution}}$ For plants sampled in (0) plots
4. $\frac{\text{Dry weight (P) plots}}{\text{Dry weight (SP) plots}}$	d. $\frac{\text{L.A. (no-S) solution}}{\text{L.A. complete solution}}$ For plants sampled in (P) plots
1 and 2 were estimates of the response to phosphorus without and with sulphur. Similarly, 3 and 4 were estimates of the sulphur response without and with phosphorus.	The ratios of a and b were intended to give an estimate of the phosphorus stresses in the absence and presence of sulphur, and c and d an estimate of the sulphur stresses in the absence and presence of phosphorus resp.

Correlation coefficients were first calculated between each of the corresponding numbers in the two columns. Two coefficients were, therefore, obtained for the phosphorus responses, one without and one with sulphur applied in the field (1 versus a, 2 versus b respectively), and similarly for the sulphur response, one without and one with phosphorus (3 versus c, 4 versus d respectively). It was found that the correlations for the response to each element were not altered significantly by the presence of the other element. This may be of some practical importance, and is in line with the results of the glasshouse work, in which it was found that phosphorus and sulphur stresses can be identified when they occur together. The relationships between the combined field responses to phosphorus and sulphur (X_2), each calculated over all the responses without and with the other element, and the glasshouse leaf area responses on day 7 (X_1), were straight lines given by the following equations.

Phosphorus response : $X_2=11.16X_1 - 9.36$ $r=0.582(P<0.01)$
(1 and 2 versus a and b)

Sulphur response : $X_2= 5.52X_1 - 4.39$ $r=0.714(P<0.001)$
3 and 4 versus c and d)

The lines were fitted taking into account the errors for both the field and the glasshouse assays. Statistical analysis showed that these correlation coefficients could be improved, if variability in the field sampling of plants for the glasshouse assay can be reduced. To investigate this further, 12 of the 21 sites were continued during the 1964 season. Greater care was taken in the field sampling. With other refinements applied to the 1964 series it is hoped to confirm and, if possible, to improve the field results for 1963.

4.1.4 Some considerations on the place of the present approach in the assessment of the nutrient status of plants.

The carrying capacity of a pasture depends on the quality of the herbage and the plant yield per unit area. Pastures in southern Australia are largely grown for grazing purposes. The amount of fodder harvested for conservation is only a fraction of the total pasture production. In contrast to cash or fruit crops, yield criteria are, therefore, not often available to evaluate the effects of fertilizer applications. In the early stages of pasture improvement, when nearly any amount of fertilizer would give a response, the increased animal production would be a rough, but probably adequate yardstick. However, this would hardly appear a satisfactory basis to assess fertilizer responses in further advanced stages. Even if satisfactory methods for soil or plant analysis were available, there is no way, except by weighing of pasture cuts, by which fertilizer applications can be assessed in terms of growth responses of the pasture. Because the present approach is based on the comparison of growth responses induced in the actual crop plant, it could be used to assess the effectiveness of fertilizer applications.

A second consideration of practical importance is, that there are many situations in which it is essential to determine, not so much the quantity of a fertilizer to apply, but to identify what nutrient element, if any, limits plant growth at a given time. There are good reasons to believe that with the elimination of phosphorus and in some areas sulphur, as major deficiencies, other elements may become limiting for plant growth. Of these, potassium and boron (Spencer, unpublished) may be quoted as recent examples.

A third aspect that has been kept in mind is the possibility of achieving a quantitative assessment of the nutrient status of subterranean clover with respect to the economically important elements phosphorus and sulphur. Particularly in the marginal range, or where deficiencies of both occur, methods of soil or plant analysis may not be satisfactory.

It was stated in the Introduction (Section 1), that the work presented here was prompted by the need to find ways of overcoming some of the disadvantages of field trials, including their cost, as a diagnostic tool. It is in this context that the usefulness of the present approach should be considered. It is in a sense an attempt to short cut conventional field trials by the use of a "micro-field experiment". It has in common with the field experiment, that use is made of the responsive capacity of the plant to the removal of a nutrient stress. Shortcuts were achieved by removing the limitations placed on the response potential of the plant by low temperatures and by the movement of applied nutrients through the soil to the roots. This was achieved by transferring plants to nutrient solutions in a more favourable environment. An earlier identification of the response was achieved by using the changes in leaf area induced by the transfer, because they were a more sensitive index of response than dry weight. The present approach to the assessment of the nutrient status of plants is, therefore, not primarily intended as an alternative to leaf or soil analysis techniques, but rather as one that may be used to identify deficiencies, and to assess and evaluate growth responses to nutrient applications as a possible shortcut for field experiments. It could be an alternative to leaf or soil analysis techniques in situations where they do not apply, are not available, or where they need confirmation.

It may be useful to mention a few of the practical difficulties encountered in the early stages of the work. It was found essential to shade the plants from direct sunlight during the first hours after transfer. In the 1964 series of field samplings, the plants were placed in the appropriate nutrient solutions in the afternoon, and the leaf area ratings carried out early in the following morning. The leaflets of subterranean clover tend to close up somewhat during the hours of greatest radiation. It was far easier to measure the areas earlier in the morning, when the leaflets were lying almost perfectly flat in one plane. This also made the rating procedure less tiring, and therefore reduced the chance of errors. A considerable reduction in paper and computational work was achieved in the later stages, by recording ratings directly on punchcard details and by the subsequent electronic processing of the data.

Another improvement that could conceivably eliminate the ratings, would be by recording the leaf area per plant during the response period photographically (Williams, unpublished). Film and exposure could be so chosen, that the leaves on the negative appeared almost transparant against a black background. It would then be comparatively simple to relate the amount of light passing through the leaves to their area. Preliminary observations indicated that the amount of overlapping by the leaves, at least in the young stages of growth, is not serious. The growth habit is a rosette, and subterranean clover could lend itself easily to such a procedure. This will be investigated further.

4.1.5 Possible avenues for further development.

Dry weight responses following the removal of nutritional stresses are the end result of a considerable chain of processes. It is conceivable that by taking the response after the removal of a stress further forward to its point of initiation, the element that had caused the stress may be identified even earlier than by changes in leaf area. Because the respiration rate is a measure of the plant's metabolic activity, it was used in a series of experiments designed to investigate the possibility of obtaining an earlier identification of stresses caused by phosphorus or sulphur.

In the present context, changes in respiration rates, as well as those in leaf area or in any response index, would be of little value without a basis for comparison. Plants raised at different phosphorus or sulphur levels were therefore, as before, transferred to complete solutions and to solutions without the previously varied element. At the desired stage of growth, leaves of comparable age were cut off at the joint with the petioles, and after obtaining fresh weights, placed in Warburg respirometer flasks. Respiration rates were recorded as oxygen uptake/g fresh weight/hour at 28° C.

Table 11 shows that the effect of low phosphorus pretreatments was apparent in somewhat lower respiration rates after transfer to complete solutions. The effect was smaller at 22 hours than at 18 hours after transfer. Plants transferred to solutions without phosphorus showed much greater differences between pretreatment levels. The respiration rates were lower the greater the phosphorus stress at the time of transfer.

TABLE 11

EFFECT OF PRETREATMENT ON OXYGEN UPTAKE (microlitre/g fresh weight/hour) AT TIMES SHOWN AFTER TRANSFER.

Varied pre-treatment element	Time after transfer	Pretreatment levels					
		1		2		3	
		1*	2**	1	2	1	2
Phosphorus	18 hrs	804	612	942	808	1042	1049
	22 hrs	896	608	1092	757	1120	1092
Sulphur	24 hrs	557	551	598	559	777	754
	72 hrs	560	505	642	489	750	734

* refers to respiration rates of leaves cut from plants in complete solutions

** leaves of plants in solutions without the varied element

For plants raised at low sulphur levels, differences between complete and no-sulphur solutions did not occur till much later. This was not only the result of an increase in the respiration rates of tissue taken from plants transferred to complete solutions, but also due to a decrease in respiration rates of the tissue of plants transferred to solutions without sulphur. It is clear that the changes in respiration rates induced by the transfer of sulphur deficient plants were considerably slower than those occurring after the transfer of phosphorus deficient plants. This finding may be related to the results of Section 3.2.3, where it was found that changes in the net assimilation rate over the first three days after the transfer of sulphur deficient plants were considerably smaller than those over the first interval after transfer of phosphorus deficient plants.

In another experiment plants raised at a low phosphorus level were transferred to complete solutions, and to solutions without nitrogen, phosphorus, sulphur, potassium, or boron respectively. After 24 hours the respiration rates of leaf tissue taken from plants transferred to the complete solutions and to the other solutions containing phosphorus were very similar, with an average of 726 microlitre/g fresh weight/hour. The respiration rates of leaves of plants in the solutions without phosphorus were much lower at 592 microlitre/g fresh weight/hour.

These results suggest that further exploration of this kind of approach may be worth while, and it may even be possible to induce responses in tissues instead of in whole plants. Essential to the success of such a technique would be the possibility to identify responses in terms of comparative changes.

4.2 Growth changes during the adjustment from nutrient stresses.

The comparison of the relative rates of increase in leaf area (R_A) and in dry weight per plant (R_W) of Figure 21 clearly showed that the differences in leaf area between the test solutions with and without the varied element were relatively greater than the corresponding dry weight differences. Figure 21 also showed that in the solutions without the varied element, both R_A and R_W were lower the greater the stress had been before transfer. This was the case in the experiments with different phosphorus, sulphur, potassium, or boron pretreatments. The transfer of deficient plants to complete solutions resulted in higher relative rates of increase in leaf area than in dry weight (Figure 21). In other words, the reason why in the present approach the comparisons of induced leaf area differences were a better basis for the assessment of the nutrient status of the plant than those in dry weight, was the relatively small effect of differences in the pretreatment nutrient supply on the rates of leaf area expansion after transfer to complete solutions. In the experiment with five sulphur pretreatment levels, for example, the differences in

R_A over the 7 day period after transfer to complete solutions between S_1 and S_5 plants was 20 per cent., while the corresponding difference in R_W was nearly 50 per cent. The results for phosphorus (Figure 21) were similar. A comparison of the slopes of the lines representing the relative changes in leaf area in complete solutions for plants raised at different potassium (Figure 8) or boron levels (Figure 12), also reveals that the rates of leaf area increase induced by the removal of a nutrient stress were not greatly affected by the severity of the stress. A quantitative relationship between the nutrient status of the plant and the leaf area differences induced by the transfer to test solutions with and without the varied element, therefore, depended on the fact that leaf area expansion in the solutions without the varied element was smaller the greater the stress at the time of transfer, and on the relatively small effect of stress levels on the rates of leaf area expansion after transfer to complete solutions.

Evidence from experiments in which it was possible to distinguish between leaves and petioles existing at the time of transfer and those formed afterwards showed that, of the assimilates produced over the 7 day recovery period, very little was recovered in the existing aerial parts. More than half was present in the new leaves formed after the removal of a stress. This was apparent for both phosphorus and sulphur, and was more pronounced the greater the stress at the time of transfer (Section 3.2.2). It is suggested that the comparatively rapid increase of R_A after the removal of a nutrient stress was related to the preferential distribution of assimilates to new leaf tissue.

To what extent the existing leaves contributed to the production of new assimilates during recovery cannot be decided with certainty. An analysis of R_W into its two components, the net assimilation rate E_A, and the ratio of leaf area to plant dry weight (A/W), showed that an important factor in the smaller relative growth rates with decreasing pretreatment levels was a reduction in E_A (Section 3.2.3). As the area of the new leaves three days after transfer to complete solutions of plants raised at a low phosphorus or sulphur level was only about 10 per cent. of the total area per plant, the changes in the net assimilation rate that occurred before day 3 would have been due largely to changes in the assimilatory capacity of leaves existing at the time of transfer. It was in this respect that the responses differed between sulphur and phosphorus. The changes in E_A presented in Figure 25 suggested that the contribution of the old leaves to the growth changes over the first interval after transfer to complete solutions for plants raised at deficient sulphur levels was small compared with those for plants raised at low phosphorus levels. In spite of the less severe stresses imposed by the sulphur pretreatments than those of the phosphorus levels in the phosphorus experiment, E_A over the first interval after transfer of sulphur deficient plants to complete solutions was the same as for corresponding plants transferred to solutions without sulphur. This was in contrast to the differences in E_A over the first interval after transfer of phosphorus deficient plants to complete solutions and to solutions without phosphorus. A marked rise in E_A for the plants recovering from sulphur stresses occurred between the first and the second interval. Concurrent with the rise in the net assimilation rate, the area of new leaves increased from 9 per cent. on day 3 to 33 per cent. of the total area-per plant on day 7. Whether the old leaves contributed much to the rise in E_A over the second interval or not, cannot be determined from the data available. It has been noticed in this and other experiments with previously sulphur deficient plants, that the disappearance of chlorosis of the leaves may take as long as 3-5 days, depending on the degree of stress. It has also been shown (Section 4.1.5) that the respiration rates of leaf tissue taken from plants recovering from sulphur stresses had changed little as late as three days after transfer to complete solutions. By contrast, the respiration rates of leaf tissue taken from previously phosphorus deficient plants had increased to nearly the same value as for leaves taken from plants that were not deficient, within 24 hours after transfer to complete solutions. In both cases the leaves sampled were those existing at the time of transfer. It is therefore suggested that, even over the period of 3-7 days after the removal of a sulphur stress, the contribution to the rise in E_A by the old leaves may have been smaller than the contribution to E_A by the old leaves after the removal of a phosphorus stress.

Whatever the exact mechanism of the induced changes may have been, it is reasonable to conclude that the assimilatory capacity of the leaves was reduced under conditions of phosphorus or sulphur stresses. The indications are that the recovery in the assimilation rate after removal of a phosphorus stress was comparatively rapid, even in existing leaves. The impairment in sulphur deficient leaves appeared more severe with little sign of recovery, at least during the first three days after addition of sulphur, in spite of a relatively milder stress than in the phosphorus experiment. The differences in the response between the two elements is probably related to the different roles of the two elements in the metabolism of the plant.

The findings of Hanson, Barrien, and Wood (1941) may be relevant to the present results. These authors found that chloroplast protein contained more sulphur per unit of nitrogen than cytoplasmic protein. Kylin (1953), in later work, subjected wheat plants to a sulphur starvation, and suggested that chloroplast protein was broken down while the synthesis of cytoplasmic protein still took place. A similar trend was observed by Ergle and Eaton (1951) in sulphur deficient cotton plants. Ergle (1954) found a marked decrease in the chloroplast content of the new leaves, and particularly in the chlorophyll content of the chloroplasts, after omitting sulphur from nutrient solutions applied to cotton plants. While in nitrogen deficient plants nitrogen is translocated to younger leaves as a result of proteolysis in older leaves, a reassimilation of protein sulphur did apparently not occur. Other evidence also shows that chlorosis accompanies sulphur deficiency, which usually appears first in younger leaves. This has been shown by Eaton in a number of crops, for example, in soybeans (1935), in sunflowers (1941), and in tomatoes (1951).

Pirson (1955) suggested on the evidence of preliminary experiments, that sulphur deficiency causes disturbances in photosynthesis from which the plant only recovers slowly. He also quotes evidence for reduced rates of photosynthesis under conditions of phosphorus deficiency. However, reactivation during recovery experiments generally appears to be fairly rapid, even under conditions of a severe deficiency.

The results of the present experiments are in line with those quoted above. A reduced net assimilation rate of sulphur deficient plants (Table 6) could conceivably have been due to a decrease in chlorophyll content, or perhaps to a disruption of chloroplasts. The time required for the synthesis of chlorophyll, or the repair of chloroplasts, could explain the delay of at least three days after the removal of a sulphur stress before a noticeable rise was observed in the net assimilation rate. Pirson (1958) suggested that a relation between the photosynthetic capacity of the leaf and sulphur nutrition may be indirect through the protein level and the chlorophyll content of the chloroplasts. Because phosphorus is more involved in energy transfer processes, no structural disorganization would occur under deficiendy conditions. The recovery in the metabolism after the removal of a phosphorus stress would, therefore, also be comparatively rapid.

Watson (1952) concluded in a review that nutrition has a greater influence on leaf area than on net assimilation rates and that the beneficial effects of fertilizer applications are therefore to be ascribed mainly to an increase in the leaf area index. Although the effects of nutritional stresses on net assimilation rates may be smaller than on leaf areas, they are certainly not negligible. It was shown in the experiments of Section 3.2.3 that plants raised at a deficient phosphorus level (P_2) showed a yield reduction of 30 per cent. compared with plants raised in full solutions (P_3). Plants raised at a deficient sulphur level (S_2) showed a reduction in yield of 15 per cent. compared with plants raised in full solutions (S_3). In both experiments, even after transfer to complete solutions, net assimilation rates of the P_2 and S_2 plants were well below those of the plants that were not deficient. To emphasize this point, the following comparison shows E_A over the first three days after transfer to complete solutions.

Pretreatments:

P_2	P_3	S_2	S_3
0.75	0.98	0.64	1.04 mg/cm^2/day

De Wit (1958), and de Wit, Dijkshoorn, and Noggle (1963) state even more explicitly, that the net assimilation rate is "only affected in cases of more extreme growth reductions". Although interpretation of extreme growth reductions may be subject to debate, the 3Q per cent. yield reduction found on day 0 for the P_2 plants, which was mentioned above, would be well within the limits of practical possibilities. The yield reduction of 15 per cent. for the S_2 plants can certainly not be called excessive.

The results presented point to the need to prevent interferences by suboptimal nutrient supplies in experiments concerned with the effects of light and other environmental factors on plant growth.

4.3 The *movement and distribution of phosphorus*.

After the removal of phosphorus or sulphur stresses, phosphorus and sulphur were rapidly taken up, and distributed to all plant parts. Phosphorus uptake over the second period (3-7 days) after transfer of phosphorus deficient plants to complete solutions, was only a fraction of that taken up over the first interval (0-3 days) (Section 3.2.4.).

Of the phosphorus taken up over the first interval, more than half moved to the leaves and petioles existing at the time of transfer to complete solutions (Table 8). Approximately 25 per cent. of the phosphorus taken up remained in the roots. The evidence presented showed that, over the second interval after transfer to full solutions, nearly half of the phosphorus previously accumulated in the old aerial parts of the plants raised at the lowest phosphorus level was translocated to newly developing leaves and petioles. A smaller retranslocation from old to new aerial parts over the second interval was established for plants that had been less deficient.

Williams (1948) considered that the rates of uptake of nitrogen and phosphorus in an experiment with oats grown at three levels of phosphorus were governed by the external supply of the nutrient and the demand for nutrients set up by the development of the plant. Each vegetative organ of a plant passes through phases of intake, relatively constant content, and of export. He considers that each organ has a capacity to accumulate nutrients, and constitutes a potential source for younger organs. Although the demand for phosphorus and nitrogen is usually met in part, if not entirely, by absorption from the medium, the rate of uptake by the roots is restricted to the extent that phosphorus can be more readily available from sources inside the plant. Williams (1948) illustrated this point with the phosphorus content of the inflorescences in his experiments. He found that the phosphorus deficient oat plants derived 30 per cent. of their inflorescence phosphorus from other plant parts, but those grown at a high phosphorus level derived no less than 93 per cent. of their inflorescence phosphorus from other plant parts. The external supply for the latter was still plentiful, but an abundant and more accessible supply was available in the plant due to senescent breakdown of protoplasm in leaves and roots.

A similar kind of situation may have existed in the present experiment. It is suggested that the phosphorus accumulated in the old leaves and petioles constituted a more readily accessible source of phosphorus for the new leaves and petioles developing over the second period than did the external supply.

Evidence that phosphorus in the leaves was freely available for retranslocation to newly developing leaves was also found when plants raised at an adequate phosphorus level were transferred to solutions without phosphorus (Table 9). All the phosphorus present in the new leaves and petioles seven days after transfer to the no-phosphorus solutions had been derived from old leaves and petioles. In contrast to the results for the sulphur experiment, no phosphorus was lost from the roots; in fact they increased somewhat in phosphorus. The general trends were not greatly altered by progressive starvation. Even after transfer of the plants raised at the lower phosphorus level to solutions without phosphorus, no phosphorus was lost from the roots, and the small quantities of phosphorus in new leaves and petioles had all been derived from old aerial parts. This is clear from the absolute phosphorus contents (mg) in the parts of the plants raised at the intermediate phosphorus level, on day 0, and on day 7 after transfer to solutions without phosphorus:

	Day 0	Day 7
Old leaves	0.036	0.023
Old petioles	0.015	0.008
New leaves	-	0.009
New petioles	-	0.005
Roots	0.054	0.054

There was more phosphorus lost from the old parts than could be accounted for in new parts. This may have been due to analytical errors in the estimation of the small quantities of phosphorus in the new petioles and leaves, and possibly also to some loss of phosphorus to the solutions.

There is abundant and almost unanimous evidence that phosphorus is freely mobile and available for redistribution in the plant. This has been established by injecting radioactive phosphate into bean leaves (Biddulph 1941), by application of radioactive phosphorus to leaves (Bukovac and Wittwer 1957; Koontz and Biddulph 1957), and by following the distribution patterns in the plant after a period of uptake of radiophosphorus (Biddulph et al. 1958). Most of the results show conclusively that the movement of phosphorus is largely directed from older to younger expanding tissues. This was also found in the present experiments. The absence of any net losses of phorphorus from the roots, both after the removal of a stress and with increasing phosphorus stress, was in marked contrast to the results of the sulphur experiment, in which the root sulphur was the first sulphur source to be mobilized in the initial stages of a sulphur stress.

4.4 The movement and distribution of sulphur.

Table 7 (Section 3.2.4) showed that the uptake of sulphur by plants raised at suboptimal sulphur supplies was nearly the same over the second interval as over the first interval after transfer to complete solutions. This was in marked contrast to the much smaller uptake of phosphorus by previously phosphorus deficient plants during the second interval compared with the first interval. Of the sulphur taken up during the first interval after the removal of the sulphur stress, more than half moved to the old aerial parts, and only about 10 per cent. to the new top parts. The remainder was recovered in the roots. With the appearance of new leaves in the second interval during recovery relatively more was translocated to the new top parts. However, the old leaves and petioles continued to accumulate sulphur. The old parts of the plants raised at the lowest sulphur level received as much as 25 per cent. of the sulphur taken up during the second period.

In view of the continued accumulation of sulphur in the older leaves and petioles during the later stages of recovery from sulphur stresses, it appears unlikely that significant amounts of sulphur in the leaves and petioles developing over that period were derived from the sulphur previously accumulated in the older top parts. Although it is possible that earlier accumulated root sulphur was translocated to the leaves and petioles developing during the second interval, the relatively powerful demand for sulphur in the roots (Table 8) suggests that the external supply was a more accessible source than root sulphur.

The results showed that in progressive stages of sulphur starvation, the demand for sulphur by the new leaves was first met by sulphur mobilized in the roots (Table 9), and later by sulphur derived from older petioles. There was no evidence of any significant mobilization of sulphur in the old leaves, even when plants already deficient were placed in solutions without sulphur. This is clear from the following comparison of sulphur contained (mg) in plant parts on the day of transfer of sulphur deficient plants (S_2) to solutions without sulphur, and seven days afterwards.

	Day 0	Day 7
Old leaves	0.058	0.057
Old petioles	0.030	0.012
New leaves	-	0.012
New petioles	-	0.006
Roots	0.042	0.046

The same results were found for plants raised at a lower level (S_1) and then transferred to solutions without sulphur, thus showing that, in subterranean clover at least, the sulphur in the leaves is the least mobile source in the plant.

Reports on the mobility of the sulphur in the plant are somewhat conflicting. Wood (1942) considered that the translocation of sulphur depends on the sulphur status of the tissue and on the presence of meristematic or other tissues rapidly utilizing sulphates. Sulphur in the leaves of plants raised at adequate nutrient levels was considered as relatively immobile. Noticeable changes in the sulphur content of leaves would occur only under conditions of rapid synthesis of sulphur compounds, or under deficiency conditions. Thomas et al. (1941) showed with radiosulphur that, under certain conditions, sulphur can be translocated as sulphate to younger plant parts when needed for growth. Thomas, Hendricks, and Hill (1950) applied sulphate to some of the leaves of lucerne, and found a movement through the stems to the crowns, and then upward again to untreated parts of the tops. Biddulph, Cory, and Biddulph (1956), also working with radiosulphur, showed that a portion of the sulphur taken up by the roots of bean plants remained available for re-utilization in other plant parts. They found that export of sulphur from the leaves depended on the age of the leaves. Export from immature leaves was low, or did not occur at all. In later work Biddulph et al. (1958) placed bean plants with their roots in solutions containing radiosulphur. After a period of an hour the plants were transferred to non-radioactive solutions. Over the next four days of growth, sulphur was mobile, but became eventually fixed in younger leaves. Kylin (1953) placed young deseeded wheat plants in solutions containing radiosulphur for a period of three days. At the end of this period the first leaf was half formed. The plants were then placed in solutions with or without sulphur for a period of 24 days. Autoradiographs showed strong concentrations of radiosulphur in the first leaves of both treatments, a low concentration in the second leaf, and no radiosulphur in the third leaf at the end of the 24 day period. A considerable concentration of radiosulphur was found in the roots of the plants in the no-sulphur solutions. At the end of the 24 days half of the sulphur in the shoots of the plants in the solutions with sulphur was present as sulphate. In the shoots of the plants in the solutions without sulphur, nearly one third of the sulphur was present as sulphate. In spite of the sulphate present in the shoots of the plants in the solutions without sulphur, deficiency symptoms were apparent in the younger leaves.

It appears from this brief review that sulphur is probably not quite as immobile as was previously thought. However, most of the evidence suggests that once sulphate is captured in growing leaves, or in other tissue exercising a demand for sulphur, it is not easily released or available for redistribution, even under conditions of a declining sulphur supply.

This was apparent in Kylin's results, and also to some extent during the later stages of the experiments of Biddulph and coworkers. In the present experiments, no net losses of sulphur were found from leaves at any time during the seven day period after transfer. With increasing sulphur starvation root sulphur was the first source of sulphur that was mobilized, followed by sulphur in the older petioles. The results presented do not preclude the possibility that some of the sulphur accumulated in the "old leaves" during the first three days after transfer to complete solutions was retranslocated to other tissues prior to the harvest on day 3. However, if retranslocation did occur over the first interval, the amounts were probably small in comparison with the retranslocation of phosphorus after the removal of phosphorus stresses (Section 3.2.4, and 4.4).

One aspect which, in the opinion of the present author, has not received sufficient attention in most of the literature quoted, is the effect of the nutrient status of the plant and the effect of the sulphur supply in the substrate on the distribution patterns of sulphur in the plant. The nutrient solutions for plants raised at optimum sulphur levels, and also the complete test solutions of the present experiments, contained 4 p.p.m. sulphur. This level was adequate for optimum growth during a period of 2-3 weeks under the existing experimental conditions. This quantity was only one sixteenth of the sulphur in the Hoagland solutions used in many of the experiments referred to above. Plants grown with an abundance of sulphate could conceivable show entirely different, and possibly less restricted, distribution patterns than plants raised at less luxurious, though still adequate, levels of supply. Another explanation for some of the conflicting results may be the differences between species used in the different experiments.

The reconcile at least some of the conflicting evidence on the mobility of sulphur, careful quantitative studies would be required of the effect of changes in the sulphur supply, and of differences in the nutrient status of the plant on the uptake and distribution of sulphur.

SUMMARY

The experimental work presented in this paper was carried out to provide the basis for an approach to the assessment of the nutrient status of plants that would overcome some of the disadvantages associated with field experiments as a diagnostic technique. It aimed at achieving a more rapid removal of nutritional stresses than is normally obtained by application of nutrients to the soil, and at achieving an earlier recognition of the induced growth changes by using a more sensitive index of response than dry weight. Use was made of the differential changes in leaf area induced over short periods of time after transfer of plants to appropriate nutrient cultures. These cultures included complete nutrient solutions and solutions without a different element.

In the first series of experiments (Section 3.1.1, Figures 3–15) subterranean clover plants (Trifolium subterraneum L., var. Mt. Barker) were raised at several levels of phosphorus, sulphur, potassium, or boron, and then transferred to complete solutions and to solutions without the previously varied element. Differences in leaf area over a period of seven days after transfer were small for plants raised at optimum levels. Plants raised at deficient levels showed differences in leaf area between the two solutions which were often detectable as early as three days after transfer. A quantitative relation between the nutrient status of the plant at the time of transfer and the induced leaf area differences was found in the experiments with plants raised at several phosphorus, potassium, or boron levels. No relationship existed between the nitrogen status of plants and the leaf area differences induced by transfer to complete solutions and to solutions without nitrogen. However, when plants were well nodulated at the time of transfer, differences in leaf area between the two solutions were small. This suggested that leaf area differences induced by transfer to complete solutions and to solutions without nitrogen could be used to assess the adequacy of the supply of symbiotic nitrogen for plant growth at the time of transfer.

In the second series of experiments (Section 3.1.2, Figure 16) plants grown at a deficient supply of phosphorus, sulphur, potassium, calcium, or boron respectively, were transferred to complete solutions and to solutions without one of these elements. Increases in leaf area over the 7 day period after transfer to complete solutions and to solutions lacking elements other than the deficient one were generally similar, and were always greater than the increases in leaf area of corresponding plants transferred to solutions lacking the deficient element.

The third group of experiments (Section 3.1.3, Figures 17–19) was concerned with the possibility of detecting multiple deficiencies. When plants raised at suboptimal supplies of two elements were transferred to complete solutions each without

one of the elements concerned, the induced leaf area differences depended on the relative severity of the two stresses. When the two elements had been about equally deficient before transfer, the leaf area increases of plants in the two solutions, each without one of the elements concerned, were smaller than of the plants in the complete solutions. When the deficiencies had been of unequal severity, the leaf area differences induced by the transfer were greatest between plants in the complete solutions and plants in the solutions lacking the element that had been in shortest supply. This pattern of leaf area responses induced by the transfer was demonstrated most clearly in an experiment with plants raised at three phosphorus times three sulphur levels.

The fourth series of experiments (Section 3.1.4) was concerned with the effects of the stage of development (Fig. 20, Table 1) and of differences in temperature (Tables 3 and 4) on the leaf area changes following the transfer to solutions with and without the previously varied element. The results indicated that leaf area differences may be affected by differences in the stage of development when the nutrient supply before transfer becomes increasingly deficient, due to the exhaustion of an insufficient, but available pool of supply. However, a comparison of leaf area differences induced by transfer to nutrient solutions of plants sampled in field experiments early and late in the growing season did not show any trends that could be related to differences in the stage of development. Within limits, the effect of temperature before or after transfer was small compared with that of differences in the nutrient supply before transfer.

Field experiments were carried out to discover possible quantitative relationships between the leaf area differences induced by transfer to appropriate nutrient solutions of plants sampled early in the growing season in the experimental sites and field responses of clover yield to applications of fertilizers containing phosphorus or sulphur. Correlation coefficients between the leaf area differences after transfer to solutions and field responses were 0.582 ($P<0.01$) and 0.714 ($P<0.001$) for phosphorus and sulphur respectively.

The differential growth changes induced by the transfer to nutrient solutions were expressed as leaf area differences, because they were relatively greater than differences in dry weight. This was found in all experiments, and was related to the fact that the relative rates of leaf area increase depended less on the nutrient status of the plant at the time of transfer than the relative rates of dry weight increase (Section 3.2.1-.3). The evidence showed that the recovery from nutritional stresses was accompanied by increases in leaf area which were, relatively speaking, not much smaller than for plants grown with an adequate nutrient supply. The leaves existing at the time of removal of the stress contributed relatively little to the renewed leaf expansion. During recovery from stresses assimilates were preferentially distributed to new aerial parts, which was more apparent the greater the stress at the time of transfer to complete solutions.

Relative rates of dry weight increase during recovery were markedly lower the greater the stress at the time of transfer to complete solutions. In experiments with plants raised at different phosphorus and sulphur levels, it was shown that the lower relative growth rates after transfer of deficient plants to complete solutions were related to reductions in net assimilation rates (Table 6, Figure 25). The recovery in the assimilatory capacity of the leaves appeared to be slower after the removal of sulphur stresses than after the removal of phosphorus stresses. This was suggested to be related to the different functions of the two elements in the metabolism of the plant.

The growth changes during the recovery from phosphorus and sulphur stresses were accompanied by marked differences in the uptake and distribution of phosphorus and sulphur in the plant (Figure 26, Tables 7 and 8). The initial uptake and distribution of phosphorus during the first three days of the recovery from phosphorus stresses was largely directed to aerial parts existing at the time of transfer. This was followed by a considerable retranslocation from these parts to newly developing leaves. During this phase relatively little phosphorus was taken up from the solutions. The initial uptake and distribution of sulphur after the removal of sulphur stresses was also directed to existing aerial parts. The roots also appeared to exercise a relatively powerful demand for sulphur. Concurrent with the appearance of new leaf tissue during the period of 3-7 days after transfer to full solutions, nearly as much sulphur was taken up by the plant as over the first three days. No net losses of sulphur from existing aerial parts were found during the second interval, and it was suggested that sulphur in the solutions was a more accessible source to meet the demands for new growth than the sulphur present in other plant parts.

SAMENVATTING

Het onderzoek beoogt de basis te vormen voor een bepaling van de voedingstoestand van de plant, die althans enkele van de bezwaren verbonden aan veldproeven als een diagnotisch middel zou kunnen overkomen. In deze methode werd ten eerste gestreefd naar een snelle opheffing van groeibeperkingen veroorzaakt door tekorten in de voeding. In de tweede plaats werd getracht, door gebruik te maken van een gevoeliger maatstaf dan de droge stof is, te komen tot een snelle identificatie van de beperkende voedingsfactoren. De methode berustte in principe op het meten van verschillen in de toename van bladoppervlakten van planten na overplanting in bepaalde voedingsoplossingen. Deze oplossingen bestonden uit volledige oplossingen, en andere waarbij telkens een verschillend voedingselement was weggelaten. De bladoppervlakten werden gedurende een periode van zeven dagen na overplanting gemeten.

In de eerste serie proeven werden jonge klaverplanten (Trifolium subterraneum L., var. Mt. Barker) gekweekt op voedingsoplossingen met verschillende concentraties aan fosfor, zwavel, kalium, borium, calcium, en stikstof. Vervolgens werden ze overgeplant op volledige voedingsoplossingen en op oplossingen waaruit het gevarieerde element was weggelaten (Fig. 3-15). Gedurende de proefperiode van zeven dagen na de overplanting waren de verschillen in bladoppervlakten gering indien planten vooraf niet deficient waren. Planten die voor de overplanting deficient waren, vertoonden duidelijke verschillen in bladoppervlakten die in vele gevallen al na drie dagen meetbaar waren. Een kwantitatief verband tussen de voedingstoestand van de plant en de veroorzaakte verschillen in bladoppervlakte werd vastgesteld in de proeven met verschillende voorbehandelingen met fosfor, kalium, en borium. Geen verband werd gevonden tussen de stikstof toestand van de plant en de verschillen in bladoppervlakte veroorzaakt door de overplanting naar volledige oplossingen en naar oplossingen zonder stikstof. Planten gekweekt van zaden die geënt waren met Rhizobium bacteriën vertoonden echter geringe verschillen na overplanting naar oplossingen met en zonder stikstof. Dit duidde sterk op de mogelijkheid, dat de door overplanting veroorzaakte verschillen in bladoppervlakte gebruikt zouden kunnen worden om na te gaan of de stikstof voorziening door symbiose voldoende is.

In de tweede serie proeven werden planten deficient in fosfor, zwavel, kalium, calcium, of borium overgeplant naar volledige voedingsoplossingen en naar vijf andere oplossingen uit elk waarvan een der bovenstaande elementen was weggelaten (Fig. 16). Gedurende de periode van zeven dagen na de overplanting, waren de verschillen in bladoppervlakte tussen de planten in de volledige oplossingen en de overeenkomstige planten in de oplossingen zonder een van de niet-deficiente elementen gering. De toename in bladoppervlakten in de zojuist genoemde oplossingen waren in alle proeven groter dan van de overeenkomstige planten in de oplossingen zonder het deficiente element.

In de derde groep proeven werd de mogelijkheid onderzocht om veelvoudige deficienties te identificeren (Fig. 17-19). De verschillen in bladoppervlakte die veroorzaakt werden door het overbrengen van planten deficient in twee elementen naar volledige oplossingen en naar oplossingen elk zonder een der twee elementen, bleken af te hangen van de sterkteverhouding der twee deficienties. Wanneer het tekort aan de twee elementen voor de overplanting ongeveer gelijk was, was de toename in bladoppervlakte in de volledige oplossing groter dan in de andere twee oplossingen. Indien het tekort van de twee elementen niet even groot was gedurende de voorbehandeling, waren de verschillen in bladoppervlakte na de overplanting het grootst tussen de planten in de volledige oplossing en de overeenkomstige planten in de oplossing zonder het meest deficiente element. Verschillen tussen de volledige oplossing en de oplossing zonder het minst deficiente element waren kleiner, naarmate de verhouding tussen de twee deficienties gedurende de voorbehandeling verder uit elkaar lag. De verschillen in bladoppervlakten na de overplanting vertoonden een vrij goede overeenstemming met de Wet van het Minimum. Dit werd duidelijk gevonden in een proef, waarin planten behandeld waren in voedingsoplossingen met drie fosfor en drie zwaveltrappen.

De vierde serie proeven betrof de invloed van het ontwikkelingsstadium (Fig. 20, Tabel 1), en van de temperatuur (Tabellen 3 en 4) op de veranderingen in bladoppervlakte na overplanting naar volledige oplossingen, en naar oplossingen zonder vooraf gevarieerde voedingselementen. Verschillen in bladoppervlakte tussen de oplossingen met en zonder het gevarieerde element werden beinvloed door het groeistadium, wanneer de voorziening met het betrokken element voor de overplanting voortdurend geringer werd, zoals bijv. door de uitputting van een beschikbare, maar beperkte hoeveelheid. In dat geval zou een meer ontwikkelde plant een groter gedeelte van de beperkte voorraad uitgeput hebben dan een minder ontwikkelde plant, onder overigens vergelijkbare condities, en dus ook meer gebrek lijden. Overplanting naar oplossingen met en zonder het betrokken element veroorzaakten dan ook grotere verschillen in bladoppervlakte voor de meer ontwikkelde planten. Een vergelijking tussen planten overgebracht uit veldproeven in het begin en tegen het einde van het groeiseizoen naar de desbetreffende voedingsoplossingen, gaf geen enkele aanduiding dat verschillen in ontwikkeling onder veldomstandigheden van groot belang zouden kunnen zijn. Binnen zekere grenzen waren temperatuursinvloeden op de veroorzaakte verschillen in de bladoppervlakte na de overplanting klein, vergeleken met de invloed van verschillen in de voedingstoestand voor de overplanting.

Veldproeven werden uitgevoerd ten einde na te gaan of kwantitatieve betrekkingen bestonden tussen verschillen in bladoppervlakte veroorzaakt door het overbrengen naar daarvoor bestemde voedingsoplossingen van vroeg in het groeiseizoen in de proefveldjes uitgegraven planten, en de klaveropbrengsten in de proefveldjes ten gevolge van de toediening van fosfor en zwavel bevattende kunstmeststoffen. Correlatie coëfficienten tussen verschillen in bladoppervlakten na overplanting, en oogstverschillen in de veldproeven waren voor zwavel en fosfor respectievelijk 0.582 ($P<0.01$) en 0.714 ($P<0.001$) (Sectie 4. 1.3).

De groeiveranderingen veroorzaakt door de overplanting naar voedingsoplossingen werden uitgedrukt als verschillen in bladoppervlakte, omdat deze relatief groter waren dan verschillen in droge stof, hetgeen dus een vroegere identificatie van de veranderingen mogelijk maakte. De betrekkelijk grotere verschillen in de bladoppervlakte dan in de droge stof, hielden verband met het feit, dat de relatieve toename in de bladoppervlakte (R_A, cm^2/cm^2/dag) na overplanting in volledige oplossingen minder afhankelijk was van de voedingstoestand van de plant dan de relatieve toename in droge stof (R_W, mg/mg/dag) (Sectie 3.2.1-.3). Planten die voor de overplanting deficient waren vergrootten hun bladoppervlakte in een relatief niet veel geringere mate dan niet deficiente planten, terwijl de overeenkomstige verschillen in de droge stof toename aanzienlijk groter waren. Gedurende de herstelperiode na het opheffen van het fosfor of het zwavelgebrek werden de nieuwe assimilaten voornamelijk verwerkt in de vorming van nieuw bladweefsel. Dit verschijnsel was duidelijker naarmate de deficientie ten tijde van de overplanting groter was.

De relatieve toename in droge stof (R_W) na het overplanten naar volledige oplossingen was kleiner naarmate de voormalige deficientie groter was (Tabel 6). Dit was duidelijk waarneembaar gedurende de eerste drie dagen na de overplaatsing. Ook gedurende het tweede gedeelte (3-7 dagen) der herstelperiode was dit nog duidelijk, maar niet in zo sterke mate als voorheen. Een belangrijke factor in de lagere waarden van R_W voor voormalig deficiente planten, vergeleken met niet-deficiente planten, was een verminderde netto assimilatie snelheid (E_A, mg/cm^2/dag). Het herstel in de assimilatie capaciteit van de bladeren bleek langzamer te verlopen na het opheffen van zwavel dan van fosforgebrek, hetgeen verband zou kunnen houden met de verschillende functies van de twee elementen in het metabolisme van de plant (Fig. 25).

De groeiveranderingen gedurende het herstel van fosfor- en zwavelgebrek gingen gepaard met belangrijke verschillen in de opname en distributie van deze twee elementen in de plant. De opname en distributie van fosfor gedurende de eerste drie dagen van het herstel van fosforgebrek, bewoog zich voornamelijk naar reeds bestaande bladeren en bladstelen. Een groot gedeelte van het in deze delen aanwezige fosfor werd daarna (3-7 dagen) verplaatst naar pas gevormde bladeren en bladstelen. In de planten met grote tekorten verloren de oudere bladeren en bladstelen zelfs 50 procent van hun fosfor. De opname van fosfor uit de voedingsoplossing gedurende deze tweede periode was gering vergeleken met die gedurende de eerste drie dagen. De opname en distributie van zwavel gedurende het herstel van zwavelgebrek was aanvankelijk ook naar de reeds bestaande bladeren en bladstelen gericht, terwijl de wortels ook een betrekkelijk belangrijk gedeelte van de opgenomen zwavel vasthielden. Gedurende het verschijnen van nieuwe bladeren in de tweede periode (3-7 dagen), was de zwavelopname door de plant bijna even groot als daarvoor. De oudere bladeren en bladstelen verloren gedurende de tweede periode echter geen zwavel. Integendeel, van de door de plant in deze periode opgenomen zwavel ging ongeveer 25 procent naar de oudere bladeren en bladstelen. De gevonden verschillen tussen het transport van fosfor en zwavel in de plant zouden erop kunnen wijzen, dat de oudere bladeren in het eerst genoemde geval een meer beschikbare fosforbron voor nieuwe bladgroei vormde, terwijl gedurende het herstel van zwavelgebrek dit niet het geval is; de zwavel in de voedingsoplossing is dan meer beschikbaar dan die in oudere plantendelen.

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