

AN EXPERIMENTAL STUDY OF THE INFLUENCE  
OF THE MICROELEMENTS ON THE UPTAKE OF  
MACROELEMENTS BY PLANTS

A. F. EL KHOLI



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## CONTENTS

	Page
1. INTRODUCTION . . . . .	9
1.1. Essential microelements . . . . .	9
1.2. The function of microelements . . . . .	10
1.3. Interrelations of microelements among each other . . . . .	12
1.4. Micro- and macro-elements interrelationships . . . . .	14
2. SCOPE OF THE PRESENT INVESTIGATION . . . . .	18
3. CHOICE OF THE EXPERIMENTAL TECHNIQUE . . . . .	19
3.1. Design of experiments . . . . .	19
3.2. Plant material . . . . .	20
3.3. Culture medium . . . . .	20
4. MATERIALS AND METHODS . . . . .	23
4.1. Experimental design . . . . .	23
4.2. Nutrient solutions . . . . .	23
4.3. Experimental technique . . . . .	25
4.4. Sampling and chemical analysis . . . . .	26
5. RESULTS OF THE B, Mn, Cu, Zn AND Fe EXPERIMENTS . . . . .	28
5.1. Visible symptoms . . . . .	28
5.2. Significant treatment effects . . . . .	28
5.2.1. Dry matter production . . . . .	32
5.2.2. The uptake of macroelements . . . . .	33
5.2.2.1. The uptake of nitrogen . . . . .	34
5.2.2.2. The uptake of phosphorus . . . . .	36
5.2.2.3. The uptake of potassium . . . . .	37
5.2.2.4. The uptake of calcium . . . . .	39
5.2.2.5. The uptake of magnesium . . . . .	39
5.2.2.6. The uptake of sodium . . . . .	40
5.3. Discussion and conclusions of the results of the B, Mn, Cu, Zn and factorial experiments . . . . .	40
6. CHOICE OF THE SECOND EXPERIMENT . . . . .	42
7. THE B- K- Cu EXPERIMENT . . . . .	44
7.1. Visible symptoms . . . . .	44

7.2.	Significant treatment effects . . . . .	45
7.2.1.	Dry matter production . . . . .	47
7.2.2.	The uptake of nitrogen . . . . .	50
7.2.3.	The uptake of phosphorus . . . . .	51
7.2.4.	The uptake of potassium . . . . .	51
7.2.5.	The uptake of calcium . . . . .	51
7.2.6.	The uptake of magnesium . . . . .	52
7.2.7.	The uptake of sodium . . . . .	52
8.	<b>GENERAL DISCUSSION . . . . .</b>	53
	<b>SUMMARY AND CONCLUSIONS . . . . .</b>	56
	<b>SAMENVATTING EN CONCLUSIES . . . . .</b>	59
	<b>ACKNOWLEDGEMENTS . . . . .</b>	62
	<b>LITERATURE . . . . .</b>	63
	<b>APPENDICES . . . . .</b>	69

The author was awarded the degree of Doctor of Agricultural Sciences, State Agricultural University, Wageningen, The Netherlands, on a thesis with the same title and contents, on 21st June, 1961.

The author is, "Head of the Microelements Laboratory, Plant Nutrition Section, Ministry of Agriculture, Guiza-Orman, Egypt, U.A.R."

# 1. INTRODUCTION

## 1.1 ESSENTIAL MICROELEMENTS

At the end of the 19th century, the classical list of ten elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and iron, had been recognized as the only elements required for plant growth. Nowadays, it is a wellknown fact that other nutrients are also essential for plant development. The criteria of essentiality had been comprehensively discussed by ARNON, 1950a, 1953 and 1958. It was stated that an element is only to be regarded as essential if the plant fails to develop normally on a medium from which it had been rigidly excluded. Because of the complexity of the plant-soil system, the definite evidence confirming that a particular microelement is essential for plant growth, was usually gained by evidence obtained from purified sand or water cultures.

In the last few decades of the present century, the so-called rare, minor or trace-elements: boron, manganese, copper, zinc and molybdenum, had proved to be indispensable nutrients. ARNON 1950b suggested that these essential elements, including iron, should be called 'micronutrients' in analogy to the term 'macronutrients'.

This new list of the fifteen essential elements tends to be increased, as the ability to perform accurate experiments, by using more purified nutrient media, is increased. According to SCHARRER, 1955 such elements as aluminium, bromine, chromium, gallium, lithium, nickel, strontium, titanium, vanadium, tungsten, and others, may, in some cases, be beneficial to plant growth. BEESON, 1958 reported that cobalt and iodine are of importance in the case of pastures which normally provide these elements to the animal diets.

As a matter of fact, microelements are required only in very small amounts. This is illustrated by table 1, which compares the quantities of macro- and micro-nutrients

TABLE 1 Nutrients removed by crops (kg/ha). (Compiled from Landbouw Gids, 1959)

	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO	MgO	Mn	B	Cu	Mo	Fe	Zn
cereals	62	30	62	12	8	0.225	0.01	0.025	0.002	1.000	0.225
pulses	112	29	55	28	12	0.077	0.031	0.035	0.021	0.616	0.077
potatoes	83	38	153	6	13	0.045	0.062	0.047	0.006	0.360	0.045
sugar beets	190	77	326	96	63	0.350	0.250	0.090	0.010	2.800	0.350
clover	102	24	85	75	24	0.150	0.040	0.035	0.024	1.200	0.150
lucerne	94	25	110	79	9	—	—	0.046	—	—	—

removed by various crops. Nevertheless, shortage of a particular micronutrient may lead to a reduction in the plant growth and to the development of deficiency symp-

toms. Considerable attention has been directed to the microelements, due to the fact that an excess of these elements may also be toxic, whereby usually characteristic toxicity symptoms are manifested by the affected plants. For microelements, in general, the region between deficiency and toxicity is much smaller than for macroelements. It may be stated that different plants vary greatly in their requirements for a particular microelement. According to ARNON, 1953 and 1958, beans require more boron than barley; so alfalfa is capable to absorb its zinc requirements from a medium that has an extremely low Zn content. Corn, growing on the same medium, shows clear deficiency symptoms. It was also observed by WILLIAMS and VLAMIS, 1957, that lettuce and tomato were less susceptible to manganese toxicity than barley.

## 1.2 THE FUNCTION OF MICROELEMENTS

It has been pointed out that the results of workers in the field of microelement research have shown that B, Mn, Cu, Zn, Fe, and Mo are essential plant nutrients. Absence of any of these elements in the soil will result in serious reductions in growth and production.

With regard to the function of these micronutrients in plant metabolism, comparatively little is known. HEWITT, 1951; McELROY and NASON, 1954; GAUCH, 1957; and others discussed the possible role of microelements in biochemical and physiological processes in plant and animal and pointed out that there is a great similarity between the behaviour of these elements in organisms and in enzyme systems. The idea that micronutrients play a catalytic role in the biological reactions has received a general agreement. However, the mechanism of these enzymatic systems and the part governed by microelements is not yet clear. McELROY and NASON have reported that the majority of enzymes either contain or require one or more metals in order to become activated. Among these metals are the essential micronutrients such as Mn, Cu, Zn and Fe, and apparently the non-essential micronutrients (Ni, Co, and others). According to these workers boron does not belong to either of the above mentioned groups of elements.

*Boron:* In this connection the review of SKOK, 1958, discussing the role of boron in the plant cell, is of great interest. He claims that the information given by ALEXANDER, 1942; DUGGER et al, 1957; and others clearly shows that both the presence or absence of boron may alter some enzymatic reactions in different ways. The difficulty in assessing the nature of reactions between the borate ion complex and the enzyme system may be due to the complexing property of the borate ion. It also appears that boron, as opposed to metals, has no function in the oxidation-reduction system; this oxidation-reduction system arises when metals, acting in electron transfer systems by changing of their valence.

Other functions of boron have been recorded. The effect of boron on the transpiration of plants was observed by BAKER et al., 1956. They noticed that the transpiration rate in boron deficient bean plants was remarkably less than that of normal plants. The reduction in transpiration was ascribed to three factors: (1) a decreased rate of water

absorption as a result of boron-deficiency, (2) an increase of non-functional stomatas in boron-deficient leaves, (3) higher concentrations of pectin and pentosan, resulting in a higher osmotic pressure in boron-deficient plants.

REED, 1947, described morphological and histological structures of plant organs in relation to boron deficiency. Apart from a retardation of growth and a die-back of both root and apical buds, apparently the bundle sheath and the sieve tubes of the phloem of the boron-deficient plants were disrupted and blocked the normal conduits for the transportation of energy-rich materials in leaves. Reed also found that inorganic phosphates were trapped in the necrotic material and in the disrupted phloem of the boron-deficient plants. Moreover, cells of leaves of the affected plants showed signs of a disturbed metabolism.

The function of boron in the translocation of sugars and on the carbohydrate metabolism had been postulated by GAUCH and DUGGER, 1953 and 1954, on the hypothesis that a sugar-borate complex might facilitate the passage of sugar through cell membranes.

The manifested symptoms of boron deficiency would rather be due to a decrease of the sugar transport. This hypothesis is not inconsistent with the results obtained by SKOK, 1957, and those of McILRATH and PALSER, 1956. Although these workers found that the addition of sugars does not cure boron-deficient plants, SKOK 1958 concluded that a probable relationship between boron and sugar translocation does exist. This relation appears to be of an indirect nature and not due to the formation of a boron-sugar complex.

*Copper:* The function of copper and its importance for the activation of oxidase enzymes, such as tyrosinase, polyphenol oxidase, monophenol oxidase, laccase and systems oxidizing ascorbic acid, had been discussed by MULDER, 1950; HEWITT, 1951; and other investigators.

*Zinc:* It is possible that zinc acts, in activating enzymes such as carbon anhydrase, hexokinase and dehydrogenase. Zinc plays a role in the synthesis of heteroauxines as it has a regulating function in the formation of tryptophane (SKOOG, 1940; TSUI, 1948).

*Manganese:* The activity of manganese as a cofactor in enzymatic reactions has been reported by several workers. HEWITT, 1951; and MULDER and GERRETSEN 1952, indicated that manganese might be involved in the carbohydrate and nitrogen metabolism. MULDER and GERRETSEN discussed the important part played by manganese in the photosynthesis of green plants. It may be mentioned here, that double and multiple activation and the similarity of the behaviour of bivalent ions, such as  $Mg^{++}$ , on the activation of enzymes which contain  $Mn^{++}$  (MCELROY and NASON, 1954), may complicate the elucidation of the specific functions of manganese in enzymatic reactions. For example, the rôle of manganese with regard to the reduction of nitrate as recorded by LEEPER, 1941; JONES et al, 1949; HEWITT et al, 1949; and others, cannot be ascertained since nitrate reduction involves a number of successive steps that are governed by different enzymes.

Both HEWITT 1951 and PIRSON 1958 do not share this opinion. It may be stated, however, that this problem has not yet been settled for manganese.

*Iron:* The importance of iron in chlorophyll formation and related phenomena, such

as chlorosis, have been reviewed comprehensively by HEWITT, 1951; BROWN, 1956; GAUCH, 1957; and GRANICK, 1958. The importance of iron in relation to cytochrome systems of the respiratory system has been also reported. It has been established that all enzymatic systems that depend on iron, involve porphyrin structures. In this connection, the well known fact that magnesium is a constituent of chlorophyll might be recalled.

GRANICK, 1948, noted that Mg-protoporphyrin is a possible precursor of chlorophyll. According to BROWN, 1956, several metals can be incorporated into the porphyrin-ring. Iron porphins or haemo compounds and magnesium porphins or chlorophylls are of great importance. HEWITT, 1951; HILL, 1949; and ARNON, 1949, suggest that copper and other micronutrients may have a catalizing effect on the formation of iron porphyrin and consequently on chlorophyll formation.

It is a common observation that deficiencies of micronutrients are always accompanied by chlorosis. It is known that visual deficiency symptoms may vary considerably among different plants. They are known as interenal or diffuse chlorosis, white tips, gray speck, scorching, mottling, ... etc. All these cases have one feature in common, i.e. the reduction and/or partial destruction of chlorophyll. It is not yet understood, however, why an excess of micronutrients may also induce chlorosis. Although an interaction between iron and other element is suggested for an explanation of this feature. These facts seem to suggest that internal and external factors other than iron activity or iron availability in the soil might be responsible for the inducement of chlorosis. This seems to be in agreement with the recent results of BROWN et al, 1959, who concluded that iron-chlorosis in the field could be corrected by means of the application of Fe-chelates, as well as by an optimal fertilizer policy and by improving cultural practices.

### 1.3 INTERRELATIONS OF MICROELEMENTS AMONG EACH OTHER

Interrelations between micronutrients have received the attention of several workers. In this respect, the antagonistic effect between manganese and iron was considered as a major problem in plant nutrition.

SOMERS and SHIVE, 1942, stated that the iron-manganese ratio in the culture medium, rather than the iron and manganese concentrations was the main factor controlling plant growth in this respect. They found that in the case of soybeans, the ratio between iron and manganese, corresponding to optimal growth, should be situated within a narrow range around 2 irrespective of the total concentrations of iron and manganese in the substrate. If this ratio was appreciably higher than 2, soybean plants showed iron toxicity symptoms which were identical to manganese deficiency symptoms.

Manganese toxicity was found to be identical to iron deficiency and could be produced when the iron-manganese ratio in the culture solution was below 2. Plant analysis had shown that high concentrations of soluble (active) manganese in the plant tissues

were invariably associated with low concentrations of soluble (active) iron. These results were partially criticized by BERGER and GERLOFF, 1947, who stated that manganese toxicity in potato plants was entirely different from iron deficiency.

Also MORRIS and PIERRE, 1947, concluded that the iron-manganese ratio in the culture solution was not a controlling factor in the growth of lespedeza plants. Moreover, it was observed by MORRIS and PIERRE, 1947, that manganese toxicity in lespedeza plants was remarkably alleviated by increasing the iron supply up to 1 p.p.m. Reduction of manganese toxicity was ascribed to the pronounced decrease of manganese content rather than to an increase of absorbed iron.

Nevertheless, the results of both SOMERS and SHIVE, 1942, and MORRIS and PIERRE, 1947, indicate a definite relationship between iron and manganese. They agree in their conclusion that high levels of iron may decrease the manganese uptake.

SIDERIS and YOUNG, 1949, also found that a high manganese supply may induce iron deficiency. In highly magniferous Hawaiian soils, chlorotic pineapple plants became healthy after spraying with ferrous sulphate solutions. The induction of chlorosis by a high manganese supply could be explained on the assumption that manganese may substitute iron in porphyrin, which thus becomes inactivated prior to its conversion to chlorophyll. This hypothesis was supported by the results obtained by WEINSTEIN and ROBBINS, 1955.

In further studies, SIDERIS, 1950, using radioactive iron ( $^{59}\text{Fe}$ ), showed that in cultures supplied with manganese, the translocation of iron from roots to leaves was considerably impeded. It was suggested that iron remained in the roots in combination with certain protein fractions of the cells. TOTH and ROMNEY, 1954, applied radioactive manganese ( $^{54}\text{Mn}$ ) to soybeans in a culture solution and showed that high levels of iron reduced the manganese content of the leaves, while an increase of manganese was found in the roots.

Similar results were advanced by BOLLE-JONES, 1955, who concluded that applications of iron reduced the uptake of manganese by potato plants. Increased iron supply decreased the concentration of manganese of the laminae, stems and petioles, but increased that of the roots. Extra potassium or phosphorus generally reduced the quantity of manganese which was retained by the roots.

Factors other than iron may have an effect on the manganese uptake. In culture solutions, TOTH and ROMNEY, 1954, had found that high levels of either molybdenum or nickel, reduced the absorption of  $^{54}\text{Mn}$  by soybeans. In sandy loam soil, the same authors observed that high levels of copper increased the manganese uptake of different plants. The effect of molybdenum however was erratic. It was also stated that the soil pH is a dominant factor affecting manganese uptake. A decrease of soil pH resulted in an increase of manganese absorption in the case of all plants.

The uptake of iron and manganese appears to depend on the nature of the nitrogen applied. SIDERIS and YOUNG, 1949, found for pineapple plants that the nitrates had no effect on the content of iron and manganese of the plant tissues. With ammonium as a source of nitrogen the iron concentration was greater than that with nitrate-nitrogen. The manganese uptake was greatly reduced when ammonium was supplied as nitrogen source.

The interactions of copper, manganese and iron were comprehensively reviewed by ERKAMA, 1950. In sterile cultures it was found that the iron content of peas was low in the copper-deficient plants, while in manganese-deficient plants the iron content was high. These results suggest an antagonism between manganese and iron, and a synergism between copper and iron. It was also found that extremely high concentrations of manganese were found in copper-deficient peas, that originated from copper-deficient seed. This would seem to indicate an antagonism between copper and manganese. Copper and manganese react differently to iron. With increasing copper supply, the iron content of the plant sap decreases, while in the protoplasm it increases. Manganese supply resulted in an increase of ferric iron in the vacuole sap, and a reduction of iron in the protoplasm.

With regard to copper-iron relationships, it was shown by BROWN and HOLMES, 1955, that copper deficiency resulted in an accumulation of iron in corn plants, especially in the nodes. It is not justified to apply these findings to other plants, since it was found that some soybean varieties and wheat differed from corn, with regard to the effect of copper on the absorption and utilization of iron.

#### 1.4 MICRO- AND MACRO-ELEMENTS INTERRELATIONSHIPS

It is of interest to investigate whether micronutrients have an effect on the uptake of macronutrients.

It is well known that both deficiency and excess of micronutrients lead to a depression of yield and sometimes to a serious damage of plants, even when macroelements are adequately supplied. The question may be put whether microelements effect the ability of plants to absorb macronutrients from a culture medium, or influence the assimilation of those ions by the plant.

In the literature, quantitative information on the relationships between micro- and macronutrients are scarcely found. The few available relationships were mainly described for the cases where macroelements had an effect on the uptake of micronutrients. Even though, they are by no means clear.

For example, the outstanding problem of micronutrients deficiencies induced by an excess of lime, suggest that there might be an effect of calcium on the uptake of micronutrients. GISIGER, 1950, however, showed that a high pH instead of the high Ca-ion concentration is the cause of an induced boron deficiency. SJOLLEMA and HUIDIG, 1909; and GISIGER, 1950, found that a high pH was responsible for a reduction of available manganese in the soil, since the gray speck disease of oats could be induced by the application of calcium carbonate, potassium hydroxide and calcium hydroxide, all resulting in an increase of the pH of the soil.

With peanut plants, RICH, 1956, found a significant negative correlation between the manganese content of the plant leaves and the pH, the exchangeable calcium and magnesium content of the soil. The manganese content in the plant was positively correlated to exchangeable and to easily reducible manganese in the soil. These observations were also reported by DE GROOT, 1956.

For iron, similar results were obtained by McGEOGE and BREAZEALE 1956, rye and barley seedlings absorbed iron as readily from calcareous soils as from non-calcareous soils, but there was a greater immobilization of iron in the plant tops in the case of the former.

WEAR, 1956, studying the effect of  $\text{CaCO}_3$ ,  $\text{CaSO}_4$  and  $\text{Na}_2\text{CO}_3$  on the uptake of zinc by sorghum, showed that the uptake of zinc decreases when  $\text{CaCO}_3$  or  $\text{Na}_2\text{CO}_3$  are supplied, while an increase of zinc uptake is observed after an application of  $\text{CaSO}_4$ . Accordingly the conclusion was drawn that a reduction in zinc uptake could be attributed to a pH effect and not to a Ca-ion effect.

When the pH effect was eliminated by using controlled culture media, the relation of calcium to the uptake of micronutrients by different plants was investigated, but no definite conclusion could be drawn.

The Calcium-boron relationship, has received considerable attention from several workers.

According to PURVIS and DAVIDSON, 1948, there exists a functional relationship between boron and calcium inside the plant. A high intake of one of these nutrients will increase the requirement for the other. MCILRATH and DE BRUYN, 1956, found that an increased calcium supply decreased the content of both soluble and total boron in siberian millet at high boron levels in the nutrient solution. Similar results were also obtained by BRENNAN and SHIVE, 1948, who found that in the case of tomato plants, increments of calcium accentuate boron-deficiency at a low level of boron, whereas a decrease of boron-toxicity was observed at a very high level of boron in the substrate. At intermediate levels of boron, calcium exerted little influence on boron absorption. HERNÁNDEZ-MEDINA and LUGO. LÓPEZ, 1958, concluded that the total boron content of pine-apple plant tissues depends on the level of boron, irrespective of the level of calcium. Also BRENNAN and SHIVE, 1948; REEVE and SHIVE, 1944; MARSH and SHIVE, 1941; and others, had arrived at the conclusion that the calcium content of plants was largely independent on the boron level, and primarily determined by the calcium concentration in the substrate.

MCILRATH and DE BRUYN, 1956, however, found that the soluble and total calcium content of siberian millet increased with an increasing boron supply.

For blue lupine, HENDERSON and VEAL, 1948, also found that the presence of boron promotes the uptake of calcium.

It has been shown that several different views exist in the literature. According to the author, this apparent discrepancy might disappear if the plant yield had been taken into consideration. The negative relation between the increments of calcium supply and the boron content of the plant, observed by BRENNAN and SHIVE, 1948 and by MCILRATH and DE BRUYN, 1956, is in fact in agreement with the above mentioned view of PURVIS and DAVIDSON, 1948. By increasing the dose of calcium, the plant tends to produce more dry matter and therefore requires more boron.

When the yield increases, the relative values per unit weight, which are given as indications for nutrient contents, may decrease. In this connection, it should be borne in mind that requirements for micronutrients are very minute. Moreover, the plant growth is very sensitive to a lack or excess of micronutrients, which will result in a

reduction of the dry matter production. For these reasons the fluctuation in the rate of growth, caused by deficiency or excess of micronutrients, may affect the relative values of the absorbed micro- and macro-nutrients.

Other factors may also interfere, such as the genetic properties of different plants in relation to their requirements of calcium and boron.

A relationship between phosphorous and micronutrients, especially iron and zinc has also been suggested. Phosphate in nutrient solutions may precipitate iron and, thus, reduce the iron availability to plants. The pH of the solution, together with other micronutrients, such as manganese and copper, may interfere in the iron absorption and/or affect the translocation of iron in the plant.

REDISKE and BIDDULPH, 1953, and BIDDULPH, 1953(a), using radioactive iron ( $^{55}\text{Fe}$ ) found that most of the iron applied to beans, in the presence of relatively high levels of phosphorus (0.001 M), failed to reach the plant leaves and caused a diffuse chlorosis. At pH 7.0 and at a low phosphorus supply (0.0001 M), chlorotic mysophyll and precipitated or immobile  $^{55}\text{Fe}$  along the veins could be observed.

At a pH of 4 the plants, receiving the same low level of phosphorus (0.001 M), produced normal, healthy leaves with a uniform distribution of  $^{55}\text{Fe}$  in the leaf tissues.

FOSTER and RUSSELL, 1958, showed that the application of ferric iron in water cultures, before or simultaneous with phosphate, reduced the transfer of phosphate from roots to plant shoots. The effect is more marked in barley than in rye.

Recently, TWYMAN, 1959, found that there was a significant drop in phosphate concentration in the leaves of tomatoes growing in a culture solution, when the iron supply was increased. The addition of iron in four applications at four day intervals, resulted in a higher phosphorus concentration in the leaves than when the same quantity of iron was applied every 8 days or in one single application.

BINGHAM and MARTIN, 1956, found that an excess of phosphate, such as  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , reduced the absorption of zinc and copper by citrus plants, whereas no effect of phosphate on manganese and iron absorption was found.

Similar results were obtained by LONERAGAN, 1951, with flax. High phosphate applications induced severe zinc-deficiency and increased the response to zinc applications. At all high phosphate-treatments an increase in phosphorus content of the plant was associated with a reduction in the zinc content of the plant.

Using radioactive zinc ( $^{65}\text{Zn}$ ), WOLTZ et al, 1953, observed that the concentration of zinc in the tops of soybean plants was reduced after an addition of phosphate (800 lb./acre  $\text{P}_2\text{O}_5$  as  $\text{KH}_2\text{PO}_4$ ).

The application of limestone appeared to be more effective than phosphate in reducing the uptake of zinc. On the other hand, BOAWN et al, 1954, did not find any effect of phosphate on the uptake of either applied or native soil zinc by bean plants. Doubling the concentration of phosphate in the plant tissues failed to produce zinc deficiency symptoms or to reduce the yield of dry matter.

The interaction between phosphorus and iron may influence the translocation of zinc within the plant.

THORNE and WIEBE, 1957, and BIDDULPH, 1953(b), noted that, with an excess of phosphorus in the culture solution, zinc precipitated along the veins. When the iron con-

tent was high, however, zinc precipitated to a lesser extent, probably because phosphate was precipitated by the excess of iron.

At a low phosphate content no pronounced precipitation of zinc along the veins could be observed. In this case zinc appeared to be uniformly distributed in the leaf.

Copper may also interfere in the phosphorus-iron relation. According to BROWN et al., 1955, high doses of phosphorus did not appreciably affect the absorption and utilization of iron by soybeans and rice unless high doses of copper were also given. With another variety of soybeans and with wheat, increasing rates of phosphorus and copper applications, did not affect the absorption of iron.

## 2. SCOPE OF THE PRESENT INVESTIGATION

It is clear that optimal levels of micronutrients are essential for normal plant growth and production. From the foregoing literature it is obvious that the uptake of micro-elements depends on soil conditions, and that the transport in the plant is governed by ionic interaction inside the plant. It is also obvious that these microelements are involved in enzymatic reactions, although their specific function is still obscure. The complicated mechanism of the nutrient uptake and metabolism has not yet enabled the assessment of suitable techniques for the study of causal relationships. The elucidation of the functions of micronutrients in plant growth however, is a wide field for future research. It is evident that the study of the interactions among micronutrients and the study of the effect of micronutrients on the behaviour of macroelements are of great importance.

As the effect of micronutrients on the uptake of macronutrients by the plant has not yet been investigated in a systematic way, an experiment was designed with the object of determining the effect of boron, manganese, copper, zinc, and iron and their interactions on the uptake of nitrogen, phosphorus, potassium, calcium, magnesium and sodium.

Three kinds of indicator plants were chosen, namely: oats, lucerne and tomato. The results of the three successive experiments which were conducted in 1959, could be used as a guide for detailed investigations of some interesting aspects. An elaborated experiment was designed in 1960 mainly to study the effect of a wide range of boron applications at low, medium and high levels of potassium and copper on the uptake of macroelements by lucerne plants.

### 3. CHOICE OF THE EXPERIMENTAL TECHNIQUE

#### 3.1 DESIGN OF EXPERIMENTS

The investigations with regard to the effect of microelements on the uptake of macroelements, may be approached in various ways. A relatively simple experimental design, would be the study of different levels of a particular microelement on the uptake of macroelements. The advantage of such an experiment, apart from its simplicity, would be the fact that the appearance of any deficiency or toxicity symptoms could easily be attributed to the absence or presence of a particular microelement. The set up of an experiment according to these principles would have two disadvantages. The influence of microelements on the uptake of macroelements is as yet entirely unknown, any experimental design would therefore require a great number of treatment continuations. Moreover the interactions between the microelements as related to macroelement uptake can not be determined in this way.

The few results of various workers have shown, that the action of the microelements on nutrients uptake is a controversial matter. Most of the microelements may interact with each other and with macroelements.

Prior to detailed investigations it is essential to obtain a general idea on the problems which are involved. In order to obtain this general picture of the effect of microelements and their interactions on macroelement uptake by plants, a factorial design would seem to be the most obvious approach. In principle low, medium and high levels of microelement applications should be compared. Using the most important elements, viz., boron, manganese, iron, copper and zinc, it is evident that such an experiment has to include  $3^5 = 243$  treatment combinations. If this would be replicated 4 or 5 times, respectively 972 or 1215 experimental plots would be required. This number is much too high to handle in one single experiment in a greenhouse and moreover much time would be involved in the chemical analysis of so many samples. It is possible, however, to carry out a  $3^5$  factorial experiment and at the same time reduce the number of experimental plots considerably, by arranging the treatment combinations in such a way that some of the higher order interactions become confounded with subunits (blocks) of the experiment. This kind of experimental design has the disadvantage that the higher order interactions may be attributed to either treatment or subunit (block) effects. The advantage, however, is the fact that a great number of treatment combinations can be tested from the results obtained from a relatively small number of experimental plots. The sacrifice of some higher order interactions, which are difficult to interpret would seem to outweigh the advantage of obtaining a general picture of all main effects and first order interactions with a relatively small number of experimental plots. It is therefore that this approach was chosen as a first step. It is likely that the results of such an experiment will indicate the cases which require more extensive investigation. Depending on the nature of these subsequent detailed investigations, a suitable experimental design would have to be chosen again.

In general it may be said in this respect that such a detailed experiment would include a relatively great number of levels of a particular microelement together with a few levels of some other elements.

### 3.2 PLANT MATERIALS

The requirements of micro- and macro-elements will not be equal for different plant species. Several workers have shown that some plant species are sensitive to deficient or toxic levels of microelements whereas other species are less sensitive. For the present investigation it was considered of importance whether mono- and dicotyledones differ in their reactions to microelements. Within the dicotyledones, leguminosae occupy an important place. They differ considerably from other dicotyledons in respect to their nitrogen nutrition. For this reason, oats was selected as a test crop for the monocotyledons, for dicotyledones tomato and the legume lucerne.

Although it is known that great differences obtain in each category between plant species and varieties, it was thought that for a preliminary trial the large amount of work involved in including more plant species, would not be justified and should be deferred to subsequent detailed investigations.

The growing period of the experimental plants was kept short. With regard to the practical application of the results it would be of advantage to dispose to the final yields of the test crop. It may be expected however that any serious microelement deficiency or toxicity condition of the plant would be accompanied by secondary effects which will be responsible for a change in the uptake pattern of the macroelements. It was therefore decided to carry out the investigation on normal plants at a young stage of development. In this way, the expenditure for the required quantities of quartz sand, chemical and demineralized water were kept at a minimum.

It is evident that only small differences in macroelement uptake may be expected on account of the microelement treatments at such a young stage of development. The experiment should therefore be designed to enable the detection of small differences in macroelement uptake. The final design and lay out of the experiment were intended to cope with this object.

### 3.3 CULTURE MEDIUM

It is of the utmost importance that the culture medium does not modify the nature of the treatments. Unfortunately, soil interferes strongly with the applied nutrients. It is well known that microelements may become unavailable to plants on account of fluctuations in pH and microbiological activity. The latter effect is related to the nature of the organic matter in the soil. Soil as a medium is therefore a too complex medium for the present investigations.

By means of water cultures it is possible to maintain a relatively constant nutrient medium, provided the solutions are frequently refreshed. In addition, all cultures have to be aerated regularly. The amount of work involved in watercultures is considerable

and particularly in the case of microelement investigations because impurities will have to be removed from large quantities of water and chemicals.

The most suitable approach would seem to be by means of a sand culture technique. Highly purified quartz sand will not react with any micro- or macro-element of the culture solutions. Moreover, the great porosity of sand assures the roots of a well aerated medium. If the sand is supplied daily with culture solutions to a constant water content, relative small quantities of highly purified water and chemicals are required. For these reasons, a sandculture technique was chosen for the present investigations.

TABLE 2  $3^6$  factorial design in 81 units ( $\frac{1}{3}$  replicate) used for the three successive experiments of 1959, with oats, lucerne and tomato. Numerals 0, 1 and 5 indicate the level of the corresponding element in each combination

blocks:	1	2	3	4	5	6	7	8	9
pot nos.:	1-9	10-18	19-27	28-36	37-45	46-54	55-63	64-72	73-81
B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe	B Mn Cu Zn Fe
5 1 0 5 1 5 0 0 1 0	5 0 1 1 5 5 0 1 0 5	5 0 1 0 0 5 0 1 0 5	5 5 1 5 5 0 0 5 1 0	5 5 1 5 5 0 0 5 1 0	5 5 1 0 5 0 1 5 0 0	5 5 1 0 5 0 1 5 0 0	5 5 1 0 5 0 1 5 0 0	5 5 1 0 5 0 1 5 0 0	5 5 1 0 5 0 1 5 0 0
0 1 1 1 0 1 0 5 5 1	0 0 5 5 1 0 0 5 5 1	0 1 0 0 5 0 1 0 5 1	5 1 5 1 0 5 0 5 0 5	5 1 5 1 0 5 0 5 0 5	0 0 5 1 0 5 0 5 0 5	5 0 0 5 5 1 0 5 0 5	5 0 0 5 5 1 0 5 0 5	5 1 5 1 1 5 1 0 5 0	0 5 1 0 0 5 1 0 5 0
1 5 0 1 5 0 5 0 5 5	1 0 0 5 0 5 0 5 5	1 0 0 5 0 5 0 5 5	5 1 5 1 0 5 0 5 5	5 1 5 1 0 5 0 5 5	0 0 5 1 0 5 0 5 5	5 0 0 5 5 1 0 5 0 5	5 0 0 5 5 1 0 5 0 5	5 1 5 0 1 5 1 0 5 0	1 0 1 5 5 1 0 5 0 5
1 0 1 5 5 1 5 1 1 1	5 5 0 0 5 5 0 0 5 5	1 0 0 1 1 1 5 0 0 0	0 5 0 1 0 5 5 5 1 1	0 5 0 1 0 5 5 5 1 1	5 1 1 0 5 0 1 0 5 0	5 1 1 0 5 0 1 0 5 0	5 1 1 0 5 0 1 0 5 0	5 5 0 1 1 5 0 1 0 5 0	0 5 5 5 0 1 1 0 0 1
0 5 5 5 0 1 1 0 0 1	5 1 5 5 5 0 0 5 5 5	0 0 5 5 5 0 0 5 5 5	0 5 5 1 1 5 5 5 1 1	0 5 5 1 1 5 5 5 1 1	1 5 1 5 0 0 5 5 5 1	1 5 1 5 0 0 5 5 5 1	0 0 0 0 1 5 5 5 1 1	0 0 0 0 1 5 5 5 1 1	0 0 0 0 5 1 0 5 1 0
0 0 0 0 0 5 1 1 5 0	0 5 1 5 0 0 5 1 5 1	1 5 5 0 1 1 5 5 0 1	1 0 1 1 0 1 1 0 1 0	1 0 1 1 0 1 1 0 1 0	1 1 0 5 5 0 1 1 0 5 5	0 1 5 5 1 0 1 1 0 5 5	0 1 5 5 1 0 1 1 0 5 5	0 1 1 5 5 0 1 1 0 5 5	0 0 0 0 0 5 1 0 5 1 0
5 5 1 0 1 0 1 5 1 5	1 5 5 1 5 0 1 5 5 1 0	1 1 1 5 1 0 1 1 1 5 1	0 1 1 0 1 1 0 1 1 0 1	0 1 1 0 1 1 0 1 1 0 1	0 1 0 5 1 5 0 1 0 5 1 5	0 0 1 1 1 5 0 1 0 5 1 5	0 0 1 1 1 5 0 1 0 5 1 5	0 1 0 5 0 0 1 0 5 1 0	5 5 1 0 1 0 1 5 1 5
1 1 5 0 5 5 5 5 0 0	0 1 0 1 1 0 1 1 0 1 5	0 5 1 1 5 1 0 1 1 1 5	5 1 0 1 5 1 0 1 1 1 5	5 1 0 1 5 1 0 1 1 1 5	0 1 5 0 0 1 5 0 0 1 5 0	1 0 5 0 0 1 5 0 0 1 5 0	1 0 5 0 0 1 5 0 0 1 5 0	5 1 0 0 0 1 1 1 1 5	1 1 5 0 5 5 5 5 0 0
5 0 5 1 1 0 0 1 0 5	1 1 1 0 0 1 1 1 0 0	5 5 0 5 0 5 5 0 5 5 0	1 1 5 5 0 5 5 0 5 5 0	1 1 5 5 0 5 5 0 5 5 0	5 0 0 0 1 1 0 1 0 1 0 1	1 1 0 1 0 1 0 1 0 1 0 1	1 1 0 1 0 1 0 1 0 1 0 1	1 0 0 0 5 0 1 0 0 5 0	5 0 5 1 1 0 0 1 0 5

TABLE 3 ( $7 \times 3 \times 3$ )-factorial design, with 2 replicates, completely randomised in one block, (experiment of 1960). Numerical figures from 1 to 7 indicate the level of the corresponding element in each combination

pot nos.	1-9	10-18	19-27	28-36	37-45	46-54	55-63	64-72	73-81	82-90	91-99	100-108	109-117	118-126
B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu	B K Cu
1 2 1 4 1 3 3 1 1	4 3 1 5 2 2 1 3 3	5 2 2 1 4 2 1 7 3 3	5 2 1 4 2 1 7 3 3	3 3 2 7 2 3 1 1 1	3 3 2 7 2 3 1 1 1	3 3 2 7 2 3 1 1 1	3 3 2 7 2 3 1 1 1	3 3 2 7 2 3 1 1 1	3 3 2 7 2 3 1 1 1	2 3 1 6 1 1 2 3 1	2 3 1 6 1 1 2 3 1	2 3 1 6 1 1 2 3 1	2 3 1 6 1 1 2 3 1	2 3 1 6 1 1 2 3 1
1 2 2 2 2 2 7 3 1	6 2 3 5 1 2 4 2 3	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	5 1 2 4 2 3 3 3 2	3 2 1 7 1 3 5 3 2	3 2 1 7 1 3 5 3 2	3 2 1 7 1 3 5 3 2	3 2 1 7 1 3 5 3 2	3 2 1 7 1 3 5 3 2
2 1 1 6 2 3 7 3 3	4 1 3 3 2 1 2 1 1	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	3 2 1 2 1 1 1 3 3	4 1 1 4 1 1 4 1 1	4 1 1 4 1 1 4 1 1	4 1 1 4 1 1 4 1 1	4 1 1 4 1 1 4 1 1	4 1 1 4 1 1 4 1 1
6 3 3 7 1 1 6 1 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	1 1 1 5 1 1 1 3 2	5 3 1 5 3 1 5 3 1	5 3 1 5 3 1 5 3 1	5 3 1 5 3 1 5 3 1	5 3 1 5 3 1 5 3 1	5 3 1 5 3 1 5 3 1
3 2 3 3 3 1 1 1 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	7 1 2 1 1 3 2 2 2	4 3 3 4 3 3 4 3 3	4 3 3 4 3 3 4 3 3	4 3 3 4 3 3 4 3 3	4 3 3 4 3 3 4 3 3	4 3 3 4 3 3 4 3 3
1 3 2 4 3 3 7 1 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	7 2 1 2 3 1 2 2 3	5 1 1 6 1 1 6 1 1	5 1 1 6 1 1 6 1 1	5 1 1 6 1 1 6 1 1	5 1 1 6 1 1 6 1 1	5 1 1 6 1 1 6 1 1
5 1 2 4 2 1 2 2 3	5 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	4 3 2 6 3 2 6 3 2	4 3 2 6 3 2 6 3 2	4 3 2 6 3 2 6 3 2	4 3 2 6 3 2 6 3 2	4 3 2 6 3 2 6 3 2
2 1 3 4 1 2 1 2 1	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	6 2 2 7 3 2 6 1 3	7 1 2 7 1 2 7 1 2 7	7 1 2 7 1 2 7 1 2 7	7 1 2 7 1 2 7 1 2 7	7 1 2 7 1 2 7 1 2 7	7 1 2 7 1 2 7 1 2 7
6 3 1 3 3 2 6 3 2	4 1 1 1 2 3 2 4 1 1	1 2 3 2 4 1 1 1 2 3	2 2 1 3 2 4 1 1 1 2 3	3 3 1 2 3 2 4 1 1 1 2 3	3 3 1 2 3 2 4 1 1 1 2 3	5 2 1 2 3 3 3 1 2 2 1	5 2 1 2 3 3 3 1 2 2 1	5 2 1 2 3 3 3 1 2 2 1	5 2 1 2 3 3 3 1 2 2 1	2 2 1 3 3 2 6 3 2	2 2 1 3 3 2 6 3 2	2 2 1 3 3 2 6 3 2	2 2 1 3 3 2 6 3 2	2 2 1 3 3 2 6 3 2

## 4. MATERIALS AND METHODS

### 4.1 EXPERIMENTAL DESIGNS

The first experiment (tabel 2) was designed to determine the effect of 3 levels of B, Mn, Cu, Zn and Fe on the uptake of macroelements.

The levels comprised:

1. no application,
2. normal application,
3. excess, i.e., five times the normal dose.

Treatment combinations were grouped in 81 units and subdivided into 9 blocks. These blocks were confounded with higher order interactions. All main effects and two factor interactions were not confounded and could therefore be calculated. As  $3^5$  factorial combinations would require 243 units, the present lay out comprises  $\frac{1}{3}$  of a replication (COCHRAN and Cox, 1957). This design was used successively with oats, lucerne and tomato as test crops. It will be shown, that as a result of the first experiment the conclusion was drawn that boron, copper and potassium interactions was one of the problems which required a more extensive investigation.

Therefore, a second experiment (table 3) was laid down, which comprised 2 replications of the factorial combinations of 7 levels of boron, 3 levels of copper and 3 levels of potassium. All treatments were completely randomized in one block. Only lucerne was used as a test crop for the second experiment.

### 4.2 NUTRIENT SOLUTIONS

Nutrient solutions based on 'Long Ashton' formula (HEWITT, 1952), were used for both experiments.

Table 4 shows the salts which were employed together with the concentrations of the microelements corresponding to the various treatments of the first experiment. The concentrations of nutrients of the second experiment are given in tabel 5.

All nutrient solutions were prepared from 'pro analysi' (Merck) grade chemicals and demineralized water of a conductivity of 0,7–0,4 micromhos  $\text{cm}^{-1}$ . Also the quartz-sand was of 'pro analysi' grade (BROCADES).

Sand and water were analysed spectrochemically to check for the presence of impurities. Only very small amounts of microelements were present. Demineralized water (prepared with Dowex 2 and Dowex 50) which was supplied in relative large quantities appeared to contain most of the impurities, i.e. 0.0002 ppm Mn, 0.001 ppm Cu and 0.0001 ppm Fe. The zero level of application of these elements thus received minute amounts of Mn, Cu and Fe.

An analysis of double distilled water from a pyrex still, showed Mn, Cu and Fe levels of the same magnitude as the demineralized water. Consequently there was no reason to use the relative expensive distilled water instead of the cheaper demineralized water.

TABLE 4 Composition of the nutrient solutions for the  $3^5$  factorial experiments with oats, lucerne and tomato

A - constant nutrients							
element Conc.				source Conc.			
K	2	m.e./liter		KNO <sub>3</sub>	2 m.e./liter		
N	10	m.e./liter		Ca(NO <sub>3</sub> ) <sub>2</sub> . 4H <sub>2</sub> O	8 m.e./liter		
Ca	8	m.e./liter					
PO <sub>4</sub>	4	m.e./liter		NaH <sub>2</sub> PO <sub>4</sub> . 2H <sub>2</sub> O	4 m.e./liter		
Na	1.3	m.e./liter		MgSO <sub>4</sub> . 7H <sub>2</sub> O	3 m.e./liter		
Mg	3	m.e./liter		(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> . 4H <sub>2</sub> O			
Mo	0.02	p.p.m.					

b - variable nutrients							
levels				source			
	0	1	5				
B	-	0.5	2.5	p.p.m.	H <sub>3</sub> BO <sub>3</sub>		
Mn	-	0.5	2.5	p.p.m.	MnSO <sub>4</sub> . 4H <sub>2</sub> O		
Cu	-	0.05	0.25	p.p.m.	CuSO <sub>4</sub> . 5H <sub>2</sub> O		
Zn	-	0.1	0.5	p.p.m.	ZnSO <sub>4</sub> . 7H <sub>2</sub> O		
Fe	-	5	25	p.p.m.	FeC <sub>6</sub> H <sub>5</sub> O <sub>7</sub> . 3H <sub>2</sub> O (Ferric citrate)		

TABLE 5 Composition of the nutrient solutions applied to the  $7 \times 3 \times 3$  Boron, Copper, Potassium experiment with lucerne. The salts were the same as in tabel 4, except:

a) for the K<sub>1</sub> level: 1.0 m.e./liter KNO<sub>3</sub> and 1.0 m.e./liter NaNO<sub>3</sub> for compensation of N.  
b) for the K<sub>3</sub> level: 2 m.e./liter from both KNO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub> were added.

A - constant nutrients							
element concentrations							
N			10	m.e./liter			
Ca			8	m.e./liter			
PO <sub>4</sub>			4	m.e./liter			
Na			1.3	m.e./liter			
Mg			3	m.e./liter			
Mo			0.02	p.p.m.			
Mn			0.5	p.p.m.			
Zn			0.1	p.p.m.			
Fe			5.0	p.p.m.			

B - variable nutrients							
levels							
	1	2	3	4	5	6	7
B	0.0	0.01	0.05	0.5	1.0	2.5	5.0
Cu	0.0	0.05	0.5				p.p.m.
K	1.0	2.0	4.0				m.e./L



PLATE 1 Model of the Polyaethylene pots and bottles, used for the experiments. From left to right: pot before planting, bottle containing the nutrient solution, pot after planting

### 4.3 EXPERIMENTAL TECHNIQUE

Polyaethylene pots of 11 cm diameter and a height of 7 cm, were filled with 400 gr. of purified quartz sand. A glass tube of 1 cm diameter and a height of 7 cm was stuck in the centre of the pot. The demineralized water and culture solution were stored in polyaethylene flasks. Approximately 50 seeds of the test crops were sown in each pot. The moisture content of the sand was kept at 60 % of its water holding capacity.

All pots received 20 ml of culture solution containing the elements which were not included in the experimental treatments. During the germination period water losses due to evaporation were compensated with demineralized water.

The actual experiment started one to two weeks after sowing, when the pots were placed in a green house and received culture solutions corresponding to the experimental treatments. Each pot was kept at a constant moisture content by means of daily additions of culture solutions. A total quantity of 250 ml was supplied to each pot during the course of the experiment.

At the final stage of the experiment the plants only received demineralized water during approximately two weeks. At the end of the experiment hardly any salt was left in the sand cultures as was controlled by conductivity measurements.

The duration of the germination and treatment periods for both experiments have been tabulated in chronological order in tabel 6. Plate 1 demonstrates the types of containers and flasks that were used for the experiment.

TABLE 6 Chronological data of the experiments.

During the 1st stage of growth the plants received demineralized water plus 20 ml nutrient solution containing the constant components only.

During the 2nd stage of growth the plants received 250 ml nutrient solutions according to the corresponding treatments.

During the 3rd stage of growth the plants received demineralized water only.

year	indicator plant	sowing date	1st stage		2nd stage		3rd stage		harvesting	age in weeks
			from	to	from	to	from	to		
1959	oats	7/4	8/4	16/4	17/4	26/4	27/4	10/5	11/5	5
	lucerne	24/6	25/6	9/7	10/7	7/8	8/8	26/8	27/8	9
	tomato	17/8	18/8	31/8	1/9	25/9	26/9	5/10	6/10	7
1960	lucerne	8/5	9/5	18/5	19/5	30/5	31/5	12/6	13/6	5

It is evident that the duration of an experiment whereby a fixed volume of nutrient solution is supplied to a growing crop, whereby the moisture content of the sand is kept constant, will depend on the intensity of the evapotranspiration of the pots, i.e. on the growth rate of the crop, the temperature and the relative humidity in the green-house. This explains why the experiment with oats, lucerne and tomato lasted resp. 5, 9 and 7 weeks.

#### 4.4 SAMPLING AND CHEMICAL ANALYSIS

The shoots of the plants were removed and dried at 70 °C and the total dry matter production per pot was determined. The dry samples were ground and stored in stoppered plastic containers. Subsamples were taken for the analysis of nitrogen, phosphorus, potassium, calcium, magnesium and sodium (SCHUFFELEN et al. 1961).

##### CHEMICAL METHODS

The dry material was digested with concentrated sulphuric acid and hydrogen peroxide.

0,3 g of the dry material was transferred to a 50 ml 'Termax' calibrated flask, together with 3 ml  $H_2SO_4$  conc. and gently heated on a hot plate. As soon as the mixture started to foam, 1 to 2 drops  $H_2O_2$  (30 %) were added. After 10–15 minutes, when the fuming had stopped, 5–10 drops of  $H_2O_2$  were added in order to obtain a colourless extract. The extracts were then heated for another 10–15 minutes at 300 °C. After cooling, the extract was made up with distilled water to a volume of 50 ml. Aliquots from this extract were taken for the chemical analysis of N, P, K, Ca, Mg and Na.

##### Nitrogen

Nitrogen was determined according to a modification of the micro-Kjeldahl method. After a steam distillation, ammonia was taken up in 10 ml 1 % boric acid solution and titrated with 0.01 N potassium bi-iodate, using a mixed indicator of bromocresol-green and methylred in aethanol (0.15 g bromocresolgreen + 0.1 g methylred in 200 ml ethanol).

##### Phosphorus

Phosphate was determined colorimetrically. A 0.5–1 ml aliquot of the extract was transferred to a test-tube and diluted to 4 ml with distilled water. 1–2 ml reducing agent (0.5 g metol (p-methylaminophenol sulphate) + 2.5 g sodium sulphite + 75 g sodium-bi-sulphite in one liter aqua dest.) were added. After shaking, 1.0 ml ammonium molybdate-sulphuric acid solution (25 g ammonium molybdate + 250 ml 10 N  $H_2SO_4$ , made up to one liter with aqua dest.) was added and the content of the tube was well mixed. After 20 minutes, 2 ml of sodium acetate solution (340 g/L) was added. As the maximum colour development was attained, the blue colour was measured at a wavelength of 600  $\mu$ , using a 'Beckman' spectro-photometer. A calibration graph was prepared from standard solutions of known phosphate concentrations, including a blank.

##### Magnesium

Magnesium was determined colorimetrically. 0.5–1 ml aliquot was transferred to a test tube and diluted to 2 ml with 4 %  $H_2SO_4$ . 1.5 ml of a mixture containing sodium carboxymethylcellulose and titan-yellow were added (75 g glycerine + 25 ml 0.5 % carbocel + 40 ml 0.1 % titan-yellow in aqua dest. + 8 ml 200 ppm Mn in Morgan solution + 2 ml 200 ppm Mg in Morgan solution). The components of the mixture

were kept separately and mixed prior to the determination of Mg. After shaking, 1.0 ml 10 N KCN alkali solution (40 g NaOH + 5 g KCN in 100 ml aqua dest.) is added. In order to obtain clear mixtures, the KCN-alkali solution was filtered before use. The test tubes were kept in the dark during one hour. The orange-red colour was measured by means of the 'Beckman' spectro-photometer at a wavelength of 540  $\mu$ . A Magnesium standard serie in 4%  $H_2SO_4$  solution was used for the preparation of a calibrated graph.

#### Calcium, Potassium and Sodium

Ca, K and Na were determined with a flame-photometer. For calcium 1.0 ml aliquot was pipetted into a test tube and made up to 10 ml with  $MgCl_2$  in 0.4%  $H_2SO_4$  solution.  $MgCl_2$  is added to eliminate the effect of the interfering sulphate and phosphate.

For the potassium and sodium determinations, a 1.0 ml aliquot was made up to 10 ml with dist. water. The standard serie was prepared in 10 ml 0.4%  $H_2SO_4$  solution.

## 5. RESULTS OF THE B, Mn, Cu, Zn AND Fe EXPERIMENTS

### 5.1 VISIBLE SYMPTOMS

Although some abnormal colour development in the leaves could be observed, it was impossible to classify the symptoms. This may be due to the fact that in a factorial experiment, the effect of one element may mask that of another. In general, it may be expected that the small quantities of microelements which were present in the seeds, in the chemicals and in the demineralized water provided an adequate supply to the young plants.

Toxicity symptoms were found in the case of high boron applications (2.5 ppm B) to lucerne. At the early stages, the leaf edges showed a white margin which gradually extended and became scorched when time progressed. The centre of the leaves remained green. The various stages of toxicity symptoms in lucerne leaves are demonstrated in plate 2 pictures 1, 2, 3 and 4.

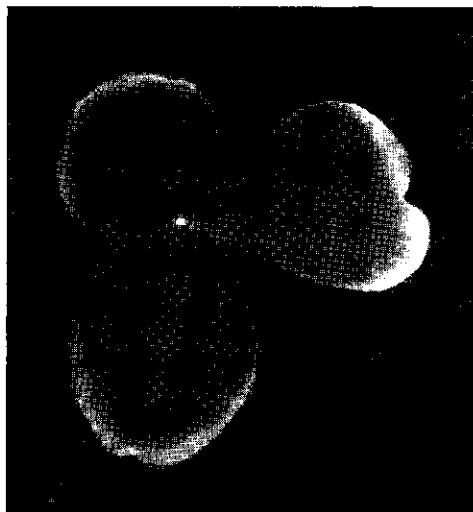
### 5.2 SIGNIFICANT TREATMENT EFFECTS

A consideration of all treatment effects, irrespective of their statistical significance would entail lengthy discussions, which might obscure the importance of significant effects. As a criterion for the importance of effects, statistical significance of the variances were compared. Only the variances which attained significance will be discussed. This procedure reduced the discussion of treatment effects considerably. It should be stressed however that the insignificance of a particular variance may not be taken as a proof that the effect does not exist. Further experimentation would be required to investigate whether these insignificant effects are of no importance for conditions other than those of the present experiment.

Prior to discussing the nature of the treatment effects, an account will therefore be given of the effects which attained statistical significance. For the calculation of the significance of treatment effects the 80 degrees of freedom were allocated in the following way:

<i>effects</i>	<i>degrees of freedom</i>
block . . . . .	8
main effects . . . . .	10
2 factor interactions . . . . .	38
higher order interactions (error) . . . . .	24

All main effects and two factor interactions may be estimated with the exception of the first order interaction between boron and iron, which was confounded with the 9 blocks of 9 units (COCHRAN and Cox, 1957). The results of the analysis of variance of the dry matter production and the uptake of macroelements, have been given in terms of the F test of significance, (tables 7, 8 and 9).



1



2



3



4

PLATE 2 Progressive stages of boron toxicity symptoms in lucerne plants

TABLE 7 Results of the analysis of variance of the ion-uptake and yield of oats as affected by micro-elements

a = nearly significant at 5% level ( $P = < 0.05$ )  
 b = significant at 5% level ( $P = < 0.05$ )  
 c = nearly significant at 1% level ( $P = < 0.01$ )  
 d = significant at 1% level ( $P = < 0.01$ )

N.B. These symbols are valid for the following tables

factors	variance ratios (F values)						
	N	P	K	Ca	Mg	Na	yield
B	3.20 <sup>b</sup>	5.00 <sup>c</sup>	< 1	< 1	2.80 <sup>a</sup>	2.06	3.09 <sup>a</sup>
Mn	1.91	< 1	2.78 <sup>a</sup>	< 1	1.65	< 1	2.95 <sup>a</sup>
Cu	< 1	< 1	< 1	1.54	< 1	< 1	< 1
Zn	3.70 <sup>b</sup>	3.32 <sup>b</sup>	2.49	3.20 <sup>b</sup>	5.20 <sup>d</sup>	2.96 <sup>a</sup>	4.02 <sup>b</sup>
Fe	1.26	3.68 <sup>b</sup>	< 1	< 1	2.1	< 1	2.16
V.C.	8.25%	7.82%	6.22%	21.69%	11.86%	19.04%	10.77%

TABLE 8 Results of the analysis of variance of the ion-uptake and yield of lucerne as affected by microelements

factors	variance ratios (F values)						
	N	P	K	Ca	Mg	Na	yield
B	1.20	< 1	5.61 <sup>d</sup>	3.04 <sup>a</sup>	< 1	1.17	2.02
Mn	1.46	< 1	< 1	< 1	< 1	< 1	1.17
Cu	1.82	< 1	2.95 <sup>a</sup>	< 1	< 1	< 1	< 1
Zn	< 1	1.40	< 1	< 1	< 1	1.19	< 1
Fe	< 1	11.87 <sup>d</sup>	< 1	4.80 <sup>b</sup>	< 1	< 1	< 1
B × Mn	< 1	< 1	< 1	< 1	< 1	< 1	< 1
B × Cu	< 1	< 1	2.80 <sup>b</sup>	< 1	1.55	< 1	< 1
B × Zn	< 1	1.96	3.41 <sup>b</sup>	< 1	2.56	< 1	1.96
B × Fe	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Mn × Cu	< 1	< 1	< 1	1.90	1.66	< 1	< 1
Mn × Zn	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Mn × Fe	1.88	< 1	< 1	2.69 <sup>a</sup>	3.61 <sup>b</sup>	< 1	2.32
Cu × Zn	< 1	1.66	1.24	< 1	< 1	1.43	1.03
Cu × Fe	< 1	< 1	< 1	< 1	< 1	< 1	1.82
Zn × Fe	< 1	1.08	< 1	1.75	2.80 <sup>b</sup>	1.62	< 1
V.C.	8.10%	11.94%	7.86%	7.60%	11.22%	29.41%	7.01%

F represents the ratio of the variance of the factor to be tested and the error variance. Table 7 compares the F values of the main effects of B, Mn, Cu, Zn and Fe on the uptake of N, P, K, Ca, Mg, Na and the dry matter production of oats. Two factor interactions have not been included in the table as none of them attained significance at  $P = 0.05$ .

TABLE 9 Results of the analysis of variance of the ion-uptake and yield of tomatoes as affected by microelements

factors	variance ratios (F values)						
	N	P	K	Ca	Mg	Na	yield
B	< 1	< 1	1.25	< 1	1.01	2.33	3.95 <sup>b</sup>
Mn	< 1	< 1	< 1	1.14	1.19	< 1	1.22
Cu	< 1	< 1	< 1	1.32	< 1	< 1	< 1
Zn	< 1	< 1	1.25	< 1	< 1	< 1	< 1
Fe	1.68	36.80 <sup>d</sup>	< 1	< 1	< 1	1.77	4.52 <sup>b</sup>
B × Mn	1.08	2.01	< 1	< 1	1.15	1.75	1.42
B × Cu	< 1	< 1	< 1	< 1	< 1	< 1	< 1
B × Zn	< 1	< 1	< 1	< 1	< 1	1.95	< 1
B × Fe	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Mn × Cu	1.05	1.74	1.01	1.07	1.04	1.01	< 1
Mn × Zn	< 1	1.88	< 1	1.49	< 1	< 1	< 1
Mn × Fe	1.23	1.24	< 1	< 1	2.74 <sup>b</sup>	3.30 <sup>b</sup>	3.10 <sup>b</sup>
Cu × Zn	< 1	1.46	1.54	< 1	< 1	< 1	< 1
Cu × Fe	1.29	< 1	< 1	< 1	< 1	< 1	< 1
Zn × Fe	1.21	3.25 <sup>b</sup>	< 1	1.83	< 1	< 1	1.26
V.C.	7.45%	6.82%	6.01%	7.21%	10.52%	10.52%	8.27%

Tables 8 and 9 give the F test of significance for tomato and lucerne. It may be seen that for both tomato and lucerne the majority of the 2 factor interactions was insignificant with respect to ion-uptake and yield.

The data of table 7, 8 and 9 have been summarized in table 10, which shows the significant main effects and interactions for oats, lucerne and tomato.

#### YIELD

The dry matter production of oats was significantly affected by Zn; none of the other microelements had any effect on the dry matter production.

In the case of lucerne, yield was not affected at all by any of the treatments. The dry matter production of tomatoes was significantly altered due to boron and iron applications.

The interaction between microelements had no effect on the yield of any of the crops, with the exception of the Mn × Fe interaction in the tomatoes.

#### NITROGEN

The nitrogen uptake was generally not significantly altered by application of micro-elements. Only boron and zinc had a significant effect on the nitrogen uptake of oats.

#### PHOSPHORUS

The phosphorus uptake was changed due to applications of iron for all plant species. With tomato, it was also affected by the interaction of iron and zinc. Boron had an effect on the uptake of phosphate by oats.

TABLE 10 Statistically significant main effects (M.E.) and interactions of micronutrients on the ion-uptake and yield of oats, lucerne and tomatoes

factors uptake	B					Mn				Cu			Zn		Fe	
	M.E.	Mn	Cu	Zn	Fe	M.E.	Cu	Zn	Fe	M.E.	Zn	Fe	M.E.	Fe	M.E.	
1 oats																
N	b										b					
P	b										b					b
K																
Ca											b					
Mg											d					
Na											b					
yield																
2 lucerne																
N																
P																d
K	d	b	b													b
Ca																
Mg										b						
Na												b				
yield																
3 tomatoes																
N																
P																
K																
Ca																
Mg										b						
Na										b						
yield	b									b						b

#### POTASSIUM

The potassium uptake by oats and tomatoes was not affected by any of the micro-element applications. With regard to lucerne it was observed that boron had a highly significant effect on the potassium uptake and also the interactions of boron with copper and zinc were significant.

#### CALCIUM

The calcium uptake by oats was changed by the zinc treatment. In the case of lucerne, the calcium uptake was affected by applications of iron. No effect on the calcium uptake was found for tomatoes.

#### MAGNESIUM

The magnesium uptake by oats was influenced by applications of zinc. In the case of lucerne and tomatoes, the main effects were all insignificant. Although the  $Mn \times Fe$  interaction was significant  $P = 0.05$  for both lucerne and tomatoes this interaction would seem of little importance because the main effects of these elements were insignificant. The same may be said for the significant  $Zn \times Fe$  interactions in the case of lucerne.

#### SODIUM

Apart from the  $Mn \times Fe$  interaction on the sodium uptake by tomatoes, no significant effects of microelements were observed.

The results of the analysis of variance have reduced the treatments to be considered, to a small number. It is obvious that only significant effects need investigation in greater detail. On the other hand the conclusion may not be drawn that all effects which did not attain statistical significance will be of no importance. From the tables of F values it may be observed that some treatments just failed to attain significance at  $P = 0.05$ , for instance the main effects of B on the Mg uptake and yield of oats and on the Ca uptake of lucerne. Similarly manganese had an effect on the K uptake and yield of oats; zinc had an effect on Na uptake of oats and Cu had an effect on the K uptake of lucerne.

As the present experiment is intended as a pilot trial, to indicate the most important effects, in the following discussion no attention will be paid to effects which were not significant. A more definite statement with regard to effects which just failed to attain significance can only be made after further extensive experimentation.

#### 5.2.1 DRY MATTER PRODUCTION

The main effects of the various levels of micronutrients on the dry matter production of the three plant species have been tabulated in table 11.

Boron reduced the yield of tomato significantly at the highest level. In the case of oats there was a slight increase in dry matter production at the normal level of 0.5 ppm B as compared with the absence of boron. When boron was in excess, the dry matter production was equal to the production when B was absent. These effects were nearly significant at  $P = 0.05$ .

Manganese application seem to reduce the dry matter production of oats, but the effect just failed to attain significance. The dry matter production of oats was significantly increased by the normal application of zinc, but dropped at the highest level of zinc application.

Iron had a significant effect on the yield of tomatoes; even the highest application of iron resulted in a further increase of the dry matter production.

The effect of the  $Fe \times Mn$  interaction on the dry matter production of tomatoes was significant. This interaction is shown by table 12.

Increasing application of iron only increase the dry matter production when manganese is absent or when it is present as an excess. At normal levels of manganese, no remarkable effect of iron on the dry matter production is found.

TABLE 11 The main effects of micronutrients on yield (g dry matter/pot)

elements	levels	tomato	lucerne	oats
B	0	0.95 <sup>b</sup>	1.4	1.81 <sup>a</sup>
	1	0.93	1.4	1.93
	5	0.90	1.4	1.81
Mn	0	0.94	1.4	1.91 <sup>a</sup>
	1	0.93	1.4	1.85
	5	0.91	1.4	1.78
Cu	0	0.92	1.4	1.85
	1	0.93	1.4	1.85
	5	0.93	1.4	1.85
Zn	0	0.92	1.4	1.87 <sup>b</sup>
	1	0.93	1.4	1.91
	5	0.93	1.4	1.76
Fe	0	0.89 <sup>b</sup>	1.4	1.80
	1	0.93	1.4	1.83
	5	0.96	1.4	1.91

TABLE 12 Mean tomato yield (g dry matter/pot)

Mn	Fe	levels		
		0	1	5
0	0.86	0.96	0.99	
1	0.95	0.93	0.92	
5	0.87	0.89	0.96	

### 5.2.2 THE UPTAKE OF MACROELEMENTS

For a comparison of the uptake of macroelements as affected by the experimental treatments, the data may be expressed in two different ways. For diagnostic purpose the contents of macroelements are usually expressed as percentages of dry matter. It is well known however that these contents will change as a function of the stage of development of the crop, i.e. will be related to the quantity of dry matter which has been produced at the time that the samples were taken.

It has also been observed that crops which are deficient of a particular element will reduce their growth to such an extent that the plant content of the deficient element remains approximately constant. STEENBJERG (1950; 1951; 1952; 1954) had demonstrated that small applications of copper to extreme Cu-deficient plants reduced the content of copper even further as the result of the diluting effect of the rapid growth after the copper application had been made. Therefore the possibility may not be excluded that

relatively low contents of elements in the shoots are found in the case of relatively rapid growth and high dry matter production. The absolute quantity of element which has been taken up, expressed in mg/pot, may still be higher than in the case where a high content coincided with a low dry matter production.

For the macroelements in oats, as an example, the correlation coefficients between the dry matter production and the two kinds of uptake data were calculated (table 13). It

TABLE 13 Correlation between:

- a) uptake as absolute values and oats yield,
- b) uptake as relative values (%) and oats yield.

The correlation coefficients were computed from the error line of the multiple covariance analysis

element	absolute values	relative values
N	+0.8618 <sup>d</sup>	-0.6582 <sup>a</sup>
P	+0.8039 <sup>d</sup>	-0.6944 <sup>d</sup>
K	+0.3423 <sup>b</sup>	-0.8285 <sup>a</sup>
Ca	+0.6440 <sup>d</sup>	+0.1887
Mg	+0.8472 <sup>d</sup>	-0.1143
Na	-0.0300	-0.5123 <sup>d</sup>

is evident from this table that the elements expressed as percentages of the dry matter content are negatively correlated with the dry matter production. On the other hand, the absolute uptake expressed in mg/pot is positively correlated with the yield. This would seem to suggest that the interpretation of uptake experiments would be complicated in the case that treatment effects would have induced considerable differences in growth, because the two methods of expression would result in entirely different conclusions. For the present experiment this difficulty fortunately does not exist as the effect of the various treatments on growth was very small, even in those cases where it reached statistical significance. This may be illustrated by table 14, where both methods of expression have been compared for the uptake of macroelements by oats. It may be seen that generally the trends in macroelement uptake as a function of the level of microelement application were similar for both methods of expression, but still there are few variations. For the presentation of the results of the uptake experiments, all data have been expressed in mg/pot, in order to have a stable base for comparisons.

#### 5.2.2.1 The uptake of nitrogen

The nitrogen uptake of tomatoes, lucerne and oats was little effected by the microelement applications as is demonstrated by table 15. In this table the main effects of the treatments have been given.

The nitrogen-uptake of oats was changed by applications of boron and zinc. Both elements applied at the normal level, increased the uptake of nitrogen. The excess dose of boron did not alter the nitrogen uptake as compared with the normal dose.

TABLE 14 Mineral contents of oats as relative (%) and absolute (mg/pot) values

elements	levels	N		P		K		Ca		Mg		Na	
		%	mg/pot	%	mg/pot	%	mg/pot	%	mg/pot	%	mg/pot	%	yield g/pot
B	0	1.84 <sup>a</sup>	33.3 <sup>b</sup>	0.35 <sup>d</sup>	6.4 <sup>c</sup>	1.07	19.3	0.33	5.9	0.125 <sup>d</sup>	2.26 <sup>b</sup>	0.58	10.4
	1	1.82	35.2	0.35	6.8	1.02	19.6	0.33	6.4	0.124	2.40	0.55	10.5
	5	1.93	35.0	0.37	6.7	1.07	19.4	0.33	6.0	0.134	2.43	0.62	11.2
Mn	0	1.84 <sup>a</sup>	35.2	0.35 <sup>d</sup>	6.6	1.02	19.5 <sup>a</sup>	0.31	6.0	0.127 <sup>d</sup>	2.43	0.54	10.4
	1	1.85	34.3	0.35	6.5	1.06	19.7	0.33	6.2	0.124	2.29	0.59	10.9
	5	1.91	33.9	0.38	6.7	1.07	19.0	0.34	6.1	0.133	2.37	0.61	10.8
Cu	0	1.86	34.5	0.35	6.5	1.05	19.4	0.31	5.8	0.129	2.38	0.58	10.7
	1	1.85	34.2	0.36	6.6	1.04	19.3	0.35	6.4	0.127	2.36	0.59	10.8
	5	1.89	34.9	0.36	6.7	1.06	19.7	0.33	6.1	0.127	2.36	0.58	10.7
Zn	0	1.84	34.5 <sup>b</sup>	0.35 <sup>b</sup>	6.5 <sup>b</sup>	1.02 <sup>b</sup>	19.1	0.34	6.3 <sup>b</sup>	0.128	2.40 <sup>d</sup>	0.55 <sup>d</sup>	10.3 <sup>a</sup>
	1	1.85	35.4	0.37	6.8	1.03	19.7	0.34	6.4	0.128	2.45	0.54	10.4
	5	1.90	33.5	0.37	6.5	1.10	19.3	0.32	5.6	0.127	2.24	0.65	11.4
Fe	0	1.89	34.1	0.38 <sup>d</sup>	6.8 <sup>b</sup>	1.07	19.3	0.35	6.3	0.132 <sup>d</sup>	2.37	0.59	10.7
	1	1.86	34.1	0.36	6.7	1.05	19.3	0.32	5.9	0.132	2.42	0.59	10.8
	5	1.84	35.2	0.33	6.4	1.03	19.6	0.32	6.1	0.120	2.29	0.58	10.6

TABLE 15 The average uptake of nitrogen as affected by different levels of micronutrients

elements	levels	absolute uptake mg N/pot		
		tomato	lucerne	oats
B	0	35.3	30.9	33.3 <sup>b</sup>
	1	35.0	30.8	35.2
	5	35.0	31.8	35.0
Mn	0	35.5	31.6	35.2
	1	35.2	31.4	34.3
	5	34.6	30.5	33.9
Cu	0	35.1	30.8	34.5
	1	34.7	30.8	34.2
	5	35.5	31.9	34.9
Zn	0	34.7	30.6	34.5 <sup>b</sup>
	1	35.0	31.4	35.4
	5	35.6	31.5	33.5
Fe	0	34.4	31.5	34.1
	1	35.3	31.1	34.1
	5	35.6	30.9	35.2

TABLE 16 The effect of the Fe × Zn interaction on the uptake of P (mg/pot) by tomatoes

Zn	Fe	levels		
		0	1	5
0	0	6.9	6.7	6.4
	1	7.3	7.1	6.0
	5	7.2	7.3	6.0

An excess of zinc reduced the nitrogen uptake significantly. All interactions between the microelements were insignificant with regard to nitrogen uptake.

### 5.2.2.2 The uptake of phosphorus

The uptake of phosphorus was highly significantly reduced by excess applications of iron with regard to all plant species. The normal level of iron results in slight decreases in phosphate uptake, but excess levels of iron reduces the P uptake to a larger extent. Interactions between iron and other microelement were insignificant with the exception of the Fe × Zn interaction in case of tomatoes (table 16).

It is evident that the reduction in phosphate uptake induced by the excess applications of iron, becomes more pronounced at higher levels of zinc. When iron is deficient or at the optimal level, zinc applications promote the uptake of phosphorus.

TABEL 17 The average uptake of phosphorus as affected by different concentrations of micronutrients

elements	levels	absolute uptake mg P/pot		
		tomato	lucerne	oats
B	0	6.8	4.8	6.4 <sup>c</sup>
	1	6.7	4.8	6.8
	5	6.8	4.8	6.7
Mn	0	6.8	4.8	6.6
	1	6.7	4.9	6.5
	5	6.8	4.8	6.7
Cu	0	6.8	4.9	6.5
	1	6.7	4.8	6.6
	5	6.8	4.9	6.7
Zn	0	6.7	4.9	6.5 <sup>b</sup>
	1	6.8	4.7	6.8
	5	6.8	4.9	6.5
Fe	0	7.1 <sup>d</sup>	5.2 <sup>d</sup>	6.8 <sup>b</sup>
	1	7.0	4.9	6.7
	5	6.1	4.4	6.4

The effect of elements other than iron was very small and only significant for boron and zinc on the uptake of phosphate by oats. Table 17 shows that normal and high levels of boron increase the uptake of phosphate. Zinc applied at a normal level also increases the phosphate uptake of oats, as compared with the lower and higher levels. None of the interactions of microelements with boron and zinc were significant.

#### 5.2.2.3 The uptake of Potassium

The potassium uptake was only significantly affected by microelement applications in the case of lucerne. Table 18 shows the effect of microelements on the potassium uptake of the three plant species. It appears that the uptake of potassium of lucerne is significantly altered by boron. Both low and high levels of boron increase the potassium uptake.

The interaction between boron and copper is demonstrated in table 19.

This interaction is significant at  $P = 0.05$  and indicates that a high application of boron at low and normal levels of copper increases the uptake of potassium; at high levels of copper, both low and high boron applications also increases the potassium uptake. Table 20, illustrates the significant interaction effect of boron and zinc on the potassium uptake by lucerne plants.

TABLE 18 The average uptake of potassium as affected by different concentrations of micronutrients

elements	levels	absolute uptake mg K/pot		
		tomato	lucerne	oats
B	0	19.0	16.0 <sup>d</sup>	19.3
	1	18.9	15.2	19.6
	5	19.4	16.4	19.4
Mn	0	19.1	16.0	19.5 <sup>a</sup>
	1	19.2	15.7	19.7
	5	18.9	15.9	19.0
Cu	0	19.0	15.7 <sup>a</sup>	19.4
	1	19.2	15.5	19.3
	5	19.1	16.3	19.7
Zn	0	19.4	15.7	19.1
	1	18.9	16.1	19.7
	5	19.0	15.8	19.3
Fe	0	19.1	15.8	19.3
	1	19.1	15.9	19.3
	5	19.2	15.9	19.6

TABLE 19 B × Cu interaction and K-uptake (mg/pot) by lucerne

B	Cu	levels		
		0	1	5
0	15.2	15.5	17.5	
1	15.3	15.1	15.4	
5	16.7	16.0	16.3	

TABLE 20 B × Zn interaction and K-uptake (mg/pot) by lucerne

B	Zn	levels		
		0	1	5
0	15.3	17.3	15.5	
1	15.4	14.9	15.4	
5	16.4	16.1	16.6	

It is obvious that at low and high levels of zinc, the uptake of potassium is increased at increasing boron applications. At the normal levels of zinc, low and high applications of boron increase the uptake values of potassium.

TABLE 21 The average uptake of calcium as affected by different concentrations of micronutrients

elements	levels	absolute uptake mg Ca/pot		
		tomato	lucerne	oats
B	0	24.8	17.0 <sup>a</sup>	5.9
	1	24.7	17.3	6.4
	5	24.4	16.5	6.0
Mn	0	25.0	16.8	6.0
	1	24.5	17.1	6.2
	5	24.4	16.9	6.1
Cu	0	25.0	17.2	5.8
	1	24.3	16.7	6.4
	5	24.6	16.9	6.1
Zn	0	24.6	16.7	6.3 <sup>b</sup>
	1	24.8	16.9	6.4
	5	24.5	17.2	5.6
Fe	0	24.8	17.5 <sup>b</sup>	6.3
	1	24.6	16.9	5.9
	5	24.5	16.4	6.1

#### 5.2.2.4 The uptake of calcium

The calcium uptake is only significantly changed by zinc and iron for oats and lucerne respectively. Interactions with any of these microelements were not observed.

As it is seen from table 21, the application of excess zinc markedly reduces the Ca uptake of oats. With lucerne plants the uptake of calcium when iron is absent, is high and is reduced by the normal dose of iron. Excess of iron results in a further decrease of the calcium uptake.

#### 5.2.2.5 The uptake of magnesium

Zinc had a significant effect on the uptake of magnesium of oats. No effect was found between the low and the normal level of zinc; excess zinc resulted in a highly significant decrease in magnesium uptake. No important main effects of any of the other microelements on the uptake of magnesium was observed (table 22).

As a matter of fact, interaction effects of manganese and iron on the uptake of magnesium by tomato and lucerne, as well as the Zn  $\times$  Fe interaction on the uptake of magnesium by lucerne were statistically significant. As the main effects of these elements are insignificant, these interactions could not be fairly interpreted. Further experimentation would be required in this case.

TABLE 22 The average uptake of magnesium as affected by different concentrations of micro-nutrients

elements	levels	absolute uptake mg Mg/pot		
		tomato	lucerne	oats
B	0	4.6	2.8	2.3 <sup>a</sup>
	1	4.5	2.8	2.4
	5	4.4	2.7	2.4
Mn	0	4.6	2.8	2.4
	1	4.6	2.7	2.3
	5	4.4	2.8	2.4
Cu	0	4.5	2.8	2.4
	1	4.6	2.8	2.4
	5	4.5	2.8	2.4
Zn	0	4.5	2.7	2.4 <sup>d</sup>
	1	4.5	2.8	2.4
	5	4.6	2.8	2.2
Fe	0	4.5	2.8	2.4
	1	4.6	2.8	2.4
	5	4.6	2.7	2.3

#### 5.2.2.6 The uptake of sodium

No significant main effect of any of the microelements was found (table 23) for any of the plant species. In the absence of significant main effects of manganese and iron on the sodium uptake by tomato, the significant Mn  $\times$  Fe interaction would not seem to be of great importance.

### 5.3 DISCUSSION AND CONCLUSION OF THE RESULTS OF THE B, Mn, Cu, Zn, AND Fe FACTORIAL EXPERIMENTS

The results of these experiments have indicated in which cases microelements have an effect on the macroelements. A great number of effects has been investigated and only in few cases could a significant change in macroelement uptake be demonstrated. Generally it may be concluded that the effect of microelements on the uptake of macroelements is small, even in the case where significant differences were obtained. The nature of the effects is not similar for all plant species which were used as test crops. In the case of oats, zinc had a pronounced effect on the yield, N, P, Ca and Mg uptake. Boron influenced the uptake of N and P of oats. The effect of zinc was not found for lucerne and tomatoes, it might be restricted to monocotyledons only. Boron on the other hand had a significant effect on all plant species, particularly on the K uptake of lucerne.

TABLE 23 The average uptake of sodium as affected by different concentrations of micronutrients

elements	levels	absolute uptake mg Na/pot		
		tomato	lucerne	oats
B	0	9.6	1.7	10.4
	1	9.4	1.6	10.5
	5	9.1	1.5	11.2
Mn	0	9.3	1.6	10.4
	1	9.5	1.7	10.9
	5	9.3	1.5	10.8
Cu	0	9.3	1.7	10.7
	1	9.3	1.6	10.8
	5	9.5	1.6	10.7
Zn	0	9.3	1.5	10.3 <sup>a</sup>
	1	9.3	1.7	10.4
	5	9.5	1.6	11.4
Fe	0	9.1	1.6	10.7
	1	9.6	1.6	10.8
	5	9.4	1.6	10.6

Iron was related to the uptake of phosphate in all tested plant species and had an effect on the yield of tomatoes.

It is striking that interactions between microelements occurred very little, even when significant main effects could be observed. This would seem to indicate that the effect of microelements on the uptake of macroelements is very specific. The absence or presence of other microelements have only small effects.

These experiments have served their purpose in a way, that a few effects have emerged which require further investigations.

These problems are:

1. the effect of Zn in the case of oats,
2. the effect of iron in the case of all plant species,
3. the effect of boron in the case of lucerne.

## 6. CHOICE OF THE SECOND EXPERIMENT

From the foregoing discussions, it was concluded that three problems would merit further investigation. As the time for experimentation was limited, a choice had to be made with regard to the problem that should be investigated in greater detail.

The effect of zinc on yield and ion uptake by oats is a very interesting problem particularly from a plant physiological point of view. In practice soil applications of zinc are not generally made, since these are very little effective in comparison to spray applications. It is considered that this problem could be investigated in detail at a later stage.

The effect of iron on phosphate uptake was observed in all plant species that were used in the present investigation. This is in agreement with the observations that have been frequently reported in the literature. Whether the effect of iron on the phosphorus uptake is of a chemical or physiological nature, is not yet known. It is known that the transport of iron from roots to shoots depends on the phosphorus status of the plant. Especially translocation studies have to be done in regard to this phenomenon. This subject would be also very interesting for future detailed investigations.

The effect of boron on the potassium uptake of lucerne seems to be an interesting problem. The first experiment showed that low and high boron levels induce a higher potassium uptake than the medium levels of boron. REEVE and SHIVE (1944) and VAN DER PAAUW (1954) observed that boron deficiency and boron toxicity is related to the level of potassium in the plant.

JACOBS & WHITE-STEVENS (1941) reported that high boron concentrations decrease the toxic effect of excess potassium in the case of melons.

JORET and MALTERRE (1937) found that the potassium content of wheat decreases when boron is applied at a high level. In 1939 these workers found the opposite in the case of sugar beets. MERILL et al (1955) observed that boron applications reduce the potassium content of the leaves of tungs (*Alniritus Montana*).

As opposed to the observations which were made by REEVE and SHIVE (1944), SARU (1933) found that the effect of boron deficiency was alleviated when potassium was applied at the high level.

Apart from the effect of potassium on the incidence of the boron deficiency or boron toxicity which is a controversial matter, the changes in boron content cannot be interpreted easily.

REEVE and SHIVE (1944) found an increase in boron content when potassium was given at high rate. CHAPMAN et al. (1939) and WALLACE and BEAR (1949) reported a decrease of the boron content when the potassium status of the environment was increased. From the work which was carried out by VAN DER PAAUW (1954) it appeared that the boron content of sugar beets was only little influenced by the potassium status of the soil.

Very few data have been reported on the effect of boron on the uptake of macro-elements by plants. MUHR (1942) investigated the effect of boron on the chemical

composition of, various crops. He concluded that an insufficient supply of boron resulted in an increase of the N, Ca, Mg and Fe contents. It was found that the effect of boron on potassium uptake was relatively small. Similar work was done by PARKS et al. (1944) who stated: 'the fact that differences in boron supply have resulted in differences in plant composition with respect to nearly all the elements examined suggests that boron may be a component of one or more interactions, or that complex interactions involving more than two elements may exist.'

This short statement suggests that there seems to be a relation between boron and potassium although the nature of this relation is not very clear. It was therefore decided to investigate the nature of the boron potassium relationships in greater detail.

The first experiment showed that the relation between potassium and boron was highly significant. The technique appeared suitable for this kind of investigation and could therefore be applied to a second experiment.

The results of the first experiment showed that besides main effects of B, interactions of  $B \times Cu$  and  $B \times Zn$  were significant at  $P = 0.05$ . It was therefore decided to include copper as a treatment in the second experiment. This experiment was carried out in the spring of 1960. The factorial combinations of 7 levels of boron, 3 levels of potassium and 3 levels of copper were examined. The design of the experiment is shown in table 3.

## 7. THE B-K-Cu EXPERIMENT

During the course of the second experiment the appearance of visible symptoms was recorded.

As opposed to the previous experiment, the design of the present experiment allowed for an interpretation of all kinds of symptoms. Prior to discussing the effect of the treatments on the uptake of macroelements, the visible symptoms will be considered.

### 7.1 VISIBLE SYMPTOMS

#### SYMPTOMS DUE TO BORON

Pronounced symptoms could be observed in all cases where boron was applied in excess (levels  $B_6$  and  $B_7$ , resp. 2.5 and 5.0 ppm. B).

The leaves of the plant had white edges which were very brittle. At a latter stage scorching and desiccation of the leaves were observed. These symptoms have been demonstrated by plate 2, photographs 1, 2, 3, 4, and were observed at all potassium levels. This is opposite to the observations which were made by WALLACE and BEAR (1949) who only found boron toxicity symptoms when the potassium levels were low. At low levels of boron ( $B_1$  and  $B_2$ , resp. 0 and 0.01 ppm) discoloured light yellow edges of the leaves were observed. The scorching and desiccating effect which was obtained in the case of boron toxicity was absent at low levels of B. The resemblance of boron deficiency and boron toxicity symptoms complicates the diagnosis based on visible symptoms.

#### SYMPTOMS DUE TO COPPER

All plants which were deficient of copper showed a slight discolouration of the leaves at all levels of potassium. This effect of copper could not be observed when B was at low and high levels, being entirely masked by the boron symptoms. Excess of copper did not result in the appearance of any symptoms. High copper applications tended to reduce the effect of boron toxicity. The absence of deficiency symptoms when all microelements were at a low level and the fact that an excess of a particular micro-element reduces the toxic effects of other microelements would seem to indicate that an optimal balance between these elements is essential. This aspect has been already discussed in the literature review. The diagnosis of deficiency or toxicity based on visible symptoms is therefore rather complicated.

#### SYMPTOMS DUE TO POTASSIUM

The well known potassium deficiency symptoms, characterized by white spots, were only observed at the lowest level of potassium (1 m.e.K/liter). These symptoms have been described in detail by EKSTEIN (1937) and CLARTÉ (1951).

## 7.2 SIGNIFICANT TREATMENT EFFECTS

The results of the analysis of variance have been given in table 24. The effect of boron has been subdivided in a linear and a quadratic effect. The linear effect represents the difference between the 1st and 7th level of boron. The quadratic effect allows for the systematic deviations from the linear effect. The F-test in table 24 clearly shows that boron and potassium have significant effects on the yield and the uptake of macro-elements by lucerne, while the effects of copper and all first order interactions of boron, potassium and copper were insignificant. It may also be seen that the linear effect of boron was significant with respect to the yield and the N, K, Ca, Mg and Na uptake. The quadratic effect of boron was significant for yield, N, P and K uptake. The significant effects resulted from this experiments have been summarised in table 25. When the results of the first and second experiment with lucerne were compared, some common features and some differences could be observed.

TABLE 24 Variance ratios indicating the main effects and interactions of the treatments on the yield and the ion-uptake of lucerne (*l* = linear; *q* = quadratic)

factor	variance ratios (F values)						
	N	P	K	Ca	Mg	Na	yield
B <sub>l</sub>	8.96 <sup>a</sup>	1.52	18.03 <sup>d</sup>	12.35 <sup>d</sup>	6.84 <sup>d</sup>	6.66 <sup>c</sup>	51.34 <sup>d</sup>
B <sub>q</sub>	9.26 <sup>a</sup>	4.98 <sup>b</sup>	6.66 <sup>c</sup>	< 1	< 1	< 1	5.09 <sup>b</sup>
K	6.90 <sup>d</sup>	3.55 <sup>b</sup>	1429.0 <sup>d</sup>	17.03 <sup>d</sup>	62.54 <sup>d</sup>	209.47 <sup>d</sup>	84.78 <sup>d</sup>
Cu	< 1	< 1	< 1	< 1	< 1	1.87	< 1
B <sub>l</sub> × K <sub>l</sub>	< 1	1.05	< 1	1.64	2.00	< 1	< 1
B <sub>q</sub> × K <sub>l</sub>	< 1	< 1	< 1	< 1	< 1	< 1	< 1
B <sub>l</sub> × Cu <sub>l</sub>	1.87	< 1	< 1	2.09	< 1	< 1	< 1
B <sub>q</sub> × Cu <sub>l</sub>	< 1	1.70	< 1	1.89	< 1	< 1	< 1
K <sub>l</sub> × Cu <sub>l</sub>	< 1	< 1	< 1	< 1	< 1	< 1	< 1
V.C.	9.59%	9.76%	9.04%	8.97%	10.93%	18.26%	7.12%

TABLE 25 Significant main effects (M.E.) and interaction of microelements and their effect on the yield and the ion uptake of lucerne

factor	B			K			Cu
	M.E.	K	Cu	M.E.	Cu	M.E.	
effected elements							
N	d				d		
P	b				b		
K	d				d		
Ca	d				d		
Mg	d				d		
Na	b				d		
yield	d				d		

The results of the first experiment indicated that the boron level only effected the uptake of potassium. The second experiment, however, showed that significant differences in the uptake of all macroelements were observed. The interactions between copper and boron on the uptake of potassium which attained significance at  $P = 0.05$  in the first experiment could not be observed in the second experiment.

These differences between the two experiments, may have been due to the following factors:

1. Differences in ion uptake depending on the growing season. The first experiment was carried out in the summer of 1959 while the second was laid down in the spring of 1960. It is known that the ion uptake of a particular plant species will be effected by the nature of the season (ARNON and HOAGLAND (1940)).
2. Unfortunately the same variety could not be used for both experiments. During 1959 the variety 'Chartain Villiers' was used. In 1960 this variety was not available and was replaced by 'Du Piots'.
3. The range of concentrations of boron and potassium investigated in 1960, was more extended than that of 1959.

The significant potassium-boron relation which was demonstrated by the first experiment, was confirmed by the second experiment. The main effects of boron, potassium and copper on the yield and the uptake of macroelements by lucerne are shown by table 26 and figures 1, 2 and 3.

TABLE 26 Mean value of macronutrient uptake and yield as affected by the different levels of B, K and Cu (Main effects)

element	conc.	levels	absolute uptake mg/pot						yield g/pot
			N	P	K	Ca	Mg	Na	
p.p.m.									
B	0.00	1	26.88 <sup>d</sup>	5.00 <sup>b</sup>	17.35 <sup>d</sup>	18.177 <sup>d</sup>	3.98 <sup>d</sup>	3.27 <sup>c</sup>	1.478 <sup>d</sup>
	0.01	2	25.15	4.88	16.43	18.276	3.83	3.37	1.443
	0.05	3	26.43	4.92	17.67	18.177	3.95	3.39	1.463
	0.5	4	26.02	4.89	17.19	17.863	3.91	3.23	1.435
	1.0	5	25.95	4.79	17.11	16.893	3.65	2.91	1.384
	2.5	6	27.40	5.02	18.39	17.313	3.68	3.15	1.331
	5.0	7	28.73	5.20	19.04	16.933	3.73	2.96	1.281
me/l.									
K	1	1	26.26 <sup>d</sup>	4.82 <sup>b</sup>	9.72 <sup>d</sup>	18.34 <sup>d</sup>	4.30 <sup>d</sup>	4.65 <sup>d</sup>	1.272 <sup>d</sup>
	2	2	25.87	4.95	15.25	18.15	3.87	2.73	1.380
	4	3	27.83	5.10	27.82	16.50	3.28	2.17	1.553
p.p.m.									
Cu	0.00	1	26.78	4.91	17.60	17.61	3.81	3.11	1.412
	0.05	2	26.75	4.97	17.69	17.88	3.84	3.32	1.407
	0.5	3	26.42	5.00	17.50	17.49	3.80	3.11	1.387

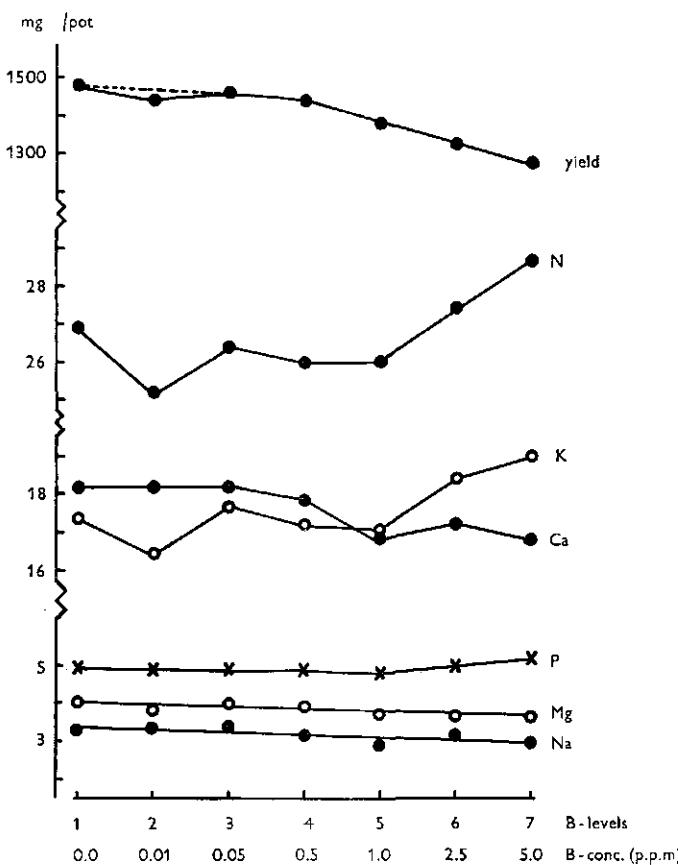


FIG. 1 Main effects of boron levels on the ion-uptake and yield of lucerne

Since the interaction effects are not significant, the corresponding data have not been given in tables. The interaction effects between boron and potassium could be deduced from the graphs (fig. 4a and b).

#### 7.2.1 DRY MATTER PRODUCTION

Table 26 and figure 1 show that an excess of boron decreases the yield. This decrease in yield is only observed at high levels of boron ( $B_6$  and  $B_7$ ). There is no difference in yield for the first four levels of boron. The critical value would seem to be somewhere around 0.5 ppm. B. The yield depression is evident at all potassium levels (fig. 4a) and is independent of the level of copper (fig. 3). In all these cases the addition of boron excess is accompanied by an increase in potassium and nitrogen uptake. This increase of N and K uptake is not of such a magnitude that excess levels are reached. The increase of potassium and nitrogen is entirely within the variation that can be found in normal growing plants.

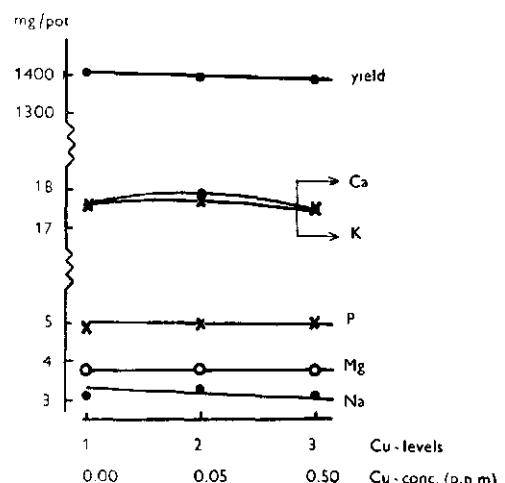
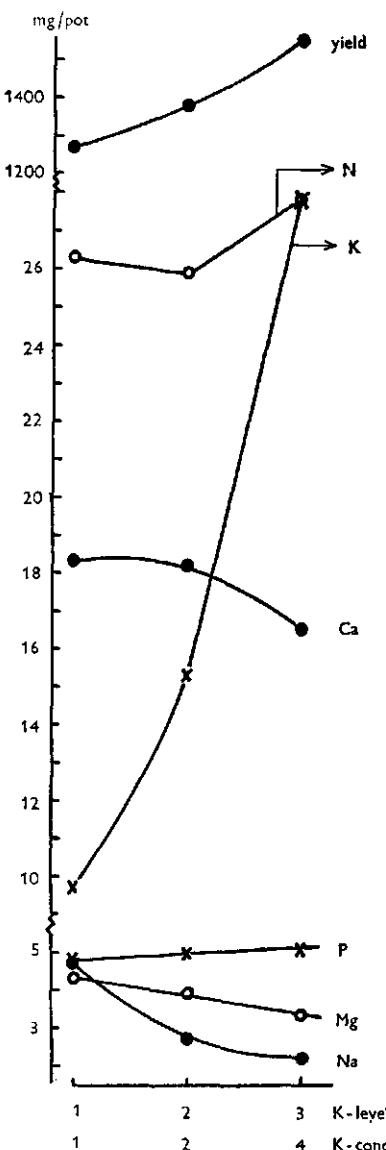


FIG. 3 Main effects of copper levels on the ion-uptake and yield of lucerne

FIG. 2 Main effects of potassium levels on the ion-uptake and yield of lucerne

It seems likely that at the low levels of boron, the boron concentration in the plant has not yet decreased to such an extent that yield depressions became evident. It may be expected however that during the course of a longer growing period the present level of boron in the medium would have been inadequate and would have resulted in a considerable yield depression. The increase in yield at high potassium levels, is normal and does not require any discussion.

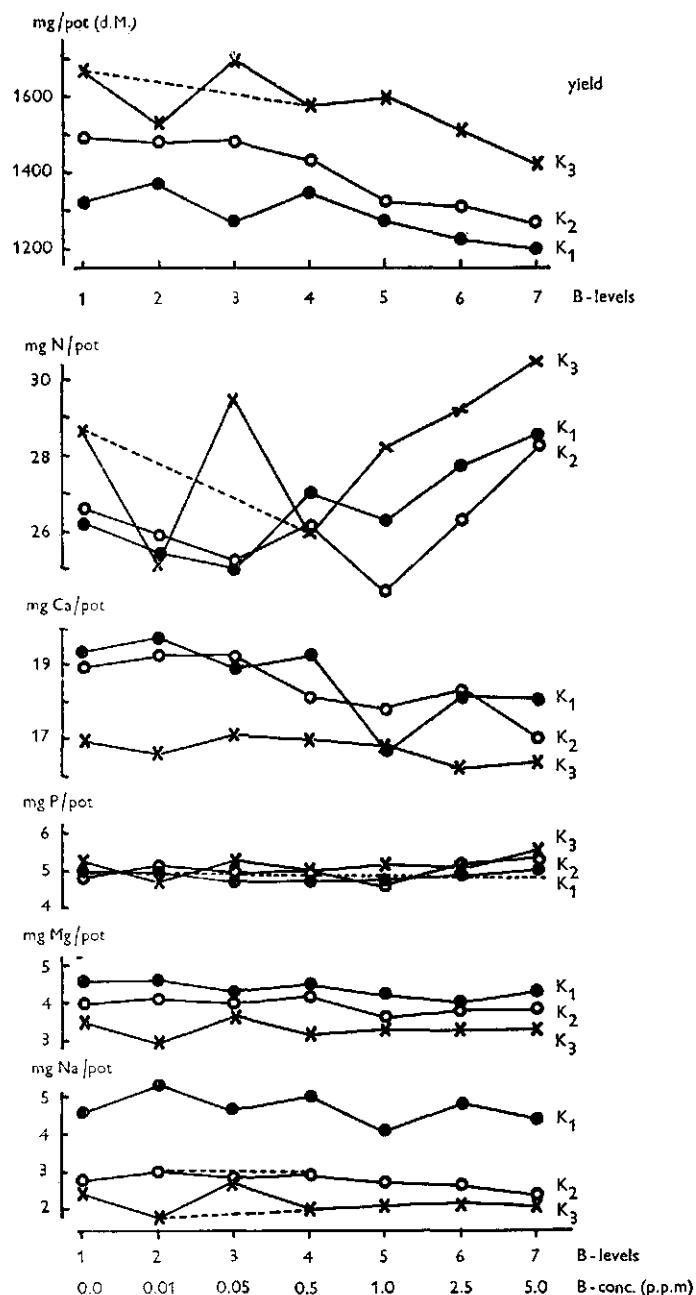


FIG. 4a Effects of B × K interaction on the yield and the uptake of nitrogen, calcium, phosphorus, magnesium and sodium by lucerne plants

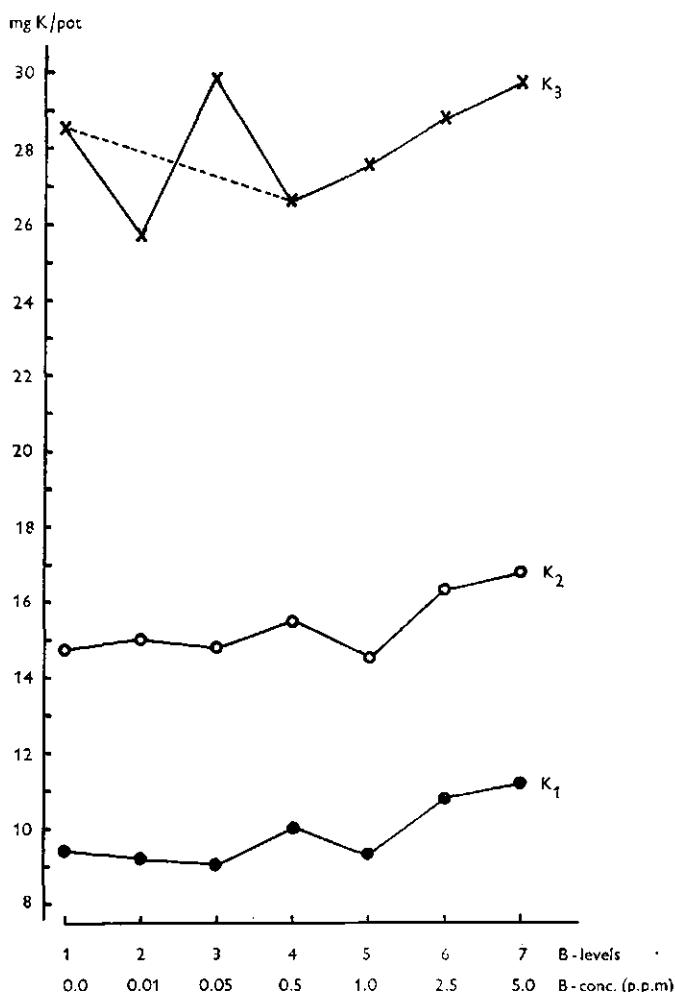


FIG. 4b Effects of B  $\times$  K interaction on the potassium uptake by lucerne

Although it may be expected that high potassium levels depress the effect of an excess of boron, it was found that the visible symptoms of boron toxicity were equally evident at low and high levels of potassium.

The level of copper had no effect on yield and on the macroelement uptake (table 26, fig. 3).

#### 7.2.2 THE UPTAKE OF NITROGEN

Additions of an excess of boron increase the uptake of nitrogen (table 26, fig. 1). The effect of low boron applications on the uptake of nitrogen is small. Also at the medium levels of boron the uptake of nitrogen was not remarkably effected.

At levels higher than 1 ppm. B, the uptake of nitrogen increased steadily (fig. 4a) and seems to become more pronounced at high potassium levels.

In the literature little is known about the effect of boron on nitrogen uptake. MEYER et al. (1960) indicate that boron is involved in the assimilation of nitrogen by the plant. It was suggested that boron has a function in the protein synthesis and therefore boron deficiency may result in an accumulation of carbohydrates and ammonia. This accumulation may be responsible for the damage of the apical tissues. Although these observations are not related to the uptake of nitrogen, they serve to illustrate the fact that boron is related to the function of nitrogen in the plant.

In the present experiment it may be observed that the boron treatments affected the uptake of N and K in a similar way (fig. 1).

The uptake of nitrogen is also related to the level of potassium. High levels of potassium are accompanied by a high uptake of nitrogen (table 26, fig. 2).

Accepting the hypothesis that boron may have an effect on the permeability of cell membranes (LEHR, 1940), it may be inferred that boron has a direct effect on the N and K uptake.

#### 7.2.3 THE UPTAKE OF PHOSPHORUS

Boron had very little effect on the uptake of phosphate. No linear effect did obtain and only a small quadratic effect was significant. This quadratic effect may be seen as the slightly lower P uptake when B was applied at 1.0 ppm.

#### 7.2.4 THE UPTAKE OF POTASSIUM

The uptake of potassium in relation to the level of boron is entirely comparable to that of the uptake of nitrogen. The results of the experiment of 1959, showed that both at low and high levels of boron the potassium uptake increased. The lowest uptake of potassium occurred at a level of 0.5 ppm. boron.

Similar results were obtained in 1960. A higher potassium uptake was found when the boron level was higher than 1 ppm. This increase in potassium uptake was independent of the level of potassium. The lower levels of boron had only a small effect on the potassium uptake.

The significant effect of the interaction between boron and copper which was observed in 1959 was not found in 1960. As no main effect of copper was present in both experiments, this interaction is of limited importance.

It is obvious that there is a pronounced effect of potassium level on the uptake of potassium (fig. 2). This effect needs not to be discussed in any detail.

#### 7.2.5 THE UPTAKE OF CALCIUM

The calcium uptake by lucerne is affected by the boron levels of the medium. At low boron concentrations (up to 0.5 ppm.) the effect on Ca uptake is not very great. At higher concentrations of boron the calcium uptake decreases (table 26, fig. 1).

This decrease in Ca uptake is more pronounced at the two lowest potassium concentrations and of less significance at the highest level of potassium. It is known that the uptake of calcium depends on the potassium concentration in the nutrient medium.

This could also be observed in the present experiment. The antagonistic effect between potassium and calcium (fig. 4a) was not very evident at the lower levels of potassium ( $K_1$  and  $K_2$ ), due to the fact that potassium was replaced by sodium (see table 5). It is interesting to note that an antagonistic effect between potassium and calcium would be observed by varying the boron level only (fig. 1). This would seem to suggest that some other factors than the concentration of potassium and calcium are involved in the uptake mechanism of these ions.

#### 7.2.6 THE UPTAKE OF MAGNESIUM

The uptake of magnesium decreases with increasing boron concentration of the medium. This decrease is almost linear (table 26, fig. 1), and could be observed at all levels of potassium (fig. 4a).

The antagonism between potassium and magnesium is demonstrated in fig. 2. In addition to the  $K/Ca$  ratio there exists a  $K/Mg$  antagonism at all levels of boron. At any level of potassium an increase in boron level increases the potassium uptake and decreases the magnesium uptake despite the fact that the  $K/Mg$  ratio of the medium remained constant.

#### 7.2.7 THE UPTAKE OF SODIUM

The uptake of sodium is similar to that of magnesium. A decrease of sodium uptake was observed when the boron concentration in the medium was increased. Antagonistic effects as were observed for  $K/Ca$  and  $K/Mg$  could also be observed for  $K/Na$  (fig. 2). As the nitrogen concentration was kept constant sodium nitrate replaced potassium at its lowest level ( $K_1$ ) (see table 5).

This explains the high uptake of sodium at the low levels of potassium.

## 8. GENERAL DISCUSSION

It is well known that for normal crop growth a number of microelements are required. As soon as the concentration of these nutrients in the medium becomes too low the plant will grow less satisfactory and as a consequence microelement deficiency symptoms may appear. These symptoms are usually characteristic and may give an indication of the element that is not sufficiently available. It is also known that an excess of microelements may induce characteristic toxicity symptoms in the plant resulting in a less satisfactory growth of the crop.

Plants are able to consume quantities of macroelements in excess of what is required for normal growth. Although this luxury consumption is not economic, it is generally not toxic to the plant and does not result in a yield depression. The range between toxicity and deficiency is much smaller for the microelements as compared with the macroelements. As a consequence an unbalanced application of fertilizers is less harmful in the case of macroelements than of microelements.

Several explanations may be given for the fact, low and high concentrations of microelements depress the yield of crops. In chapter 1, a number of hypothesis with regard to the importance of microelements for plant growth, have been discussed.

The conclusion was drawn that microelements are generally involved in enzymatic processes inside the plant. Presumably these micronutrients participate in enzyme systems or have a catalytic function in enzymatic reactions. It was inferred that there existed significant interactions among microelements. It appeared difficult, however, to obtain a clear picture on the function of microelements because relevant data in the literature are scarce and confused.

The present investigations have been devoted to a special aspect of the problem, namely the effect of microelements on the uptake of macroelements. Therefore the uptake of macroelements was determined at various levels of microelements in the growth medium. This aspect was chosen to investigate whether one or more microelements would have an effect on macroelements to such an extent that the decrease in yield might be explained on account of any change in uptake of one of the macroelements. Microelements might affect both the uptake and the transport of macroelements in the plant, since all these processes are governed by enzymatic reactions which are regulated by micronutrients.

The experiments were designed to detect small differences in the uptake of macroelements, as induced by variations of levels of the micronutrients.

It is evident that extreme deficient or toxic of levels of micronutrients may be responsible for secondary effects on account of the reduction in growth and resulting in a change in macroelements uptake. Accordingly an experimental design was chosen which would enable the detection of small differences that might be induced by the various treatments. On the other hand, this type of experiment is not designed to investigate the causal relationships between microelement levels and macroelement uptake. In order to check whether different plants react in a similar way to different concen-

trations of the microelements, three plant species were taken as test crops. In the first experiment it was found that zinc had an effect on the yield and uptake of N, P, Ca, and Mg of oats. An interaction of zinc and iron was found in the case of lucerne and tomato for the uptake of Mg, and P respectively. Apparently different plants species reacted differently to the treatments. These differences among the test crops may be partly due to differences in tolerance to the zinc concentration of the medium. On the other hand if zinc would be involved in the carrier system of the ion uptake, it might have had a similar effect in the case of all crops. In order to arrive at a definite conclusion, this problem has to be investigated in detail in the future.

The applications of copper had apparently no effect on the yield and uptake of macro-elements of all crops. It is very unlikely therefore that the copper ion has a direct effect on the carrier system that is involved in macroelement uptake.

In the case of manganese, no main effect could be detected. Only the interaction between iron and manganese was significant in respect to the magnesium uptake by lucerne. Also the calcium and magnesium uptake and the yield of tomato were significantly affected by the  $Fe \times Mn$  interaction. The absence of any main effect renders the interpretation of these interactions very difficult.

Iron decreased the phosphate uptake. This effect is well known in literature. It is generally assumed that the reduction of P uptake is due to the precipitation of phosphorus ( $FePO_4$ ) in the medium or in the root, resulting in a decrease of phosphate in the leaves of the plant. Despite the fact that the transport of phosphate may be hampered by an excess of iron supply it is very unlikely that iron is related to the uptake mechanism of phosphate.

Boron had an effect on the nitrogen and phosphate uptake of oats, and on the yield of tomatoes. It was also found that in the case of lucerne, boron had a pronounced effect on the potassium uptake. In the second experiment which included an extended range of boron concentrations, the effect of boron on the yield and uptake of all macroelements has been demonstrated.

Significant interactions between  $Mn \times Fe$ ,  $Zn \times Fe$ ,  $B \times Cu$  and  $B \times Zn$  could also be detected. These interactions were of little importance since they occurred in a few instances only.

As there is very little similarity in the data, the conclusion may be drawn that micro-elements have generally no specific effect on the uptake mechanism of macroelements. The effects that could be observed are probably the result of modifications of the physiological conditions of the plant due to the biological activity of the micro-nutrients. This is also emphasized by the fact that even the significant treatment effects were very small.

An exception may be made for the element boron whose effect on the uptake of macro-elements has been investigated in greater detail. Despite the fact that there is no indication of a direct influence of boron on the carrier system of the macroelements, some indications have been obtained on the importance of the boron concentration in the medium for the uptake of macroelements. At high levels of boron the uptake of nitrogen, phosphorus and potassium increased, while that of calcium, magnesium and sodium decreased.

The importance of boron for plant growth has been discussed and various hypothesis have been already mentioned. According to LEHR (1940) the borate ion may be fixed in the cell wall as an organic complex and would be responsible for a change in the permeability of the cell wall. REED (1947) showed by means of microscopic investigations of plant cells that the transport tissue was damaged when the boron concentration was very low, resulting in a reduction of the transport of ions and carbohydrates. STUIVENBERG and POUWER (1950) observed that both auxin and boron treatment could prevent the appearance of the 'stip' in apples. EATON (1944) showed that an auxin treatment of cotton seedlings prevented boron deficiency. It was observed by SCHURFELLEN (1948) that auxin has an influence on the uptake of macroelements and he attributed this phenomenon to a permability effect.

These observations seem to suggest similar functions of boron and auxin in relation to the permeability of plasma membranes of cell walls. Consequently boron may be involved in an universal process which would be related to the ion uptake by plants. It is remarkable however that this process had an opposite effect on different ions. The results of the second experiment show that the well known antagonistic effects between potassium-calcium, potassium-magnesium and potassium-sodium could be induced by changing the boron concentration of the medium only.

Apart from a change in permeability of the cell walls other factors might therefore be involved.

Further research is required to investigate what kind of factors are of importance. The results of the experiments may be considered from another angle. It is evident that even the significant effects of microelements on the uptake of macroelements are very small. The variations in macroelement uptake are of a similar magnitude as those which can be expected in practice. These variations have a very small influence on the yield of the crop. This conclusion is of importance with regard to a practical fertilizer policy and implies that fertilizing with macroelements may be done independently of the microelement applications.

The reverse of this conclusion, however, is not true. The need for microelements will often be determined by the rate at which macroelements have been applied. The increase in yields which are obtained after the application of macroelements result in a greater demand for microelements. It is also probable that a particular favorable balance between macro- and micronutrients is essential, for instance between potassium, calcium and boron. Thus fertilizing with micronutrients will depend on the macronutrient applications and the situation of the micronutrients in the soil. It should be stressed that this procedure has been accepted in normal fertilizer practice. Experience has shown that the high applications of macroelements have revealed the importance of micronutrient deficiencies in many crops.

It is desirable that the preliminary conclusions which could be drawn from the experiments in the laboratory and the greenhouse are investigated in greater detail in field experiments.

## SUMMARY AND CONCLUSIONS

An extensive literature study showed that the present knowledge with regard to the function of microelements in plant growth is very limited. It could be assumed that the effect of these micronutrients is related to enzymatic processes that are taking place in the plants. Little information has been reported on the effect of microelements on the uptake of macroelements by plants. The present investigations were devoted to the question whether microelements have an effect on growth and macroelements uptake by crops. As a general approach, many factors were investigated simultaneously in the first experiments. The results of these experiments which yielded information of a general nature served for the selection of the problems that required further investigation.

The pilot trials which were carried out in 1959, studied the effect of boron, manganese, copper, zinc and iron, each at three levels i.e. low, normal and high. Oats, tomato and lucerne were used as test crops. The yield and the uptake of N, P, K, Ca, Mg and Na were measured. The experimental design of these experiments was a  $3^5$  factorial in 81 units ( $\frac{1}{3}$  replicate).

An elaborated experiment was carried out in 1960 using only lucerne as an indicator plant. This experiment included 7 levels of boron, 3 levels of potassium and 3 levels of copper in a factorial combination. All crops were grown in sand cultures using nutrient solutions based on the 'Long Ashton' solution. Pro-analysed 'Merck' grade chemicals, purified sand 'Brocades', demineralized water and polyethylene containers were used. Demineralized water and sand were checked spectrographically for microelement impurities.

In order to avoid secondary effects which may have a disturbing influence on the uptake of macroelements, the growth period of the plants was limited to a few weeks. After drying and weighing the shoots, subsamples were taken for chemical analysis.

### VISIBLE SYMPTOMS

The design of the first experiments that were carried out in 1959, did not allow for a systematic description of all the observed symptoms. The symptoms were of a complex nature, probably due to the fact that a great number of factors were combined in every treatment and therefore various symptoms might have been attributed to a combination of various microelement deficiencies or toxicities. The symptoms associated with the highest level of boron, were very obvious however.

In this respect lucerne appeared to be very sensitive to an excess of boron, while oats and tomato were less sensitive. Plate 2 demonstrates these symptoms of boron toxicity. In the second experiment of 1960 with lucerne both boron deficiency and toxicity symptoms could be observed. The incidence of boron toxicity symptoms was not influenced by any of the potassium and/or copper levels. At the highest level of potassium the symptoms of boron toxicity were accentuated.

## YIELD

A significant effect of zinc on the yield of oats had been found. The yields corresponding to the low and high levels of zinc were lower than that of the normal zinc level. The yield of tomato was significantly affected by boron and iron. Boron supply decreased and iron supply increased the yield. Also the Mn  $\times$  Fe interaction affected the yield significantly. In the experiment of 1959, no effect of any of the microelements on the yield of lucerne could be observed, whereas in that of 1960 it was found that the higher levels of boron reduced the yield of lucerne.

## THE ION UPTAKE

The ion uptake of the crop has been expressed in mg/pot. From a comparison between the data expressed as mg/pot (absolute values) and as a percentage of the dry matter (relative values), it was concluded that the former way of expression had some advantage over the latter.

The uptake of some of the macronutrients was significantly affected by altering the levels of the micronutrients in the culture media. In this respect the three plant species reacted differently. The differences in the uptake values as affected by the levels of a particular microelement were generally small.

By changing the potassium level in the lucerne experiment of 1960, differences in the uptake of macroelements were also observed. Generally, the uptake of all the other cations, viz., calcium, magnesium and sodium was reduced, while the uptake of anions, viz., phosphorus and nitrogen was increased when the level of potassium in the nutrient medium was increased.

In the following, only the statistically significant effect of micronutrients on the uptake of macronutrients will be summarized (see tables 10 and 25).

## NITROGEN

Increasing levels of boron increased the uptake of nitrogen of oats (1959) and of lucerne (1960).

## PHOSPHORUS

For all the three test crops it was found that the uptake of phosphorus was significantly reduced when the iron level in the growing medium was high.

## POTASSIUM

Excessive applications of boron significantly increased the uptake of potassium by lucerne (expts. 1959 and 1960). It was also significantly affected by B  $\times$  Cu and B  $\times$  Zn interactions (expt. 1959). Whereas the influence of boron on the potassium uptake was confirmed by the experiment of 1960, the B  $\times$  Cu interaction effect could not be reproduced.

## CALCIUM

The calcium uptake by oats was reduced by an excess of zinc. In case of lucerne (expt. 1960) the calcium uptake was reduced when boron was applied at a high level. In the

experiments of 1959, the uptake of calcium by lucerne was decreased by high applications of iron.

#### MAGNESIUM

Zinc decreases the uptake of magnesium by oats. With lucerne the magnesium uptake was decreased by increments of boron supply (expt. 1960).

In the experiments of 1959 the Mn  $\times$  Fe interaction significantly affected the uptake of magnesium by lucerne and tomatoe plants, while the main effects of these elements were insignificant.

#### SODIUM

With regard to the effect of micronutrients on the uptake of sodium, only the Mn  $\times$  Fe interaction in the case of tomato was significant (expts. 1959). In the experiment of 1960 the uptake of sodium by lucerne was decreased when boron was applied at high levels.

Besides the micronutrient effects, the results of the experiment of 1960 showed the well known antagonism between potassium and calcium, magnesium or sodium.

#### GENERAL CONCLUSIONS

Various effects of microelements on the uptake of macroelements could be demonstrated. The effects were different for the test crops that were used. The absence of any uniformity in the data suggests that the effects of microelements are related to changes in the general physiological status of the plant and not to the uptake mechanism of macroelements.

The effect of boron which was investigated in detail during 1960 might be related to the influence of boron on the permeability of cell membranes. It was found that an excess of boron increased the uptake of N, P, K and decreased that of Ca, Mg, and Na.

## SAMENVATTING EN CONCLUSIES

Uit een uitvoerige literatuurstudie bleek, dat de kennis van de rol die de micro-elementen bij de plantengroei vervullen nog zeer fragmentarisch is. Zij wordt in hoofdzaak toegeschreven aan invloeden die op enzymatische processen in de plant worden uitgeoefend. De literatuur geeft enkele gegevens over de invloed van macro-elementen op de behoefte aan micro-elementen. Over de invloed van de micro-elementen op de opname van de macro-elementen is weinig bekend. In deze studie werd nagegaan of deze invloed aanwezig is en of zij van betekenis is voor de groei van het gewas. Er werd uitgegaan van de gedachte, dat de meeste algemene informatie kan worden verkregen door een opzet te kiezen, waarbij vele factoren tegelijk in een oriënterende proef konden worden bestudeerd. Daarna is een facet uitvoeriger bestudeerd.

De oriënterende proef, die in 1959 genomen werd, betrof een studie van de invloed van B, Mn, Cu, Zn en Fe, in drie niveaus (laag, normaal en hoog) bij drie plantensoorten (haver, tomaat en lucerne) op de opbrengst en de opname van N, P, K, Ca, Mg en Na.

Er werd een 35 factorieel schema gekozen met 81 eenheden ( $\frac{1}{3}$  herhaling).

In de tweede proef (1960) werd slechts lucerne als proefgewas gebruikt. Hierbij werden 3 kalium-, 3 koper- en 7 borium-niveaus van het milieu als objecten gekozen.

De planten groeiden in een zandcultuur, waaraan de 'Long Aston' voedingsoplossing werd toegevoegd. De chemicaliën waren Merck p.a..., het zand gezuiverd zand 'Bro-cades', de potten waren van polyethyleen. Als water werd gedemineraliseerd water gebruikt. Kwartszand en water werden spectrografisch op hun verontreiniging met micro-elementen gecontroleerd. De groei van de planten werd tot enkele weken beperkt om secundaire effecten tengevolge van gebrek of overmaat aan micro-elementen zoveel mogelijk te voorkomen.

De geoogste planten werden gedroogd, gewogen en gemalen en onderzocht op hun gehalte aan N, P, K, Ca en Mg.

### SYMPTOMEN

In 1959 waren tengevolge van de proefopzet geen studies over gebreks- en overmaats-verschijnselen mogelijk. De verschijnselen waren van complexe aard, daar in elke behandeling een groot aantal factoren waren gecombineerd. Alleen B-overmaatverschijnselen waren duidelijk waarneembaar bij het hoogste B-niveau. Lucerne was gevoeliger voor de B-overmaat dan haver en tomaat. In 1960 werden bij lucerne zowel B-gebrek als B-overmaatsverschijnselen waargenomen. Plaat 2 geeft een afbeelding van de B-overmaatsverschijnselen. Het optreden van deze verschijnselen had op alle K- en Cu-niveaus plaats. Verhoging van het K-niveau in de oplossing versterkte optreden van de verschijnselen van boriumovermaat.

### OPBRENGSTEN

Bij haver werd een significant effect van Zn op de opbrengst gevonden. De opbrengst was bij het lage en het hoge niveau lager dan dat bij het middelste Zn-niveau.

De opbrengst van tomaat werd significant beïnvloed door B en Fe. Boriumtoevoeging verlaagde, ijzertoevoeging verhoogde de opbrengst.

Een interactie Mn  $\times$  Fe op de opbrengst werd waargenomen.

In 1959 werd bij lucerne geen invloed van een der onderzochte elementen op de opbrengst gevonden.

In 1960 werd gevonden, dat bij de hogere B-niveaus de opbrengst lager lag dan die bij de lage en midden-niveaus.

### IONENOPNAME

De ionenopname is weergegeven als mg per pot. Uit een vergelijkend onderzoek bij haver, werd geconcludeerd dat deze wijze van uitdrukken beter was, dan die in de gehalten per plant. Een verandering van het gehalte van micro-elementen van het milieu had een significant effect op de opname van enkele macro-elementen. De drie plantensoorten reageerden echter zeer verschillend. De gevonden variaties in de opname van macro-elementen was in het algemeen zeer klein.

Veranderingen van het kalium-niveau van het milieu in de lucerne proef van 1960 beïnvloeden de opname van K, Mg, Ca, Na en ook van P en N.

Verder zullen alleen significante verschillen worden besproken.

### STIKSTOF

Een toename van het borium-niveau vergrootte de opname van stikstof bij haver (1959) en bij lucerne (1960).

### PHOSPHAAT

Bij de drie gewassen werd bij stijgende ijzer-gehalten van het milieu een afname van de phosphaatopname gevonden.

### KALIUM

Bij lucerne werd in 1959 geconstateerd dat bij een toename van het boriumgehalte in het milieu de kaliumopname toenam. Tevens werden voor dit gewas interacties B  $\times$  Cu en B  $\times$  Zn op de kaliumopname waargenomen.

In 1960 werd de invloed van B bevestigd, de interactie B  $\times$  Cu kon toen niet worden vastgesteld.

### CALCIUM

Bij haver werd de calciumopname gedrukt door overmaat Zn. Bij lucerne had in 1960 een vermindering van de Ca-opname plaats bij hogere borium-niveaus. In 1959 werd bij lucerne de Ca-opname verminderd bij een hoog Fe-niveau.

#### MAGNESIUM

Zn drukte de magnesiumopname bij haver. Bij lucerne werd de Mg-opname verminderd door stijgende B-giften (1960).

In 1959 werden interacties aangetoond bij lucerne en tomaten voor Mn  $\times$  Fe en bij lucerne voor Zn  $\times$  Fe.

Hoofdeffecten van deze elementen waren echter niet significant.

#### NATRIUM

Voor de opname van Na werd slechts een interactie Mn  $\times$  Fe bij tomaten waargenomen.

In 1960 werd bij lucerne een vermindering van de natriumopname bij hoge B-niveaus gevonden.

In het experiment van 1960 werd naast de beïnvloeding van de ionenopname door de micro-elementen het bekende antagonistische van kalium met Ca, Mg, Na, waargenomen.

#### CONCLUSIES

Verschillende effecten van de micro-elementen op de opname van de macro-elementen konden worden aangetoond.

Deze effecten waren bij de gebruikte gewassen zeer verschillend. Het ontbreken van een uniformiteit in de resultaten leidt tot de conclusie, dat het effect van de micro-elementen meer gezocht zal moeten worden in een wijziging van de algemene fisiologische toestand in de plant, dan in een specifiek effect op het opname-mechanisme der macro-elementen. Het effect van borium, dat op alle onderzochte elementen in 1960 bij lucerne werd gevonden, is mogelijk in verband te brengen met een invloed van B op de permeabiliteit van de cellen.

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APPENDIX 1 Chemical composition and yield of oats (expt. 1959) as affected by different combined levels of micronutrients, (on dry matter bases)

potNo.	treatments					N	P	K	Ca	Mg	Na	yield
	B	Mn	Cu	Zn	Fe	%	mg/100 g	%	mg/100 g	mg/100 g	mg/100 g	g/pot
1	5	1	0	5	1	2.20	372	1.24	250	133	835	1.49
2	0	1	1	1	0	1.92	339	1.09	334	134	685	1.79
3	1	5	0	1	5	2.00	319	1.09	334	140	618	1.87
4	1	0	1	5	5	1.80	306	1.05	292	130	584	1.89
5	0	5	5	5	0	2.00	346	1.30	250	129	835	1.33
6	0	0	0	0	0	1.88	313	0.85	334	145	401	1.87
7	5	5	1	0	1	2.04	386	1.00	458	175	484	1.45
8	1	1	5	0	5	1.92	279	1.07	334	114	584	1.91
9	5	0	5	1	1	1.80	326	0.95	334	138	518	2.02
10	5	0	0	1	0	2.04	359	1.10	334	144	735	1.85
11	1	0	5	5	1	1.96	332	1.07	334	143	601	1.88
12	0	5	0	5	5	2.12	379	1.30	250	139	902	1.24
13	1	5	1	1	1	1.92	332	1.09	250	131	635	1.98
14	1	1	0	0	1	1.92	326	1.04	334	128	601	1.97
15	5	1	1	5	0	2.12	372	1.20	375	142	668	1.85
16	0	1	5	1	5	1.88	306	1.04	334	128	651	2.04
17	5	5	5	0	0	2.08	359	1.07	375	149	701	1.74
18	0	0	1	0	5	1.92	286	1.12	292	129	668	1.84
19	5	0	1	1	5	1.92	352	1.00	375	119	418	2.06
20	0	0	5	0	1	1.76	326	1.00	334	133	401	1.91
21	1	0	0	5	0	1.88	352	1.07	250	134	651	1.82
22	5	5	0	0	5	1.96	306	1.14	250	134	701	1.82
23	5	1	5	5	5	1.96	352	1.10	250	125	401	1.89
24	0	5	1	5	1	2.00	352	1.27	250	127	701	1.64
25	1	5	5	1	0	1.96	386	1.05	375	130	334	1.93
26	0	1	0	1	1	1.88	332	1.14	167	147	635	1.80
27	1	1	1	0	0	1.76	357	1.22	401	119	652	1.72
28	5	0	1	0	0	1.96	372	1.00	334	144	384	1.93
29	0	1	0	0	5	1.76	313	0.97	334	114	501	2.02
30	5	1	5	1	0	2.20	412	1.35	375	122	752	1.47
31	1	0	0	1	1	1.76	332	1.00	250	136	367	1.94
32	0	0	5	5	5	1.88	313	1.14	250	117	601	1.76
33	1	5	5	0	1	1.92	392	1.00	458	144	367	1.93
34	1	1	1	5	1	1.76	352	1.09	375	120	752	1.64
35	0	5	1	1	5	1.80	326	0.95	334	121	334	1.97
36	5	5	0	5	0	1.96	386	1.09	292	145	768	1.59
37	5	5	1	5	5	1.92	366	1.05	292	133	835	1.63
38	0	0	0	5	1	1.92	346	1.05	250	144	768	1.90
39	5	0	5	0	5	1.96	339	1.05	250	129	584	1.91
40	1	5	0	0	0	1.84	399	1.02	250	139	735	1.99
41	0	5	5	1	1	1.72	379	1.02	250	133	668	2.02
42	1	0	1	1	0	1.92	392	1.20	250	138	835	1.56
43	0	1	1	0	1	1.80	359	1.12	250	125	818	1.52
44	5	1	0	1	5	1.76	326	0.98	292	127	685	1.95
45	1	1	5	5	0	1.68	332	0.92	334	132	651	2.11
46	1	5	1	0	5	1.76	339	0.90	334	129	718	2.05

APPENDIX 1 (continued)

pot No.	treatments					N	P	K	Ca	Mg	Na	yield
	levels					%	mg/100 g	%	mg/100 g	mg/100 g	mg/100 g	g/pot
	B	Mn	Cu	Zn	Fe							
47	5	1	1	1	1	1.80	359	0.90	334	143	668	2.12
48	0	0	1	5	0	1.88	394	1.05	484	124	668	1.91
49	0	5	0	1	0	1.84	386	1.04	417	151	451	1.75
50	5	5	5	5	1	2.04	439	1.22	334	169	835	1.47
51	1	1	0	5	5	1.72	339	0.98	292	120	718	2.00
52	1	0	5	1	5	1.76	326	0.90	334	127	367	2.10
53	0	1	5	0	0	1.88	386	1.14	250	146	785	1.72
54	5	0	0	0	1	2.04	392	1.24	167	140	852	1.55
55	0	5	0	0	1	1.76	374	0.97	484	117	384	2.00
56	5	5	5	1	5	1.84	360	0.97	401	124	685	2.03
57	5	0	0	5	5	1.76	344	0.94	334	116	668	2.03
58	5	1	1	0	5	1.72	320	0.97	367	118	584	2.09
59	1	5	1	5	0	1.80	410	0.97	401	130	618	1.95
60	0	1	5	5	1	1.88	420	1.24	367	117	718	1.49
61	0	0	1	1	1	1.64	347	0.92	334	110	601	2.10
62	1	0	5	0	0	1.72	344	0.98	334	116	551	2.18
63	1	1	0	1	0	1.80	392	1.07	401	116	334	1.74
64	0	5	5	0	5	1.72	370	1.04	317	92	635	1.73
65	5	5	1	1	0	1.84	404	1.04	384	132	635	1.99
66	1	1	5	1	1	1.80	350	0.94	367	116	618	2.07
67	1	5	0	5	1	1.88	397	1.05	334	116	584	1.97
68	0	0	0	1	5	1.80	350	1.09	367	108	351	2.00
69	0	1	1	5	5	1.76	337	0.98	334	98	301	2.04
70	5	0	5	5	0	2.16	447	1.32	284	122	752	1.45
71	5	1	0	0	0	1.68	364	0.92	367	118	351	1.97
72	1	0	1	0	1	1.64	334	0.85	334	109	284	2.09
73	1	5	5	5	5	1.92	394	1.00	334	104	334	1.81
74	0	5	1	0	0	1.96	440	1.12	484	133	468	1.53
75	5	1	5	0	1	1.88	374	1.00	401	128	367	1.94
76	5	5	0	1	1	1.88	417	1.12	401	128	418	1.72
77	0	0	5	1	0	1.72	350	0.87	367	115	334	2.03
78	5	0	1	5	1	1.76	377	0.95	334	125	484	1.96
79	0	1	0	5	0	1.72	350	0.98	401	110	334	1.93
80	1	1	1	1	5	1.92	377	1.07	367	103	334	1.82
81	1	0	0	0	5	1.60	300	0.84	334	96	301	2.14

APPENDIX 2 Chemical composition and yield of Lucerne (expt. 1959) as affected by different combined levels of micronutrients, (on dry matter bases)

pot No.	treatments					N	P	K	Ca	Mg	Na	yield
	B	Mn	Cu	Zn	Fe	%	mg/100 g	%	mg/100 g	mg/100 g	mg/100 g	g/pot
1	5	1	0	5	1	2.24	360	1.18	1.22	187	100	1.59
2	0	1	1	1	0	2.56	380	1.30	1.28	220	183	1.32
3	1	5	0	1	5	2.36	313	1.25	1.37	227	183	1.31
4	1	0	1	5	5	2.20	313	1.03	0.95	173	143	1.64
5	0	5	5	5	0	2.64	373	1.33	1.55	207	197	1.34
6	0	0	0	0	0	2.12	380	1.13	1.13	193	97	1.46
7	5	5	1	0	1	2.32	360	1.27	1.10	200	127	1.33
8	1	1	5	0	5	2.24	313	1.15	1.20	203	93	1.30
9	5	0	5	1	1	2.40	373	1.13	1.17	220	143	1.46
10	5	0	0	1	0	2.56	327	1.38	1.20	200	133	1.37
11	1	0	5	5	1	2.28	360	1.03	1.13	213	67	1.73
12	0	5	0	5	5	2.40	313	1.22	1.20	200	183	1.34
13	1	5	1	1	1	2.24	373	1.13	1.27	213	133	1.31
14	1	1	0	0	1	2.24	360	1.12	1.20	200	167	1.43
15	5	1	1	5	0	2.24	387	1.08	1.15	200	120	1.51
16	0	1	5	1	5	2.16	300	1.35	1.07	183	107	1.66
17	5	5	5	0	0	2.28	340	1.25	1.13	200	120	1.39
18	0	0	1	0	5	2.32	320	1.25	1.23	233	123	1.25
19	5	0	1	1	5	2.24	300	1.22	1.12	193	133	1.36
20	0	0	5	0	1	2.48	373	1.13	1.27	203	133	1.45
21	1	0	0	5	0	2.16	387	1.07	1.23	207	120	1.44
22	5	5	0	0	5	2.24	307	1.13	1.27	213	120	1.43
23	5	1	5	5	5	2.32	293	1.22	0.92	167	203	1.48
24	0	5	1	5	1	2.48	400	1.38	1.35	213	143	1.20
25	1	5	5	1	0	2.48	373	1.22	1.30	207	93	1.33
26	0	1	0	1	1	2.40	293	1.18	1.35	233	283	1.38
27	1	1	1	0	0	2.24	347	1.02	1.20	183	167	1.49
28	5	0	1	0	0	2.12	400	1.12	1.02	177	127	1.51
29	0	1	0	0	5	2.32	487	1.13	1.17	170	233	1.33
30	5	1	5	1	0	2.00	347	1.03	1.10	173	140	1.38
31	1	0	0	1	1	2.04	347	1.00	1.20	177	167	1.45
32	0	0	5	5	5	2.24	300	1.22	1.23	187	160	1.23
33	1	5	5	0	1	2.08	327	1.07	1.17	180	160	1.45
34	1	1	1	5	1	1.96	327	0.97	1.27	173	110	1.39
35	0	5	1	1	5	2.16	347	1.03	1.13	317	107	1.51
36	5	5	0	5	0	2.56	387	1.33	1.23	210	127	1.27
37	5	5	1	5	5	2.32	300	1.15	1.12	187	100	1.45
38	0	0	0	5	1	2.16	373	1.07	1.27	200	100	1.42
39	5	0	5	0	5	2.20	313	1.12	1.27	193	110	1.46
40	1	5	0	0	0	1.92	353	0.93	1.27	197	100	1.59
41	0	5	5	1	1	2.52	360	1.70	1.27	197	127	1.24
42	1	0	1	1	0	2.08	327	1.03	1.20	180	110	1.36
43	0	1	1	0	1	2.08	353	1.05	1.18	200	120	1.43
44	5	1	0	1	5	2.24	293	1.13	1.33	193	100	1.36
45	1	1	5	5	0	2.24	327	1.00	1.27	223	143	1.43
46	1	5	1	0	5	2.04	267	1.12	1.15	180	103	1.35

APPENDIX 2 (continued)

pot No.	treatments					N	P	K	Ca	Mg	Na	yield
	levels					%	mg/100 g	%	mg/100 g	mg/100 g	100/mg g	g/pot
	B	Mn	Cu	Zn	Fe							
47	5	1	1	1	1	2.20	347	1.05	1.22	187	108	1.45
48	0	0	1	5	0	2.08	373	1.03	1.23	187	108	1.48
49	0	5	0	1	0	2.00	300	1.03	1.13	193	147	1.39
50	5	5	5	5	1	2.00	313	1.03	1.03	183	90	1.45
51	1	1	0	5	5	1.96	267	0.97	1.20	173	77	1.46
52	1	0	5	1	5	2.08	267	1.00	1.05	183	147	1.46
53	0	1	5	0	0	2.32	400	1.12	1.17	207	110	1.51
54	5	0	0	0	1	2.24	327	1.13	1.10	190	110	1.35
55	0	5	0	0	1	2.00	347	1.03	1.30	190	43	1.31
56	5	5	5	1	5	2.28	320	1.13	1.13	190	67	1.52
57	5	0	0	5	5	2.28	287	1.22	1.17	217	90	1.34
58	5	1	1	0	5	2.24	287	1.13	1.17	180	67	1.36
59	1	5	1	5	0	2.08	407	1.12	1.30	220	67	1.48
60	0	1	5	5	1	2.16	333	1.08	1.13	190	73	1.36
61	0	0	1	1	1	2.40	333	1.18	1.27	203	147	1.37
62	1	0	5	0	0	2.28	373	1.12	1.32	213	77	1.31
63	1	1	0	1	0	2.24	373	1.03	1.17	177	90	1.46
64	0	5	5	0	5	2.00	293	1.03	1.10	163	67	1.46
65	5	5	1	1	0	2.24	380	1.08	1.23	220	67	1.37
66	1	1	5	1	1	2.24	347	1.03	1.23	197	67	1.48
67	1	5	0	5	1	2.08	387	1.07	1.20	183	83	1.40
68	0	0	0	1	5	2.12	333	1.12	1.20	213	100	1.37
69	0	1	1	5	5	2.04	320	1.00	1.13	167	73	1.44
70	5	0	5	5	0	2.24	400	1.13	1.23	187	77	1.42
71	5	1	0	0	0	2.16	387	1.13	1.25	193	67	1.52
72	1	0	1	0	1	2.16	327	1.17	1.20	197	100	1.36
73	1	5	5	5	5	1.80	313	0.97	1.22	183	83	1.54
74	0	5	1	0	0	2.00	387	1.02	1.33	207	77	1.35
75	5	1	5	0	1	2.04	353	1.13	1.10	190	53	1.40
76	5	5	0	1	1	2.12	347	1.22	1.22	183	113	1.28
77	0	0	5	1	0	2.32	387	1.22	1.23	187	83	1.37
78	5	0	1	5	1	2.24	347	1.07	1.23	197	107	1.45
79	0	1	0	5	0	2.04	387	1.03	1.33	207	67	1.38
80	1	1	1	1	5	2.16	333	1.03	1.30	193	77	1.30
81	1	0	0	0	5	2.08	320	1.12	1.13	173	67	1.48

APPENDIX 3 Chemical composition and yield of Tomato (expt. 1959) as affected by different combined levels of micronutrients, (on dry matter basis)

pot No.	treatments					N	P	K	Ca	Mg	Na	yield
	B	Mn	Cu	Zn	Fe	%	mg/100 g	%	%	mg/100 g	%	g/pot
1	5	1	0	5	1	4.04	753	2.20	2.90	503	1.00	0.89
2	0	1	1	1	0	4.04	740	2.13	2.90	527	1.12	0.87
3	1	5	0	1	5	3.88	653	2.00	2.78	507	1.07	0.86
4	1	0	1	5	5	4.08	687	2.43	2.90	527	1.08	0.85
5	0	5	5	5	0	4.20	907	2.33	3.03	527	1.07	0.83
6	0	0	0	0	0	4.24	820	2.50	3.17	593	1.17	0.80
7	5	5	1	0	1	4.08	820	2.60	3.00	600	1.29	0.84
8	1	1	5	0	5	4.36	747	2.33	3.03	510	1.10	0.87
9	5	0	5	1	1	4.08	800	2.07	2.73	483	1.10	0.93
10	5	0	0	1	0	4.08	827	2.23	2.90	513	1.15	0.92
11	1	0	5	5	1	4.08	753	2.23	2.78	520	1.22	0.90
12	0	5	0	5	5	3.92	673	2.10	2.70	487	1.13	0.94
13	1	5	1	1	1	4.08	773	2.20	2.83	537	1.09	0.88
14	1	1	0	0	1	4.16	787	2.33	2.83	527	1.02	0.86
15	5	1	1	5	0	4.24	913	2.70	3.17	640	1.08	0.77
16	0	1	5	1	5	3.60	593	2.20	2.63	483	1.13	0.94
17	5	5	5	0	0	4.08	847	2.33	3.00	460	1.07	0.84
18	0	0	1	0	5	3.52	613	1.83	2.50	493	0.97	1.03
19	5	0	1	1	5	3.68	600	1.97	2.67	510	1.03	1.02
20	0	0	5	0	1	3.68	593	2.10	2.43	487	1.38	0.96
21	1	0	0	5	0	3.92	820	2.33	3.10	537	1.03	0.81
22	5	5	0	0	5	4.24	780	2.50	3.08	537	1.03	0.81
23	5	1	5	5	5	4.00	647	2.27	2.70	550	1.09	0.86
24	0	5	1	5	1	3.76	853	2.07	2.67	467	1.09	0.90
25	1	5	5	1	0	4.08	787	2.20	2.68	487	1.02	0.92
26	0	1	0	1	1	3.84	847	2.20	2.78	483	1.00	0.89
27	1	1	1	0	0	3.60	660	2.13	2.50	453	1.09	0.98
28	5	0	1	0	0	4.24	880	2.33	2.90	520	0.97	0.82
29	0	1	0	0	5	3.68	633	2.10	2.78	513	0.97	0.93
30	5	1	5	1	0	3.92	827	2.27	2.87	560	1.03	0.89
31	1	0	0	1	1	3.76	727	2.10	2.78	537	1.07	0.92
32	0	0	5	5	5	3.76	640	1.90	2.50	507	0.97	1.01
33	1	5	5	0	1	3.84	747	2.13	2.70	493	1.03	0.87
34	1	1	1	5	1	3.52	753	2.00	2.43	483	1.17	0.92
35	0	5	1	1	5	3.36	547	2.10	2.50	443	1.13	0.94
36	5	5	0	5	0	3.92	840	2.33	3.03	553	0.95	0.79
37	5	5	1	5	5	4.08	573	1.83	2.43	483	0.95	1.00
38	0	0	0	5	1	3.84	753	1.83	2.57	517	0.83	1.04
39	5	0	5	0	5	3.92	727	2.27	2.83	483	0.90	0.88
40	1	5	0	0	0	4.16	827	2.20	2.95	493	1.00	0.87
41	0	5	5	1	1	3.76	847	2.00	2.53	517	0.90	0.94
42	1	0	1	1	0	3.92	867	2.37	2.90	480	0.90	0.80
43	0	1	1	0	1	3.36	633	1.93	2.43	517	1.02	1.06
44	5	1	0	1	5	3.76	653	1.83	2.50	483	0.89	0.98
45	1	1	5	5	0	3.48	720	1.83	2.43	513	1.13	1.06
46	1	5	1	0	5	3.28	587	1.80	2.37	533	0.89	1.10

APPENDIX 3 (continued)

treatments						N	P	K	Ca	Mg	Na	yield
pot No.	levels					%	mg/100 g	%	%	mg/100 g	%	g/pot
	B	Mn	Cu	Zn	Fe							
47	5	1	1	1	1	3.80	720	1.83	2.50	510	0.87	1.00
48	0	0	1	5	0	3.84	767	1.93	2.78	560	0.93	0.93
49	0	5	0	1	0	3.68	767	1.93	2.78	510	1.03	0.98
50	5	5	5	5	1	3.92	800	2.00	2.67	570	0.92	0.96
51	1	1	0	5	5	3.72	600	2.00	2.50	503	1.03	0.95
52	1	0	5	1	5	3.72	620	1.97	2.50	500	1.00	1.00
53	0	1	5	0	0	3.68	727	1.83	2.50	527	1.07	1.01
54	5	0	0	0	1	3.80	707	2.10	2.67	537	1.07	0.99
55	0	5	0	0	1	3.52	660	1.93	2.67	467	0.95	0.94
56	5	5	5	1	5	3.60	653	2.05	2.67	447	0.97	0.95
57	5	0	0	5	5	3.76	653	2.10	2.47	463	1.03	0.98
58	5	1	1	0	5	3.84	773	2.50	2.47	373	1.05	0.80
59	1	5	1	5	0	3.68	800	1.97	2.70	493	1.03	0.96
60	0	1	5	5	1	4.00	680	1.83	2.43	437	1.03	1.02
61	0	0	1	1	1	3.36	707	2.07	2.63	500	1.02	0.97
62	1	0	5	0	0	3.64	707	2.30	2.78	460	1.03	0.74
63	1	1	0	1	0	3.52	760	1.83	2.67	493	0.98	1.05
64	0	5	5	0	5	3.36	633	1.77	2.37	410	0.90	1.09
65	5	5	1	1	0	3.92	873	2.33	2.67	440	0.87	0.82
66	1	1	5	1	1	3.76	827	2.33	2.67	403	0.93	0.85
67	1	5	0	5	1	3.60	787	1.90	2.67	447	1.03	0.97
68	0	0	0	1	5	3.60	680	1.67	2.43	423	0.80	1.01
69	0	1	1	5	5	3.80	653	2.05	2.37	380	1.00	0.90
70	5	0	5	5	0	3.76	887	2.15	2.70	443	1.03	0.87
71	5	1	0	0	0	3.52	773	2.05	2.63	457	0.95	0.96
72	1	0	1	0	1	3.68	787	1.77	2.67	467	0.87	0.94
73	1	5	5	5	5	3.92	667	1.83	2.43	463	1.00	0.92
74	0	5	1	0	0	3.92	887	2.30	2.63	437	0.93	0.84
75	5	1	5	0	1	4.00	853	1.97	2.67	470	0.93	0.87
76	5	5	0	1	1	3.88	840	2.23	2.43	370	1.33	0.74
77	0	0	5	1	0	3.52	720	1.67	2.50	460	0.87	1.04
78	5	0	1	5	1	3.56	720	1.83	2.50	443	0.88	1.01
79	0	1	0	5	0	3.44	713	1.83	2.43	443	1.00	0.96
80	1	1	1	1	5	3.44	587	1.67	2.33	433	0.83	1.06
81	1	0	0	0	5	3.40	593	1.60	2.33	427	0.77	1.14

APPENDIX 4 Chemical composition and yield of Lucerne (expt. 1960) as affected by different combined levels of B, K and Cu, (on dry matter bases)

pot No.	treatments			N	P	K	Ca	Mg	Na	yield
	levels	B	K	Cu	%	mg/100 g	%	%	mg/100 g	mg/100 g
1	1	2	1	1.92	328	1.07	1.30	272	191	1.49
2	1	2	2	2.03	381	1.21	1.40	290	255	1.49
3	2	1	1	1.54	325	0.57	1.35	284	350	1.40
4	6	3	3	2.07	353	1.96	1.15	247	152	1.40
5	3	2	3	1.74	353	0.98	1.20	250	200	1.68
6	1	3	2	1.72	294	1.81	0.99	222	166	1.57
7	5	1	2	2.12	363	0.77	1.38	331	391	1.40
8	2	1	3	1.74	338	0.68	1.38	323	359	1.20
9	6	3	1	2.18	391	2.14	1.09	239	191	1.48
10	4	1	3	2.02	366	0.74	1.40	357	393	1.36
11	2	2	2	1.99	391	1.15	1.31	289	212	1.44
12	6	2	3	2.15	409	1.39	1.39	273	223	1.29
13	7	1	1	2.29	409	0.88	1.56	339	370	1.12
14	3	3	1	1.71	310	1.64	1.01	231	152	1.87
15	4	3	3	1.60	304	1.74	1.04	232	129	1.49
16	4	2	1	1.81	328	1.13	1.30	297	216	1.45
17	4	1	2	1.79	335	0.62	1.45	313	345	1.38
18	3	3	2	1.71	294	1.73	0.94	208	163	1.76
19	3	1	1	1.77	350	0.70	1.47	319	380	1.29
20	7	3	1	2.16	381	2.18	1.13	250	143	1.36
21	7	3	3	2.44	406	2.18	1.20	261	166	1.40
22	6	1	2	2.41	434	0.95	1.48	372	433	1.19
23	1	1	2	1.86	344	0.67	1.45	330	331	1.26
24	7	1	3	2.38	400	0.99	1.42	390	366	1.16
25	2	2	3	1.77	347	1.06	1.34	282	193	1.42
26	1	2	1	1.74	341	0.94	1.33	273	172	1.50
27	6	3	2	1.89	319	1.88	1.07	224	147	1.47
28	4	3	1	2.05	356	2.10	1.10	243	152	1.35
29	6	2	3	2.23	403	1.40	1.42	312	230	1.24
30	4	1	3	2.05	338	0.70	1.42	326	361	1.32
31	1	1	1	1.86	381	0.61	1.46	322	318	1.28
32	7	1	2	2.33	434	0.89	1.46	330	336	1.20
33	7	2	1	2.29	440	1.34	1.39	307	184	1.32
34	5	3	3	1.72	350	1.69	1.09	227	110	1.58
35	6	2	2	1.88	397	1.09	1.41	288	168	1.32
36	4	1	1	2.09	335	0.92	1.38	337	400	1.17
37	5	2	2	1.84	335	1.15	1.42	292	193	1.25
38	5	1	2	2.17	413	0.80	1.46	340	380	1.34
39	3	2	1	1.66	331	0.96	1.31	286	189	1.48
40	5	1	1	2.08	359	0.81	1.44	320	324	1.16
41	1	1	3	2.15	369	0.82	1.45	354	379	1.30
42	2	3	1	1.59	307	1.50	1.01	188	99	1.67
43	3	3	3	2.12	378	2.08	1.10	224	168	1.45
44	7	3	2	2.10	400	1.98	1.13	208	143	1.54
45	1	2	3	1.47	304	0.94	1.27	247	184	1.38
46	1	3	3	1.66	350	1.76	1.05	210	156	1.70

## APPENDIX 4 (continued)

pot No.	treatments			N	P	K	Ca	Mg	Na	yield
	B	K	Cu	%	mg/100 g	%	%	mg/100 g	mg/100 g	g/pot
47	4	2	3	1.68	362	1.02	1.31	290	179	1.48
48	2	1	1	2.02	406	0.70	1.45	357	336	1.41
49	1	3	2	1.72	310	1.69	1.01	222	136	1.49
50	2	2	2	1.67	335	1.04	1.31	296	241	1.62
51	2	2	3	1.67	313	1.01	1.18	262	211	1.44
52	1	3	1	1.72	307	1.67	1.01	194	168	1.68
53	6	1	3	2.26	394	0.86	1.34	340	428	1.27
54	2	2	1	1.69	350	0.86	1.29	276	200	1.47
55	5	2	1	1.91	344	1.16	1.29	304	244	1.36
56	3	3	2	1.73	310	1.77	1.08	229	152	1.55
57	1	1	3	1.94	391	0.70	1.44	357	361	1.30
58	4	2	2	1.86	347	1.07	1.35	293	205	1.42
59	4	1	2	2.04	394	0.75	1.37	341	375	1.43
60	3	1	1	1.75	322	0.66	1.37	330	361	1.30
61	3	3	3	1.60	325	1.66	0.99	212	165	1.71
62	1	1	2	1.89	366	0.68	1.38	347	370	1.34
63	3	3	1	1.63	270	1.75	0.97	209	170	1.63
64	4	2	1	1.82	357	1.14	1.33	290	207	1.37
65	7	2	3	2.30	397	1.31	1.40	324	207	1.16
66	5	3	1	1.58	298	1.57	1.00	204	117	1.57
67	6	3	3	1.75	325	1.72	1.00	217	110	1.68
68	4	3	2	1.44	307	1.54	1.03	207	97	1.68
69	2	1	2	1.99	397	0.70	1.51	391	537	1.50
70	7	1	1	2.54	409	1.01	1.49	411	433	1.29
71	7	2	2	2.12	378	1.36	1.29	321	198	1.24
72	5	2	1	1.79	353	1.13	1.29	297	205	1.37
73	7	3	3	2.11	394	2.18	1.10	246	159	1.40
74	1	1	1	2.11	397	0.73	1.51	362	310	1.36
75	4	2	3	2.01	357	1.02	0.97	304	207	1.33
76	3	2	2	1.87	332	1.07	1.18	297	203	1.47
77	6	2	2	1.88	412	1.15	1.36	299	182	1.32
78	6	2	1	2.00	394	1.28	1.33	299	207	1.22
79	1	2	3	1.73	304	0.79	1.02	258	133	1.46
80	1	3	3	1.66	319	1.67	1.02	197	131	1.70
81	2	3	3	1.63	325	1.66	1.08	197	127	1.55
82	3	1	2	2.46	375	0.81	1.52	355	366	1.18
83	3	1	3	1.88	378	0.61	1.48	345	350	1.24
84	1	3	1	1.87	319	1.68	1.01	229	147	1.67
85	2	1	3	1.96	384	0.66	1.43	352	361	1.33
86	5	3	3	1.72	344	1.83	1.12	216	138	1.45
87	4	2	2	1.75	353	1.08	1.28	275	196	1.40
88	4	3	2	1.55	319	1.55	1.10	192	108	1.60
89	2	3	2	1.70	297	1.98	1.09	207	140	1.29
90	5	2	2	1.81	363	1.12	1.35	258	173	1.35
91	6	1	1	2.15	373	0.78	1.44	308	359	1.26
92	6	1	3	2.19	393	0.83	1.57	320	373	1.19

## APPENDIX 4 (continued)

pot No.	treatments			N	P	K	Ca	Mg	Na	yield
	levels			%	mg/100 g	%	mg/100 g	mg/100 g	mg/100 g	g/pot
	B	K	Cu							
93	3	2	1	1.71	341	0.94	1.43	271	163	1.44
94	5	3	1	1.79	316	1.73	0.99	190	133	1.69
95	4	3	3	1.57	332	1.58	1.13	197	120	1.60
96	5	1	1	1.91	291	0.35	0.63	316	180	1.25
97	6	3	2	1.75	319	1.98	1.04	202	143	1.39
98	3	1	3	1.97	400	0.77	1.67	355	384	1.12
99	3	2	3	1.77	344	1.04	1.42	274	207	1.32
100	2	3	1	1.73	322	1.82	1.07	190	143	1.54
101	2	3	2	1.59	288	1.59	1.12	187	122	1.48
102	7	1	3	2.46	447	0.88	1.63	333	320	1.18
103	4	1	1	2.11	387	0.77	1.57	321	352	1.22
104	6	3	1	2.01	338	1.85	1.09	192	124	1.45
105	6	1	2	2.35	425	1.00	1.53	306	407	1.14
106	7	1	2	2.23	409	0.96	1.46	330	393	1.10
107	5	2	3	1.76	356	1.09	1.43	255	191	1.16
108	2	2	1	1.74	322	0.99	1.37	238	177	1.31
109	6	1	1	2.19	397	0.87	1.49	326	352	1.18
110	3	1	2	2.05	384	0.70	1.47	318	370	1.33
111	5	3	2	1.63	325	1.76	1.09	199	138	1.52
112	2	3	3	1.60	310	1.58	1.14	180	94	1.46
113	7	2	1	2.23	425	1.28	1.31	267	156	1.15
114	7	2	2	2.08	409	1.34	1.41	287	193	1.34
115	7	3	1	1.97	369	1.99	1.14	199	145	1.40
116	5	1	3	2.08	422	0.91	1.63	342	444	1.07
117	1	2	2	1.83	319	1.00	1.31	253	205	1.42
118	2	1	2	1.82	344	0.70	1.48	309	368	1.25
119	4	3	1	1.70	282	1.72	1.04	165	140	1.58
120	5	3	2	2.11	319	1.78	1.01	190	140	1.61
121	5	1	3	1.97	372	0.76	1.39	316	379	1.27
122	7	2	3	2.36	449	1.32	1.25	282	209	1.24
123	3	2	2	1.69	316	1.04	1.25	255	214	1.31
124	6	2	1	1.94	359	1.11	1.50	284	177	1.29
125	7	3	2	2.07	369	2.03	1.21	216	133	1.27
126	5	2	3	1.91	347	1.13	1.29	233	196	1.33