TRACE ELEMENTS

in

PLANT PHYSIOLOGY

WALTHAM, MASS., U.S.A.
Published by the Chronica Botanica Company

PUBLICATIONS DE L'U.I.S.B

Série A.—Compte rendu des Assemblées générales et Actes officiels. (Les Comptes rendus des Assemblées générales I (1919) à VIII (1935) sont épuisés.)

Série B.—Compte rendu des Colloques:

No. 1.—Les éléments rares dans la physiologie des Plantes (oligo-éléments).—Rothamsted, 1947.
2.—Les bases théoriques de la Nomenclature botanique et de la Systématique.—Utrecht, 1948.
3.—L'interaction entre l'oeuf et le sperme.—Milan, 1948.
4.—La Terminologie génétique et cytogénétique.—Stockholm, 1948.
5.—Les bases scientifiques d'une organisation internationale de la lutte biologique.—Stockholm, 1948.
6.—Problèmes du développement de la croissance et de la régénération du système nerveux.—Chicago, 1949.
7. Le rôle des Anaérobies dans la nature.—Bruxelles, 1949.
8.—Problèmes de l'embryologie physio-génétique.—Berne, 1949.

Série C.—Publications diverses de l'U.I.S.B. et de ses Commissions et Sections.

No. 1.—Systematic check list of Mite genera and type species, by C. D. Radford, 1950.

Toutes ces publications pourront être demandées au Secrétariat général de l'Union international des Sciences biologiques, 57, rue Cuvier, Paris-V•, France.

• LOTSYA — A Biological Miscellany •

Edited by Frans Verdoorn — Volume 3

DR. J. P. LOTSY

TRACE ELEMENTS
in
PLANT PHYSIOLOGY

A Symposium organized by the
International Union of Biological Sciences
with Support of UNESCO
at the Rothamsted Experimental Station,
Rothamsted, Harpenden, Herts., England
November 5-6, 1947

With Contributions By:—

D. I. Arnon
H. Burström
J. Erkama
L. Gisiger
E. J. Hewitt
E. A. Jamalainen
J. Lavollay
M. P. Löhnis
D. Mulder
E. G. Mulder
B. Němec
L. Seekles
F. Steenbjerg
T. Wallace

INTERNATIONAL UNION of BIOLOGICAL SCIENCES
Series B • COLLOQUIA • No. 1
UNION INTERNATIONALE des SCIENCES BIOLOGIQUES

TRACE ELEMENTS

in

PLANT PHYSIOLOGY

with a Report of the Proceedings by

T. Wallace, C.B.E., M.C., D.Sc., F.R.I.C.,
Agricultural and Horticultural Research Station, Bristol University, Long Ashton, Bristol

and a Foreword by M. J. Sirks

1950

WALTHAM, MASS., U.S.A.

Published by the Chronica Botanica Company

First published MCML
By the Chronica Botanica Company
of Waltham, Mass., U.S.A.

Authorized Agents:—

New York, N. Y.: STECHERT-HAFNER, INC.,
31 East 10th Street.
San Francisco, Cal.: J. W. STACEY, INC.,
551 Market Street.
Toronto, Ont.: WM. DAWSON SUBSCRIPTION SERVICE LTD.,
60 Front St. West.
Mexico, D. F.: AXEL MORIEL SUCRS.,
San Juan de Letran 24-116; Ap. 2762.
Caracas: SUMA S. A.,
Sabana Grande, 102, "El Recreo".
Bogota and Medellin: LIBRERIA CENTRAL.
Lima: LIBRERIA INTERNACIONAL DEL PERU,
Casa Matriz. Boza 879; Casilla 1417.
Santiago de Chile: LIBRERIA ZAMORANO Y CAPERAN,
Compañía 1015 y 1019; Casilla 362.
Rio de Janeiro: LIVRARIA KOSMOS,
Rua do Rosario, 135-137; Caixa Postal 3481.
Sao Paulo: LIVRARIA CIVILIZAÇAO BRASILEIRA,
Rua 15 de Novembro, 144.
Buenos Aires: ACME AGENCY, SOC. DE RESP. LTDA.,
Suipacha 58; Casilla de Correo 1136.
London, W. C. 2: WM. DAWSON AND SONS, LTD.,
Chief Agents for the British Empire,
Cannon House, Macklin Street.
London, W. C. 1: H. K. LEWIS AND CO., LTD.,
136, Gower Street.
Uppsala: A.-B. LUNDEQUISTSKA BOKHANDELN,
Drottninggatan 2.
Groningen: N. V. ERVEN P. NOORDHOFF,
Chief Agents for Continental Europe.
Paris, VI: LIBRAIRIE P. RAYMANN & Cie.,
17, Rue de Tournon.
Hamburg 13: BUCH- UND ZEITSCHRIFTEN-UNION mbH.,
Harvestehuder Weg 5.
Basel: BUCHHANDLUNG WEPF & CO.,
Eisengasse 5.
Torino: ROSENBERG & SELLIER,
Via Andrea Doria 14.
Madrid: LIBRERIA J. VILLEGAS,
Preciados, 33.
Lisbon: LIVRARIA SÁ DA COSTA,
100-102, R. Garrett.
Moscow: MEZHDUNARODNAJA KNIGA,
Kuznetski Most 18.
Peiping (O): FRENCH BOOKSTORE,
½ T'ai-chi-ch'ang, Ex-Legation Quarter.
Tokyo: MARUZEN COMPANY, LTD.,
6, Tori-Nichome Nihonbashi; P. O. Box 605.
Calcutta, Bombay, and Madras: MACMILLAN AND CO., LTD.
Johannesburg: CENTRAL NEWS AGENCY, LTD.,
Commissioner & Rissik Streets; P. O. Box 1033.
Sydney: ANGUS AND ROBERTSON, LTD.,
89 Castlereagh Street, Box 1516D.D. G.P.O.
Melbourne, C. 1: N. H. SEWARD, PTY., LTD.,
457, Bourke Street.
Wellington: WHITCOMBE & TOMBS, LTD.

Made and printed in the U.S.A.
Designed by Frans Verdoorn

PREFACE

by T. Wallace, C.B.E., M.C., D.Sc., F.R.I.C.

Agricultural and Horticultural Research Station, Bristol University, Long Ashton, Bristol

The symposium on trace elements held at the Rothamsted Experimental Station in November 1947 was a notable event to those who were privileged to attend, as it brought together, after the isolation of the war years, a number of workers from countries in western Europe, Great Britain and the U.S.A., who had been engaged with important investigations in this field during the war. It was also appropriate that the Rothamsted Station should have been selected by the International Union as the home of the conference, as this enabled the members to revive old memories and to meet distinguished Rothamsted colleagues, of whom must be specially mentioned Dr. Winifred Brenchley and Dr. Katherine Warington.

It is with deep sorrow that we record in this volume the death of the Chairman, Professor B. Němec, whose charm and geniality were enjoyed by all members of the conference.

Trace element problems have claimed the attention of plant physiologists for many decades and before the war the importance of deficiencies of iron, manganese, copper, zinc and boron, in restricting the production of various crops in many countries, had been recognised both by scientists and farmers. Under wartime conditions of crop production, the importance of trace elements was greatly accentuated. This was due to a number of factors: the urgent need for greatly increased production which necessitated the large scale production of special crops; intensified methods of cultivation; the extension of cropping to poorer soils; and the shortages of particular fertiliser materials.

In England, prior to 1939, trace element problems in crops were of little more than academic interest, with the exception of iron deficiency in some horticultural crops, mainly in fruit trees, and boron deficiency in sugar beet, swedes and turnips.

During the war, however, boron deficiency in sugar beet, mangolds and many brassica crops was widespread and of serious consequence, and manganese deficiency was also prevalent in arable crops over many areas of ploughed up grassland and necessitated special control measures.

Other problems were also noted which have subsequently been shown to be due to deficiencies of other trace elements: failures of cereals on Fen soils due to copper deficiency, of fruit trees due to deficiencies of copper and zinc, and of cauliflowers and broccoli due to deficiency of molybdenum.

The symposium recorded similar experiences in some of the European countries: in particular, the widespread occurrence of deficiencies of copper, boron and manganese, and the practical difficulties of curing these under wartime conditions.

Other post-war symposia and conferences in the U.S.A. and elsewhere(1,2,3) have indicated the world-wide interest in, and importance of, trace elements in food production, and the records of these should provide useful data for further studies of scientific and practical problems.

In the past, investigations of trace element problems have been mainly of two kinds: those carried out to throw light on crop failures and others undertaken to ascertain the effects on plant growth of elements other than those generally recognised as plant nutrients. Arising from the former, a vast body of practical data relating to the effects of trace elements on crops has been obtained but relatively little information of the rôles of these in plant growth has resulted, whilst in the latter studies progress has been slow because of the difficult nature of the problems involved. At the present time, it is possible to recognise by various diagnostic methods the main practical problems arising from trace elements in field crops and to provide efficient remedies for most pathological conditions associated with them (methods for controlling iron deficiency, however, are still unsatisfactory). The great need is thus for fundamental studies and for these it will be necessary to develop highly refined methods, as for example, to determine whether other elements must be addded to the present list of essential trace elements. Difficult problems in biochemistry also await solution and, in particular, it may be expected that the intensification of research on the rôle of trace elements in enzyme systems will yield a rich harvest of knowledge.

In view of the above statements, it is not surprising that the main topics discussed at Rothamsted referred to methods and to practical problems of trace elements in crop production.

The present volume records the papers presented at the conference and some of the main points arising from these that were discussed.

It was appropriate that Professor Němec, in his opening address, should have touched on some of the historical aspects of trace elements research and, in particular, made reference to the pioneer water culture experiments of Sachs and Stoeckard in Saxony, from which have developed the elaborate techniques in use today. He also referred to the large number of mineral elements that may occur in plants and to the difficulties this raises in fundamental studies. In discussing 'accumulator' plants he illustrated his remarks by exhibiting a sample of gold obtained from the ash of *Equisetum palustre* from volcanic soils in Bohemia and also referred to the concentration of droplets of metallic mercury in the seed capsules of *Holosteum umbellatum* on some soils.

Three papers dealt primarily with methods used in the diagnosis of the mineral status of plants and in the growing of plants in sand and solution cultures for fundamental studies. The writer outlined the main features of the visual diagnosis method and illustrated its application to some problems of plant nutrition. E. J. Hewitt described his sand culture methods for large-scale investigations, including the highly refined methods used in experiments on molybdenum deficiency. Subsequent to the conference these methods have been further developed and techniques are now available for all the trace elements. D. I. Arnon drew attention to the experimental difficulties in proving the essentiality of trace elements in plant nutrition, and illustrated his points from the work of Stout and Arnon on molybdenum. Two important points that arose from this discussion were the formulation of criteria of essentiality of mineral nutrients and the proposal to use the term 'micro-nutrient element' to replace such terms as 'trace element,' 'minor element' etc.

Four papers presented are of importance in focussing attention on the functions of trace elements in the metabolic processes of plants.

E. G. Mulder described his investigations relating to the effects of copper and molybdenum on *Aspergillus niger* and on higher plants, and indicated the rôle of molybdenum in the reduction of nitrate in plants and in the fixation of atmospheric nitrogen by *Azotobacter.* Similar results for nitrate have been obtained by other workers and Nicholas,(4) using Mulder's strain of *Aspergillus,* has been able to prepare standards to determine the availability of the trace elements Cu, Zn and Mo in soils, and has applied the method with success in investigating instances of deficiency of these nutrients. These studies on molybdenum have already found important applications in agriculture, in Australia, New Zealand and Great Britain.(5,6,7,8)

J. Lavollay has also used *Aspergillus niger* in his studies to determine the coefficient of action of mineral nutrients, and has been able to demonstrate quantitative interactions of nutrients and similarity of action between potassium and rubidium. This last point is of interest in connection with investigations of G. and D. Bertrand,(9) reporting the widespread occurrence of rubidium in plants.

J. Erkama described comprehensive investigations relating to the rôles and interactions of copper, manganese and iron. He concluded that Cu and Mn react differently on iron, reactions with the former occurring on the protoplasm and with the latter in the vacuole sap. Erkama, like many other investigators, believes that relationships of nutrients must be studied in attempting to determine their individual functions. In this connection the complexities of such investigations must be stressed and the difficulties that arise in distinguishing between reactions occurring in the substrate and within the plants.

H. Burström discussed experiments relating to the action of nitrogen, manganese and phosphorus on roots, in which he has been able to distinguish separate effects for each element. Following from the results obtained with manganese and nitrogen, it would appear of interest to extend the work to include molybdenum, and to examine the interactions of manganese and molybdenum in view of their special relationships in acid soils.(8)

Trace element problems in crops occurring in European countries were discussed in five contributions.

L. Gisiger described the conditions associated with crop failures in Switzerland during the war due to deficiencies of boron and manganese respectively. Overliming was of importance in causing or accentuating these difficulties. Gisiger concluded from his experiments that the damage caused by overliming results from the action of the increased concentration of the $OH^{\cdot}$ anion. The anion exerts a hydrating action on the plant roots which boron tends to counteract. Boron deficiency effects result when the $OH^{\cdot}$ anion is in excess in relation to boron. In the case of manganese, the $OH^{\cdot}$ anion is regarded as reducing the availability of manganese over an intermediate range of *p*H.

It may be noted that alternative hypotheses have been advanced to explain the lime effect in both cases.(10,11)

F. **Steenbjerg** outlined the main problems of trace elements occurring in Denmark and summarised some of the results of his extensive investigations, particularly those concerning copper. He emphasised, in particular, the shape of the curve—which is 'S' shaped—relating dry matter production of plants and their total copper content.

M. **Löhnis** reported extensive data relating to the damaging effects on *Phaseolus vulgaris* and other crop plants of excess manganese in acid soils, and endeavoured to relate the susceptibility of crops to deficiencies and excesses of the element to 'ease of absorption of manganese.' Difficulties to this explanation are, however, presented by crops such as beans (*Phaseolus*) and strawberry.

Extensive pot culture and field experiments on injurious factors in acid soils have also been in progress at Long Ashton for a number of years.(12)

The contribution of D. **Mulder**, presented by M. **Löhnis**, concerned the occurrence of zinc deficiency in fruit trees in Holland, Hungary, Denmark and Switzerland. This deficiency has also been identified in fruit trees, mainly apples, in several areas of England and seems to be a worldwide problem in fruit crops, particularly *Citrus* and apples.

Trace element problems, described by E. A. **Jamalainen**, are also of great practical importance in Finland, especially deficiency of boron, which presented very serious problems during the war when supplies of boron could not be obtained from abroad.

A point of particular interest is that boron deficiency in Finland occurs on strongly acid soils, though the effects are, as usual, increased by liming. Copper deficiency is also of importance, but manganese deficiency is of very local occurrence due to the acid reaction of most of the Finnish soils.

The paper of L. **Seekles** dealt with trace element problems of farm stock. Though not directly concerned with plant physiology, this contribution served to show the importance of plant nutrition studies in helping to solve problems of animal and human nutrition. The similarity of the patterns of the problems in plants and animals were obvious—the simple deficiency, the 'conditioned' deficiency, the excess, the interactions, the pathological symptoms and finally the rôle of the nutrients in the enzyme systems. The need for collaboration between plant and animal physiologists, to whom may also be added soil scientists and climatologists, is now well recognised, and this coordination is of vital necessity to ensure the progress that is needed to increase the world supplies of food sufficiently fast to meet the needs of the rapidly expanding world population.

References

1. Symposia on Minor Elements, 1945: Soil Science 60.
2. McCollum-Pratt Institute, 1950: Symposium on Copper Metabolism (in press).
3. British Commonwealth Specialist Conference in Agriculture, 1949: Plant and animal nutrition in relation to soil and climatic factors (in press).
4. Nicholas, D.J.D., 1950: J. Hort. Sci. (in press).
5. Anderson, A. J. and Oertel, A. C., 1946: Commonwealth of Australia C.S.I.R. Bull. 198.
6. Davis, E. B., 1945: Nature 156: 392.
7. Jones, J. O., 1950: *Ibid.* 165: 248.
8. Plant, W., 1950: *Ibid.* 165: 553.
9. Bertrand, G. and D., 1950: C. R. 230: 343.
10. Jones, H. E. and Scarseth, G. D., 1944: Soil Sc. 57: 15.
11. Quastel, J. H., 1946: The Royal Inst. of Chemistry of Gt. Britain and Ireland. Lecture on Soil Metabolism.
12. Wallace, T., Hewitt, E. J. and Nicholas, D. J. D., 1945: Nature 156: 778.

FOREWORD

by M. J. Sirks, Ph.D.

President, International Union of Biological Sciences
Genetisch Instituut der Rijksuniversiteit, Haren near Groningen, Netherlands

The promotion of international coöperation by the organization of symposia in special fields of limited scope is one of the many tasks the International Union of Biological Sciences has undertaken. Our large international congresses, important as they are for science and scientists, often do not offer enough opportunity (and they should perhaps not endeavour to do so) for extensive discussions by workers in various special fields.

The Bureau of the International Union of Biological Sciences is greatly indebted to Dr. Ogg, *the Director of the Rothamsted Experimental Station, for the kind hospitality offered by his great station to the contributors to this, our first symposium, to Professor* Wallace *of Long Ashton, who collected the papers delivered by the members and prepared the notes reporting on the discussions, and to Dr.* Verdoorn, *Botanical Secretary of the I.U.B.S. and Managing Editor of Chronica Botanica, who, with other members of the editorial board of Chronica Botanica, prepared the manuscript and illustrations for press and produced this volume in his new serial,* "Lotsya – A Biological Miscellany."

I trust that this symposium will be welcomed by those who are interested in recent advances in our knowledge of the fascinating rôle of trace elements in pure and applied biology.

The Chronica Botanica Co., International Plant Science Publishers

Noteworthy Books:—

Baldwin: **Forest Tree Seed**	$5.00
Bawden: **Plant Viruses and Virus Diseases,** *third, entirely revised edition*	6.00
Camp et al.: **International Rules of Botanical Nomenclature**	3.50
Chardon: **Los Naturalistas en la America Latina, Vol. I**	4.50
Chester: **The Cereal Rusts**	5.00
Clements, Martin and Long: **Adaptation and Origin in the Plant World**	6.00
Condit: **The Fig**	5.00
Copeland: **Genera Filicum**	6.00
Correll: **Orchids of North America**	7.50
Crafts, Currier, and Stocking: **Water in the Physiology of Plants**	6.00
Dachnowski-Stokes: **Peat** (in press)	5.00
Darwin: **A Naturalist's Voyage with the Beagle** (1839/1951) (in press) ca.	4.75
Elliott: **Bacterial Plant Pathogens** (in press) ca.	6.00
Erdtman: **Introduction to Pollen Analysis**	5.00
Ewan: **Rocky Mountain Naturalists**	5.00
Finan: **Maize in the Great Herbals**	3.00
Foxworthy: **Forests of Tropical Asia** (in press)	6.00
Frear: **Chemical Insecticides**	6.50
Frear: **Chemical Fungicides and Plant Insecticides**	5.50
Fulford: **Bazzania in C. and S. America**	5.00
Guilliermond: **Cytoplasm of the Plant Cell**	5.00
Gundersen: **Families of Dicotyledons**	4.75
Hoagland: **Inorganic Nutrition of Plants**	4.50
Honig, Verdoorn et al.: **Recent Advances in Tropical Biology and Agriculture** (in press)	6.00
Horsfall: **Fungicides and their Action**	5.00
Howes: **Vegetable Gums and Resins**	5.00
Jessen: **Botanik der Gegenwart und Vorzeit in Culturhistorischer Entwickelung** (1864/1948)	6.00
Johansen: **Plant Embryology**	6.00
Kelley: **Mycotrophy of Plants**	4.50
Knight: **Dictionary of Genetics**	4.50
Lloyd: **Carnivorous Plants**	7.00
Moldenke: **Plants of the Bible** (in press) ca.	6.00
Murneek et al.: **Vernalization and Photoperiodism**	4.50
Nickerson et al.: **Biology of Pathogenic Fungi**	5.00
Sarton: **Guide to the History of Science** (in press) ca.	5.00
Schopfer: **Plants and Vitamins**	5.00
Sirks: **The Evolution of Biology** (in press) ca.	4.75
G. M. Smith et al.: **Manual of Phycology** (shortly) ca.	6.00
Stevens: **Agricultural Plant Pathology** (in press) ca.	4.75
Verdoorn et al.: **Plants and Plant Science in Latin America**	6.00
Waksman: **The Actinomycetes**	5.00
Wallace et al.: **Rothamsted Symposium on Trace Elements**	$4.50
Wilde: **Forest Soils and Forest Growth**	5.00
Wulff: **Historical Plant Geography**	5.00
ZoBell: **Marine Microbiology**	5.00

* * *

CHRONICA BOTANICA, An International Collection of Studies in the Method and History of Biology and Agriculture (annual subscription)	7.50

Special issues of Chronica Botanica which are available to non-subscribers:—

Arber: **Goethe's Botany**	2.00
Asmous: **Fontes Historiae Botanicae Rossicae**	1.25
BIOLOGIA, An International Year-Book (per annual volume)	4.75
Browne: **A Source Book of Agricultural Chemistry**	5.00
Browne: **Thomas Jefferson and the Scientific Trends of His Time**	1.25
DeTurk (ed.): **Freedom from Want, A Symposium**	2.00
Howard: **Luther Burbank, A Victim of Hero Worship**	3.75
Jack: **Biological Field Stations of the World**	2.50
Lanjouw—Sprague: **Botanical Nomenclature and Taxonomy, A Symposium**	2.50
Merrill: **Merrilleana—Selected General Writings**	4.00
Rafinesque: **A Life of Travels**	2.50
Reed: **Jan Ingenhousz—Plant Physiologist**	3.00
Rickett: **Botanical Expedition to New Spain**	2.50
Saint-Hilaire: **Voyages au Brésil**	2.00
Stevens: **Factors in Botanical Publication and other Essays**	2.00
Vavilov: **Selected Writings** (in press)	7.50
Verdoorn et al.: **Plant Science and Plant Scientists during the Second World War** (in press) ca.	3.75
Verdoorn (ed.): **21st World List of Plant Science Institutions and Societies** (in press) ca.	4.00
Wyman: **Arboretums of N. America**	1.50

* * *

Imports which we handle for the Americas only:—

Hultén: **Plants in NW Europe**	14.50
Lanjouw: **Synopsis of Proposals**	2.00
Lindquist: **Genetics in Swedish Forestry Practice**	3.50
Meyer-Abich (ed.): **Biologie der Goethe-Zeit**	5.50
Nelson: **Introductory Botany**	3.75
Verdoorn et al.: **Manual of Bryology**	9.50
Verdoorn (ed.): **Manual of Pteridology**	11.00
Weevers: **Fifty Years of Plant Physiology**	5.00

Catalogue and Book Department List on Request

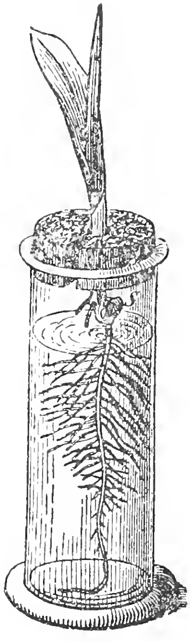
The Chronica Botanica Co., Waltham, Massachusetts, U.S.A.

CONTENTS

LIST OF ILLUSTRATIONS

* * *

LIST OF TABLES



OPENING ADDRESS

by the late B. Němec, D.Phil.,

formerly Professor of Plant Physiology, University of Prague

It is an honour and pleasure, indeed, to open this symposium on behalf of the International Union of Biological Sciences. I sincerely welcome all those attending this Symposium on Trace Elements in Plant Physiology. We are very glad our meeting is organised in close collaboration with the Rothamsted Experimental Laboratory where so much has been done to promote our knowledge of the nutrition of plants. I wish to thank Dr. Ogg, Director of the Rothamsted Laboratory, very much for his kind words of welcome.

Ninety years ago Julius Sachs began, in Prague—as private assistant of T. Purkynje—his experiments on water cultures. He studied the formation of lateral and adventitious roots and as he saw roots growing very well in spring water, he supposed it would be possible to grow plants in spring water to the ripe fruit stage. He changed the water at least twice each day and was able to demonstrate on the occasion of an Agricultural Congress in Prague (1858) surprising results of his cultures. But it had been the idea of Stöckhardt in Tharandt to dissolve salts in distilled water and so to determine the essentiality of some mineral elements for plants. Sachs, who was appointed in 1859 as assistant at Tharandt, was soon able to demonstrate that six mineral elements (S, P, K, Mg, Ca, Fe) are essential for higher plants. Water cultures have been more useful than the sand cultures of Salm-Horstmar. Plant physiologists, for a long time, believed that only six mineral elements were essential for plant nutrition. In papers from the last century, dealing with the analysis of plant ash, usually only these six elements are mentioned. In the first edition of Wolff's *Aschenanalysen* (1871) other elements are seldom mentioned.

Meanwhile in various species and in plants from different localities other mineral elements have been discovered and before the last war Bobko and Belwousow developed the hypothesis that *all* elements are present in plants though some of them in extremely small quantities. Nearly all elements have been found in plant ashes, but it has not yet been proved that they are all indispensible for the nutrition of plants. At first it was assumed that accessory elements, in small amounts, only stimulate plant growth. In the case of many elements, as Paracelsus already stated, it is really the question of the quantity present as to whether a substance acts as a poison or as a stimulant.

For water cultures refined methods of purifying salts and water and the provision of suitable vessels have been necessary. It has now been possible to prove that there are, in addition to the classical elements, still other elements essential for normal plant growth. Just twenty years ago (1927) Miss Brenchley concluded that boron in small quantities is essential for at least some of the higher plants. She concluded further (1927) that copper compounds were poisonous for higher plants except in great dilution, when the element may exert a stimulating action. Since Miss Brenchley's work was published, it has been shown that copper is essential for many species, at least in some stages of their development. Miss Brenchley also concluded (1929) that in soil cultures many plants respond to zinc salts, and it is now certain—as for manganese and molybdenum—that both copper and zinc are essential for many plants.

There are perhaps still other mineral elements (Ti, Al, Rb) essential for plant growth. But plants absorb and accumulate elements, which are very probably without importance for their nutrition. We have shown in our laboratory that many plants absorb and accumulate gold, though it is not essential for them. It can in great dilution stimulate the growth of some plants (*Zea Mays*), but plants grow well without it. The soil, where we have collected our plants (Oslany in Slovakia), contains in 100 tons only 2 g gold, but 10 kg ash of *Equisetum palustre* contains 0.61 g gold. A comprehensive paper on gold in living organisms has been published in Microchimica Acta by T. Babička (Vol. 5, 1943).

Investigations on essential trace elements are of considerable importance in agriculture. There are localities where the soil is very low in these elements and plants grown on such soils appear sick. The effect of addition of boron, copper, zinc or manganese as fertilizers is often surprising. It is thus easily understood why such trace elements are now added to synthetic fertilizers or directly to the soil. In Czechoslovakia, *e.g.*, boron and manganese are added to superphosphates.

In small countries, where we find a variety of geological formations, as in Bohemia, the soil generally contains all essential trace elements, except in some special localities. In some areas of Bohemia a corky growth in apples occurs and in Moravia and Slovakia apricot trees suffer from 'apoplexy' (sudden death). To prevent this the soil around the tree must be mixed with powdered copper sulphate or moistened with a solution of it at an early stage. Very good results have been obtained when, in planting young trees, the soil has been mixed with small quantities of powdered copper sulphate, zinc sulphate and boron.

I will not discuss the question of the rôle of micro-elements in the living plant. There are many problems and I hope they will be discussed during our meeting. I hope that our discussions will provide many valuable contributions to the manifold problems of micro-elements, both from a theoretical and practical point of view.

THE APPLICATION OF THE METHOD OF VISUAL DIAGNOSIS TO TRACE ELEMENT PROBLEMS IN PLANTS

by T. WALLACE, C.B.E., M.C., D.Sc., F.R.I.C.,
Agricultural and Horticultural Research Station, Bristol University, Long Ashton, Bristol

Introduction:—My interest in the application of visual methods to problems of the mineral nutrition of plants began in 1920, when I commenced investigations on the nutrition of fruit crops. Previous experiments by other workers over a long term of years, following the orthodox methods of field trials, had produced very meagre results and added little to the knowledge of the problems involved.

This led me to attempt to obtain some basic data by means of sand cultures and in the initial stages I was particularly interested in producing specimen plants deficient in each of the major plant nutrients. These early results showed clearly that each deficiency produced distinctive and characteristic visual effects, and later work showed that these visual results could be usefully applied to field problems of fruit crops.

The experiments were subsequently extended to embrace a wide variety of farm and market garden crops, and to include problems relating to both major and trace elements.

The particular aspects of plant nutrition which have received attention in our experiments are as follows:

1) The status of mineral nutrients in crop plants and the effects of manures and fertilisers and of cultural treatments on mineral nutrition.
2) Pathological conditions resulting from defective mineral nutrition.
3) The development of diagnostic and curative methods for use in the field.

During the course of our work it has been apparent that to solve many of the problems which presented themselves it was desirable to use a number of methods which could be used in complimentary and confirmatory rôles. These are as follows: visual diagnosis; foliage spraying and tissue injection with compounds of nutrient elements; quick chemical tests on plant tissues; chemical analysis of plants and plant parts (especially leaves); soil tests; field trials with fertilisers and special indicator plants.

To those of you who are interested in the details of these methods, I would refer you to my book "The Diagnosis of Mineral Deficiencies in Plants" (1).

The Visual Method, Its Basis and Use:—Before proceeding to discuss the application of the visual method to problems of trace elements in plants it may be useful to refer to the basis of this method and to the way in which it is applied to nutritional problems.

The visual method has as its basis the fact that when plants are deficient in any one of the essential mineral nutrients or when elements are present in excess they develop well defined and often characteristic symptoms of the deficiency or excesses. The effects produced are generally most clearly seen in the leaves, but they may also show in shoots, stems, flowers, fruits and roots. Thus leaves are usually particularly important in applying the visual method, but all parts of plants may be used, according to the particular deficiency or excess.

The symptoms produced allow of some grouping and classification but to use the method extensively a large amount of detailed knowledge for particular crops is necessary.

The symptoms are established by growing plants in solution cultures or in sand under strictly controlled conditions of nutrition and are later checked, where possible, under field conditions by means of special fertiliser plots and by instances occurring in general farm practice. In making these checks the other complementary methods are generally included. The visual method may be applied both to intensive laboratory studies in plant physiology and pathology involving mineral nutrition, and to practical field problems of crop nutrition. Examples of the former uses will be given later whilst a detailed description of the application of the method in the field is given in my book.

The Application of the Visual Method to Trace Element Problems:—The manner in which the visual method has been applied in our investigations to trace element problems in plants may be discussed under four headings:

a) Deficiencies.

b) Excesses—causing direct toxicities and induced deficiencies.
c) Relationships of trace elements to other nutrients.
d) Soil acidity effects.

Deficiencies.—Many effects of deficiencies of individual trace elements, like those arising from deficiencies of the various major elements, are characteristic and can be used for diagnostic purposes. Well known examples are 'Heart Rot' of sugar beet and mangolds, 'Cracked Stem' of celery and 'Corky Core' of apples due to boron deficiency; 'Little Leaf' of fruit trees due to zinc deficiency; and 'Wither Tip' of oats due to copper deficiency. In our investigations we have been able to distinguish many characteristic effects resulting from deficiencies of iron, manganese and boron, some of which are illustrated here today, whilst our preliminary studies with molybdenum promise to be equally fruitful of results. It should also be mentioned that in some instances more than one deficiency may be recognised visually on a single plant.

I should like to stress the point that a knowledge of these effects may give considerable guidance in laboratory studies and may also save considerable time, effort and expense in field investigations. These two points will become evident in considering some of the points which follow.

Excesses.—The effects of excesses of certain trace elements have been examined on a number of plants and here again many effects produced have been found to be characteristic and easily recognised visually for various plants. Thus excess of boron produces a very characteristic type of marginal scorch on the older leaves of many plants. Excess of manganese produces spotting of the leaves of cereals and potato; stem and petiole lesions on potato and tomato; chlorotic mottling of the leaves of sugar beet, runner bean and many of the Brassicae; and also incurling and necrotic spotting of the leaf margins, and distortion and suppression of the laminae of the leaves of Brassicae (2, 3). Excess of aluminium results in visual effects on leaves which are similar to those due to phosphorus deficiency whilst roots are characteristically stubby and lacking in fibre (10).

It has also been shown by the visual method that two distinct effects may result from excesses of trace elements, namely direct toxic effects, such as those above, and induced deficiencies of other elements. Thus it has been demonstrated that an excess of manganese, zinc, copper, cobalt, nickel or chromium, in addition to producing direct toxic effects, may also induce a deficiency of iron (4). In these instances the latter deficiency has been confirmed by painting and spraying treatments of the leaves with sulphate of iron. The problem of relationships is further discussed under *c*.

Relationships of trace elements to other nutrients.—Various relationships have been shown to exist in plants by means of the visual method between various trace elements and between individual trace elements and essential major elements, and pointers thus provided for physiological studies.

The Fe/Mn ratio, first demonstrated by Somers and Shive (5) and later by Pearse (6) and others, is perhaps the best known example. Hewitt (4) at Long Ashton has shown by the visual method that other metals, notably copper, zinc, cobalt and nickel are much more effective than manganese in inducing iron deficiency.

Interesting relationships between trace elements and major nutrient elements have also been demonstrated visually, notably Fe/K, Mn/Ca and Al/P. At varying levels of iron and potassium it has been shown on potato that, when both are at a low level, iron deficiency is evident by chlorotic tip foliage whereas, when potassium is at a high level and iron remains inadequate, the deficiency shows as a slight paling of the whole of the foliage (7). In the case of manganese and calcium it has been demonstrated that, with manganese in excess, its toxic effects are greatly decreased by a high level of calcium (3).

When aluminium is present at toxic levels the appearance of the leaves and shoots reflect the condition of phosphorus deficiency, which can be shown by chemical tests to exist in these organs (3).

Soil acidity effects.—An attempt has been made, by applying the visual method in conjunction with sand cultures, to determine the causes of various injurious effects produced in crops by soil acidity. Beginning with the viewpoint that the effects might result from deficiencies of the basic elements, calcium, magnesium, potassium and sodium, and of nitrogen and phosphorus, and of excesses of hydrogen ions, manganese or aluminium, various crops have been grown in sand culture and subjected to appropriate nutrient treatments to examine the effects of these various possibilities (2, 3, 8, 10). The visual effects produced have been compared with effects observed in crops suffering from soil acidity in the field (9). The results obtained with potatoes, cereals, various Brassicae, runner beans and sugar beet have shown how far excesses of the two trace elements manganese and aluminium are likely to be of importance in the soil acidity problem for these crops (10). Thus for potatoes neither appears of great importance, calcium deficiency being the major factor, though manganese toxicity effects are sometimes evident in the field. With cereals, excesses of both aluminium and manganese are important, particularly for barley but only slightly for oats. For many of the Brassicae manganese toxicity is a serious factor, but calcium deficiency may also be important, and the two effects may occur together. For runner beans manganese toxicity is responsible for the prevalent effect of 'Scald' (8). In the case of sugar beet, complete failures are often associated with the excess aluminium type of plant, but the mottled chlorotic condition which results from manganese toxicity is also of common occurrence, whilst calcium deficiency is only rarely observed.

Conclusion:—In introducing to you for discussion the subject of the application of the visual method to trace element problems of plants, I should like to emphasise the fact that at Long Ashton we realise clearly that the visual method has its limitations and is most effective when used in conjunction with other recognised procedures. I trust, however, that the points I have brought before you will serve to show that the visual method can provide valuable data in the investigation of many problems of plant nutrition in which trace elements are concerned.

Effects produced by deficiencies, excesses, relationships of trace elements and of soil acidity factors, described in the above text, were illustrated by numerous photographs from sand culture experiments carried out at Long Ashton Research Station.

References

1. WALLACE, T., 1943, 1944: The diagnosis of mineral deficiencies in plants—a colour atlas and guide (London: H. M. Stationery Office).
2. HEWITT, E. J., 1945: Long Ashton Ann. Report, p. 50.
3. HEWITT, E. J., 1946: Long Ashton Ann. Report, p. 51.
4. HEWITT, E. J., 1948: Nature 161:489.
5. SOMERS, I. I. and SHIVE, J. W., 1942: Plant Phys. 17:582.
6. PEARSE, H. L., 1944: Farmg. S. Africa 19:688.
7. JONES, E. W. and HEWITT, E. J., 1949: Long Ashton Ann. Report, p. 49.
8. WALLACE, T., HEWITT, E. J. and NICHOLAS, D. J. D., 1945: Nature 156:778.
9. WALLACE, T., DAVIES, W. M., NICHOLAS, D. J. D. and HEWITT, E. J., 1946: Long Ashton Ann. Report, p. 61.
10. HEWITT, E. J., 1947: Long Ashton Ann. Report, p. 82.

Points from Discussion of Paper by Prof. Wallace

Dr. Mulder:—

1) Your pictures with iron deficiency in oat plants showed a striation of the leaves. In Holland I have seen symptoms of acidity damage which was similar to these iron deficiency symptoms. They occurred in natural soil as well as in sand-peat mixtures.

2) Magnesium deficiency in acid Dutch soil is much increased by adding potassium salts and still more by adding nitrogen as an ammonium compound. Nitrates always have a beneficial effect on the magnesium supply of the plant probably because of an increased magnesium absorption.

Prof. Wallace:—

1) We also have noted symptoms resembling iron deficiency in cases of soil acidity damage, especially in oats, but when we have attempted to control the symptoms by spraying with iron solutions they have also disappeared without treatment and so our results have been inconclusive.

2) High potash conditions have also accentuated magnesium deficiency in our experience and we should expect ammonium salts to give a similar reaction from the viewpoint of NH_4 being a competing base. In sand culture experiments nitrate N has increased the intake of magnesium and, in both greenhouse and field crops, those receiving high levels of nitrogenous manuring have suffered less from magnesium deficiency than comparable low nitrogen crops.

Prof. Seekles:—

1) Damage to plants either by a deficiency or an excess of manganese at a certain *p*H value could be explained assuming a difference of activation of definite enzymes. Normally the activation graph of an enzyme shows an optimum at a definite concentration of ions of trace elements. Both less and more of the trace element lowers the enzyme activity.

2) Different trace elements may exert an influence on the

action of oxidising enzymes, that is to say on the level of the oxidation-reduction potentials. This influence is not restricted to manganese (Cu, Co, Fe etc.)

Miss Brenchley:—

1) In growing plants in sand culture, is it possible to maintain a constant *p*H? In water cultures the *p*H of an acid solution may be changed by the plant within a few hours, almost to neutral in some cases.

2) It is not easy to understand why when a sprouted potato is put into a very acid soil the shoot should so soon die from lack of calcium. One would have thought that the reserves of calcium in the tuber would have carried the shoot on for much longer.

Prof. Wallace:—

1) The *p*H of culture media in sand is changed by growing plants in a similar way to water culture.

2) We do not understand why potato sprouts die so quickly in a calcium deficient medium. Since sprouts grow healthily in air we can only assume the calcium content is sufficient for growth under those conditions but insufficient when other nutrients are added externally without further increase of Ca. The effect can be produced in sand in a neutral medium and is thus not due to specific toxins of acid soils.

Dr. Löhnis:—

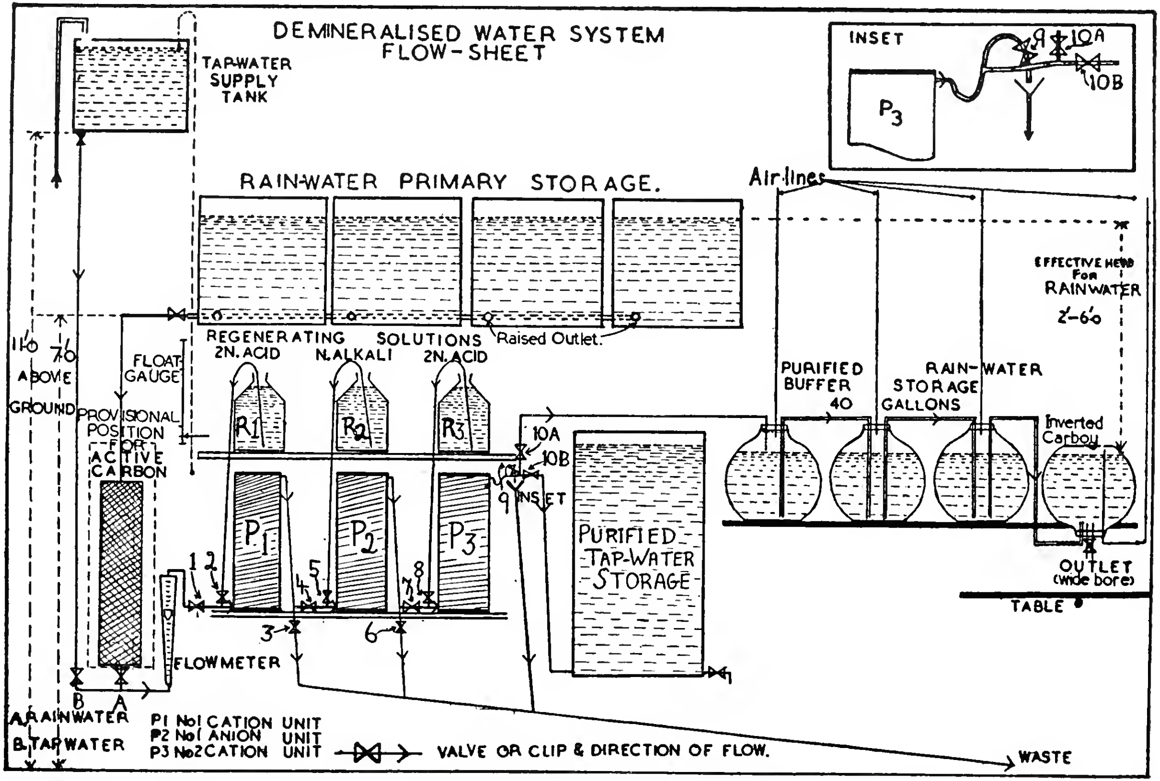
1) Has injury from excess of aluminium been noted in field crops? And if so does it go along with excess of manganese?

2) Is it more advisable to apply the direct method of estimation of micronutrients to petioles or to the leaf blades? In *Phaseolus* my results were more clearcut when blades were analysed.

Prof. Wallace:—

1) Plants of the excess aluminium type have been observed in crop failures in acid soils, *e.g.*, cereals and sugar beet. We should always expect excess aluminium condition in such instances to be accompanied by a high level of manganese.

2) In using quick tissue tests we prefer to use petioles rather than leaf laminae as the former contain less interfering pigments.



— TEXTFIGURE 1 —

LARGE SCALE SAND CULTURE METHODS FOR THE STUDY OF TRACE ELEMENT NUTRITION OF PLANTS

by E. J. Hewitt, Ph.D., B.Sc.,
Agricultural and Horticultural Research Station, Bristol University, Long Ashton, Bristol

The methods described here were specially designed to permit the study of nutritional relations and particularly trace element deficiencies of iron, manganese, boron and molybdenum in plants when it is necessary to use a large scale, required either by the scope of the experiment or by the size of the cultures themselves, as when fruit trees are used. Special problems of technique arise when large numbers of plants are to be grown simultaneously and the materials available for use are often limited owing to scarcity, cost or undesirable interaction in the experiment, but simple materials can often be suitably adapted or refined for such purposes. In their simplest form they comprise clay flower pots with a central drainage hole; a silica sand obtained from Leighton Buzzard in Bedfordshire; rain water collected from the roof of the large glasshouse used to shelter the plants and analytical reagent (A.R.) grade nutrient salts; for certain purposes, for example, molybdenum deficiency experiments, the clay pots and rain water are not effective and must be replaced by pyrex glass containers and distilled water. Each of these materials will be considered in order below.[1]

Containers:—Ten inch clay pots are used for most experiments with vegetables. Satisfactory results with deficiencies of iron, manganese or boron and all major nutrient elements are obtained if the clean dry pots are carefully painted with at least three coats of a pure bitumen solution free from these elements. The bitumen paint prevents the plant roots from absorbing nutrients from the clay which otherwise yields all the boron and calcium required

---

[1] The methods outlined in this paper have subsequently been extended to include copper, zinc and molybdenum deficiencies, and further adapted for large scale experiments. Descriptions of these will appear in a Technical Communication of the Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, England, entitled 'The Use of Sand and Water Culture Methods for Plant Nutrition' by E. J. Hewitt (*in preparation*).

for normal growth of most plants. The sand is retained in the pots by a layer of glass wool covered with a glazed porcelain filter disc or a small watch glass. These can be cleaned in acid before use.

Bitumen painted clay pots, however, are not suitable for the study of molybdenum deficiency and for this purpose five litre capacity pyrex glass beakers provided with a central drainage hole one inch diameter are most successful.

Certain plastic materials, notably polythene, are useful for making large containers or pot linings and polythene has proved suitable for work involving deficiencies of iron, manganese and boron. Care, however, must be taken to avoid the presence of harmful plasticising compounds. Contamination from catalysts and during manufacture may also occur and require consideration when plastics are used. Paraffin wax linings were found unsatisfactory owing to biological breakdown of the wax. Attempts to use wax-soluble organic mercury compounds to prevent this resulted in stimulation and increased fresh weights of crops grown in such experiments.

Sand:—The principal impurity in the sand is iron which exists mostly as ferric oxide concretions and as a stain on the surface of the grains and often occurs in minute crevices. Impurities in lesser amounts include traces of manganese, copper, zinc, boron and molybdenum, all of which occur mainly in the larger fragments present in the sand. The sand is purified first by removing the fraction containing these fragments by sieving on a 24 mesh pure copper gauze, and then treating with concentrated hydrochloric acid containing oxalic acid.

As several tons of sand must be treated, this acid process is carried out automatically in the apparatus illustrated in FIGURE 2. It is constructed in an acid resisting and inert material called "Keebush" and comprises a vessel with a conical base and a central tube with apertures around the base. A steam jet projects a short distance beyond the holes. The vessel is filled with sand saturated with acid, and steam is forced through the jet. This carries the sand and acid up the central tube and circulates the whole mixture whilst at the same time heating it strongly. The apparatus is automatic and a batch of 250 pounds of sand may be treated for several hours unattended. The treated sand is leached in large glazed vessels first with water and then with suitably purified nutrient solution until all adsorbed acid is removed.

Sand purified once in this manner is suitable for the production of manganese and boron deficiencies. Satisfactory results for iron deficiency and promising results with molybdenum deficiency have been obtained with batches of sand that have been treated twice, and the batches give uniform results.

Sand used for experiments with major nutrient elements is not treated as above but leached with water or cold 2% hydrochloric acid if required for more precise work, and particularly for the study of calcium deficiency.

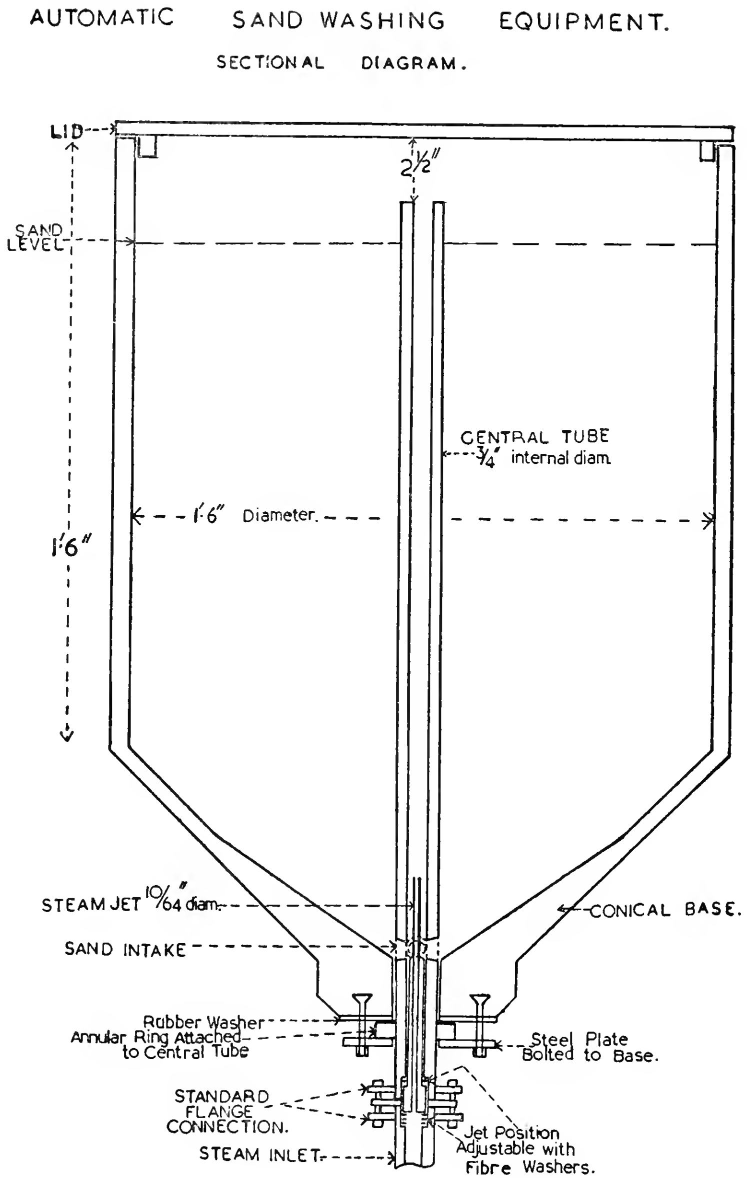
Water Supply:—An adequate supply of sufficiently pure water is essential to the technique and presents special problems for large scale work. Rain water is used for most experiments involving major element studies and is sufficiently pure for such work when collected from a clean glass roof and stored in well-weathered, galvanized tanks heavily painted with the pure bitumen solution. Rain water so stored may be purified by use of Permutit ion-exchange resins to obtain some thousands of gallons of a relatively pure water. The arrangement in use is shown in the illustration (FIGURE 1). The supply to the small glass vessels (of 50 gallons total capacity) is automatically controlled, so that flow ceases when they are full but continues slowly after water is withdrawn as rapidly as required for use. Rain water so purified compares favourably with distilled water from carefully managed heavily tinned copper stills. It contains less than 0.002 p.p.m. of iron and manganese. The boron content is about 0.004 p.p.m. and copper and zinc do not often exceed 0.006 p.p.m. This water is satisfactory for production of severe deficiency effects of iron, manganese and boron as shown in the illustrations. So far, for molybdenum deficiency work, water obtained by the redistillation from pyrex glass of water from tinned copper stills has been used. The glass stills are of the continuous action pattern.

Nutrient Reagents:—Nutrient reagents used for work with the major elements are not purified further and normally a recrystallised grade is used. When trace element deficiences are studied salts of analytical reagent grade are chosen and further purified by various means according to the element omitted from the treatment.

Those required for iron and manganese deficiencies are prepared by the alkaline phosphate adsorption method devised by Steinberg (1) adapted for large scale use by Stout and Arnon (2) and further modified here to include sodium bicarbonate before heating, which markedly increases the adsorptive efficiency of the reaction. Most reagents used for boron deficiency are purified by two or three recrystallisations during each of which an equal volume of purified alcohol is added to obtain economical recoveries of over 90%. The first recrystallisation is carried out in slightly acid solution to enhance the separation from boric acid in the mother liquor. A pure calcium nitrate is prepared from redistilled nitric acid and reprecipitated calcium carbonate.

The basal reagents required for molybdenum deficiency are first recrystallised as described above and then further purified by a new method specially designed to remove this element. The method is based on the use of 8 hydroxyquinoline for the concentration of molybdenum and other elements by co-precipitation with iron from ash solutions prior to spectrographic analysis (3). As used here nearly all the 8 hydroxyquinoline is removed by the addition of the calculated equivalent of iron as ferric chloride and molybdenum is carried down when the iron compounds are precipitated by potassium hydroxide at *p*H 5.0. Higher values for *p*H cause desorption of the molybdenum. Control experiments have shown that this method does not produce toxic effects from such traces of 8 hydroxyquinoline that may remain after precipitation. This method was devised as experiments showed that the alkaline phosphate adsorption method does not satisfactorily remove molybdenum.

Iron compounds require special attention for trace element research. When required for work on manganese problems the ferric citrate used is prepared from ferric chloride freed from manganese compounds by extraction with ether saturated with 6N. hydrochloric acid (4), (5). As this method also concentrates molybdenum compounds with the iron a different procedure is used to obtain ferric citrate for molybdenum deficiency cultures. Ferric chloride is first acidified with hydrochloric acid and shaken with toluene 3:4 dithiol in amyl acetate which extracts molybdenum. The iron in the ferric chloride is then partially precipitated with 8 hydroxyquinoline and filtered; the residual iron is precipitated as ferric hydroxide with ammonia and converted to the citrate with recrystallised citric acid.



— Textfigure 2 —

Conclusion:—The effectiveness of the technique outlined here may be judged by the visual symptoms of the deficiencies that have been produced with it in several crop plants, and some examples are illustrated here.

The use of sand culture methods admittedly adds an extra burden in the purification of this material but results suggest that this can frequently be done efficiently enough to allow of much useful work and its use has several advantages over solution cultures as a rooting medium, including its adaptability for studies where nutrients are given in solid form. Experience shows that the limiting factor at present in the use of sand may be due to the slow liberation of trace nutrients present as difficultly soluble compounds or partly occluded in crevices and which are not always completely removed in the acid treatment.

References

1. STEINBERG, R. A., 1935: J. Agr. Res. 51:413.
2. STOUT, P. R. and ARNON, D. I., 1939: Amer. J. Bot. 26:144.
3. SCOTT, R. O. and MITCHELL, R. L., 1943: J. Soc. Chem. Ind. 62.
4. HILLEBRAND, W. F. and LUNDELL, G. E. F., 1929: Applied Inorganic Analysis.
5. PIPER, C. S., 1945: Private communication.

Points from Discussion of Paper by Dr. Hewitt

Dr. Löhnis:—

Have you gained any experience as to the degree of tolerance of lucerne for excess of Mn as in the neighbourhood of Wageningen I have formerly noted that lucerne developed very poorly on unlimed soil? Mn toxicity might be involved here.

DEFICIENCIES OF MINOR ELEMENTS CAUSED BY EXCESSES

by LEO GISIGER, Dr.sc.techn.,
Eidg. Agrikulturchemische Anstalt, Liebefeld-Bern, Switzerland

The Problem:—During the world war, Switzerland was obliged to extend the area of its arable land. Furthermore, we, Swiss agronomists, had to try, by different means, such as drainage, removing stones, rounding-off of land, breaking up of pastures and clearing forests, to reclaim new areas for cultivation.

On this newly acquired arable land, crop failures often occurred in the early years. It was assumed, and the opinion soon became prevalent, that the crop failures were caused by deficiencies of trace elements. This hypothesis found strong support in the success obtained from using boron and manganese. The more difficult the supply position of boron and manganese compounds became, the more we had to consider these problems from another point of view.

In the course of time, it came to be of great practical interest to assume that the above-named difficulties were not primarily caused by a deficiency of minor elements, but by an excess of lime. The following is an attempt to show by a few experiments some of the relations existing between limed soils and the occurrence of the so-called "deficiency diseases." In addition, there are soils, which are naturally poor in minor elements as has been ascertained for boron in acid and very acid soils of cleared land. I wish especially to emphasize this latter point.

The Influence of Liming on Boron Deficiency:—*The fixation hypothesis in partial contradiction to experiments.*—The increase of growth and of yield as a consequence of liming acid and strongly acid soils is known; if excessive quantities are used, the increases obtained may be less and yields may be decreased by further dressings. In many instances complete infertility of the soil may result from excessive liming. So far this phenomenon has not been connected with an actual deficiency in one or more of the major plant nutrients, but has been explained by their partial fixation. The contents of nutritive substances in the plants grown, in experiments undertaken to study this point, showed a diminution only exceptionally. This very interesting fact seems to contradict the theory of fixation.

TRUNINGER also found no close relation between quantities of added lime and the boron content in plants. The analysis of the plants in the experiments showed a slightly higher percentage of boron. Our experiments on the influence of manuring with boron and of providing the soils with lime did not show any change in the boron content of oat-straw as a result of the lime dressings. This fact is in agreement with the results of experiments concerning the solubility of boron. HASLER could not find any increased fixation of boron from increased lime dressings.

In other tests added boron and boron originally present in the soil showed the same solubility in limed and unlimed soils. In these experiments the analysis was made after two plantings following the boron manuring and one after the liming.

On the other hand, surprising advantages were achieved by employing large quantities of phosphoric and boric acid. This fact stands in obvious contrast to our findings. Before discussing these differences, I should like specially to mention the root development as observed on limed soils. It was described by TRUNINGER. In his experiments, the roots only grew in the over-lying, unlimed soils, used as the covering layer for the seed, and did not grow into the soil limed to excess. From experience it is known that, as a general rule, the poorer a soil is in nutrients, the better the roots will develop, at least as long as the seeds continue to hold a nutrient reserve.

Without going into great detail, the experiment may be summarized as follows:—

1) The roots did not grow into the soil that was limed in excess. The root-growth seems to be prevented by a harmful factor, a superfluity of lime if you will allow me to generalise.

2) Where the plants do not grow into the soil, no certain conclusion concerning the influence of the soil nutrients upon the growth of plants can be drawn from their ash composition.

3) When a supplement of phosphoric acid or boron shows an improvement of growth and an increase of yield on overlimed soils, it may be explained as follows: by employing phosphoric acid, the supplement changes the soil and suppresses the damaging factor, that is, the acid neutralizes the strength of the alkaline reaction; by adding boron, the roots are strengthened against the injurious surplus.

The nature of the injurious effect of overliming acid soils.—In earlier tests with an acid soil, which was not quite impoverished, it was ascertained that the damage from overliming is greatly reduced, when the limed soil is leached for some time (GISIGER, 1944). Based on this experience, the following test was made: An amount of acid soil was divided into 4 parts, each of which was limed with increasing quantities, *sc.* 0.5, 3, 7 and 24 grammes CaO per kg soil. The well-mixed soils were put into large vessels and watered for several months to leach them. After drying a little the soils were put into test pots in which white mustard was sown. Before sowing, all the pots received the same P N K manuring, to which 16 different test-dressings were added *viz.:* lime in various forms and quantities; a manure with boron, manganese and potassium, the latter as a hydrate (lye). The results lead to the following conclusions:

Ist series (0.5g CaO/kg soil).—On a very acid soil growth cannot be improved by a supplementary manuring with calcium chloride and gypsum, if the anions Cl^- and SO_4^- are not washed out. By liming a very acid soil with calcium hydrate and carbonate, the same effect can be observed. The quantity of potassium hydrate used, *i.e.,* 2 grammes and 4 grammes per pot, was not enough to neutralize sufficiently the acidity of the soil. The dressing of boron and manganese employed did not show any effect, neither on the acid soil nor on that with calcium hydrate. It seems that the immediate cause of infertility in the very acid soil is not a calcium deficiency but the concentration of hydrogen-ions, which can be reduced by lime and by potassium hydrate.

IInd series.—3 grammes of lime did not suffice, after leaching the soil, to reduce the acidity enough to ensure a normal development of the mustard. The soluble calcium salts (chloride and sulfate) did not effect a noticeable change of the hydrogen ion concentration, neither in the soil nor in its water. Neither did they help the growth, which was reduced by the chloride, *i.e.,* by exchanging acid.

Calcium hydrate and carbonate in smaller and larger quantities enabled a satisfactory development of the mustard; this could not be observed on the pots with potassium lye, especially on those which received the small dose. When manuring with boron and manganese an effect cannot be expected or noticed. In the series 1 and 2 one should observe the small content of Ca-ions in the leaching water from the pots with well-developed plants.

IIIrd series (7g CaO/kg soil).—The different fertilisers did not noticeably influence plant growth. The good development of the plants will attract attention to the low content of calcium in the leaching water.

IVth series (24g CaO/kg soil).—In the pots with and without soluble calcium salts, this series generally shows satisfactory growth, but growth is noticeably reduced in comparison with series 3 (the injurious factor could not be washed out entirely). From liming with carbonate a decrease in growth is barely visible, but it is noticeable with both the small and large quantities of calcium hydrate. It may appear surprising that the boron and the manganese did not increase growth. This seems to contradict earlier results with boron manuring. It seems possible that the damage done by the calcium hydrate was too great and as a result boron proved ineffective.

In this test no relation was observed between the calcium content and the improvement of growth on acid soil; the same can be said with regard to the injury to growth on slightly alkaline soils. On the contrary, the experiment pointed to a relation between the increase and reduction in growth and the *p*H values in the soil and its water. The mustard showed an optimal growth in slightly acid or neutral soil with the *p*H values 5.8-7.2, corresponding to the *p*H values in the soil water from 6.6-7.5. The depression was noticeable at *p*H values in the soil from 7.4-7.7 and in the water from 7.7-7.8, whilst growth was noticeably retarded when the *p*H value was higher by 0.1-0.2 *p*H units. Thus it can be said that in our test soil the hydrogen ion concentration is more important for regular development than the concentration of calcium ions.

Can the damaging excess, caused by overliming, be removed?—Experience shows that liming acid soil is only followed by noticeable damage, if the liming is sufficient to saturate the soil and the *p*H value rises over 7 as shown in the following outline of the reaction in the limed soil.

$$\underset{H}{\overset{H}{\boxed{\text{Soil}}}} + 2CaCO_3 \rightleftharpoons \boxed{\text{soil}}\,Ca + Ca(HCO_3)_2$$

acid soil + calcium carbonate ⇌ soil saturated + calcium bicarbonate.

Calcium bicarbonate is a salt with a strong base and a weak acid and by its hydrolysis an alkaline reaction results according to the equation:

$$Ca(HCO_3)_2 + 2H_2O = Ca(OH)_2 + 2H_2CO_3.$$

If calcium bicarbonate could be washed out, it would be possible to reduce the injury to growth, but there would remain the well-saturated absorbent complex, which can also give rise to an unfavourable and high concentration of hydroxide (OH)-ions.

To prove the effect of leaching, the following experiment was made. An acid pasture soil was limed with increasing quantities in 3 parallel series. After liming the first and the third series were immediately planted and the third series received in addition to the general base manure of P N K, a further 31 mg of boric acid. Mustard was chosen as a test plant. The middle series was leached during a period of two months before planting. For this leaching, water was poured daily on the pots, so that from 75 to 100 cm³, dripped through, *i.e.*, in two months a total quantity of 5500 cm³ of water.

TABLE 1: *Leaching of differently limed soils:—*

Liming in g carbonate per pot	Water flowing through, in cc	Leached CaO		
		Total in mg	From manure	
			mg	%
0	5575	113	—	—
4.8	5395	173.7	60.7	2.3
9.6	5385	214.6	101.6	2.0
19.2	5535	462	349	3.2
28.8	5640	887	774	4.8
38.4	5600	1110	997	5.0
72	5540	1290	1177	2.8
144	5485	1373	1260	1.6

95 to 98% of the added lime remained in the soil; nevertheless, in the test remarkable differences in growth were observed between the leached and the unleached series without boron. The injurious effect of the higher lime dressings is particularly interesting. In the unleached series the plants in the pot with the 5th lime quantity are restricted in growth, in the pots with the next higher lime quantity the growth of the plants was almost

entirely prevented. It is possible that here and in the following pots a few plants were enabled to grow, because the injurious exchanging matter was washed into the deeper layers.

In the leached series, serious damage is shown only by the highest amount of lime.

The growth in the boron series is very similar to that in the non-leached series; it shows a quick improvement through the first lime treatments and then a sudden decrease which is moderated by boron. The leached series, surprisingly, shows better growth than the boron series.

The mustard reacts similarly to flax, as illustrated in PLATE 1 which shows three test series with increasing lime quantities on an acid soil. In the first series the mustard was sown immediately after liming. The growth shows an improvement through the small lime doses, but after the fifth dose there is a progressive decrease. In the next two series the same soil is used as in series one; the second series was treated with 31 mg of boric acid, whilst the third was leached during one winter. With this leaching a noticeable reduction of the so-called lime damage was again achieved.

Experiments with new soils led to similar results, as can be seen from PLATE 2; all the 6 pots had been filled with the same soil rich in lime.

The Mitscherlich pots (the three on the right) were well leached for a period of a fortnight. Then the same base manure of P N K was given to all pots. Into the second and the fifth pot a supplement of boric acid, and into the third and sixth a strong dose of soluble phosphate (mono calcium phosphate) was mixed. A similar advantage resulted from the leaching and from the boron dressing, the latter did not produce a further noticeable effect in the leached soil.

The illustration is clear enough without any further explanation and these experiments may be summarized as follows:

1) On very acid soils the growth can be noticeably improved by liming.

2) Injuries to the growth of plants, "sensitive" to lime, are only observed when lime is added in excess of the quantity needed for the saturation of the soil up to *p*H 7.

3) The injury is probably caused by alkaline calcium compounds, which arise from transformation. The injury can be traced to a too high concentration of hydroxide (OH)-ions.

4) These damaging compounds can be washed out almost entirely, so that such sensitive plants as flax and mustard are less hindered in growth.

5) In soil rich in calcium, if the water table is high, the enrichment

of calcium compounds will cause injuries in the root zone of the plants.

6) Boron has been employed with success for a long time against the damage by alkaline reaction but a certain amount of leaching also gives good results. In a humid climate, leaching can be effected by drainage and lowering of the water table.

The antagonism between boron and hydroxide (OH)-ions.—In accordance with certain statements in the literature, I believe that the results obtained here confirm once more the fact, that "lime injury" in plant growth is traceable to a high concentration of OH-ions in the soil. The liming causes a part of these injuries indirectly; these injuries are never a direct result of the concentration of calcium ions in the soil.

According to PALLMANN, the hydroxide (OH)-group is among the hydrophile radicals which favour hydration. We may well assume an extreme hydration (swelling) of the root tips in a strongly alkaline soil. The root tips are also exposed to the microorganisms by which they can be decomposed. As the osmotic pressure of the roots is reduced they are unable to penetrate into the soil which is oversaturated with hydroxide ions. On the other hand, when such penetration still occurs their reduced osmotic pressure makes the absorption of water difficult (SCHROPP and ARENZ).

In contrast to the hydroxide ions, boron has dehydrating properties; according to NOACK it can withdraw water from swelled gelatine. Similar observations were made by SCHMUCKER with germinating pollen. Pollen hydrates in a nutritive solution so strongly without boron that the germinating tube bursts, whereas the tube remains intact when boron is added. These facts offer an acceptable explanation of the harmful effect of the hydroxide-ions without the hypothesis of a higher boron fixation by lime.

In acid soils, overliming results in too great a concentration of OH-ions which cause the roots to swell too much. To prevent this, the roots must be able to assimilate an adequate supply of boron. The physiological balance has to be restored by a high boron dressing to provide for a strong assimilation of the element. In this case the boron does not act as a nutrient in the usual way but rather as a medicine. The higher doses of boron strengthen the plant roots against the unfavourable and harmful influence of the OH-ions. This hypothesis or theory avoids the conflicting views expressed in the earlier theory according to which the damage was ascribed to the Ca-ions and also to a fixation of boron by them.

The Causes of Grey Speck Diseases:—*Opinions and results.*—Since the fundamental research of **Hudig** it has been ascertained by innumerable experiments, that the grey speck disease can be prevented by manganese sulfate. **Samuel** and **Piper** as well as **Lundegårdh** tried to find the quantitative relations between manganese in the soil and the grey speck disease. **Lundegårdh**'s water culture experiments and his observations in the field led to the conclusion that the grey speck disease occurs mostly in soils of a neutral or a slightly alkaline reaction but not under slightly acid and strongly alkaline conditions. By the analysis of plants it was shown that the curve of the availability of soil manganese shows a minimum in the neutral sphere, coinciding with the optimum of the disease.

Results confirming these statements were reported by **Popp** and **Maschhaupt**. These authors show that the grey speck disease can be prevented by applying large amounts of quicklime, whilst similar liming with carbonate gives unfavourable effects.

Maschhaupt does not contribute the advantages of quicklime only to chemico-physical reaction, but he also takes partial sterilization of the diseased soil in account. The same theory is supported by **Gerretsen** after comprehensive experiments. On the one hand, sterilization may control micro-organisms which attack the enfeebled roots under conditions of manganese deficiency; on the other hand, the manganese assimilation by micro-organisms may be reduced as a consequence of the sterilization.

Thus the higher availability of manganese in strongly alkaline soils may be explained by the smaller biological fixation. **Samuel** and **Piper** consider 12 to 14 p.p.m. manganese in the dry matter of oats as a threshold value for the occurrence of grey speck disease. Beside this, they assume that the Ca:K relation also influences the occurrence of grey speck disease decisively.

As a result of a shortage of manganese salts for manurial purposes, the investigation of the problem of the grey speck disease became imperative in Switzerland, during the war. Some results of our experiments, in this connection, are worth noting.

Quicklime as well as boron may favour or prevent the occurrence of grey speck disease.—In support of the results, reported above, it was found in a tentative trial, that the grey speck disease occurs more severely in leached soil than in non-leached soil. From this it was concluded that the disease can either be favoured by boron or reduced by addition of OH-ions. In a further experiment using boron in increasing quantities, the result was inconclusive in so far as the grey speck disease was not produced. After having limed the soil, already given boron, with increasing quantities of quicklime, the growth of oats proved convincingly:

1) On an acid peat soil which is healthy and quite suitable for the growth of cereals, the grey speck disease cannot be produced by boron manuring alone in increasing quantities.

2) By increasing quicklime dressings the soil will at first become diseased but much greater quantities of quicklime will make it sound again. For the time being, the question remains open, whether the Ca-ions and their concentration have a special significance in this case.

3) If from 6 to 50 kg of boric acid is added per hectare (20 to 160 mg per pot) the grey speck disease increases in soils on which this disease appears. With higher boron quantities the disease decreases and does not occur, when the boron application attains 400 kgs of boric acid per hectare.

4) The chemical analysis of oat straw shows the following:
   a) with the increasing lime quantities the manganese content first decreases but shows an increase after the highest lime dose;
   b) of the boron dressings given, only the highest quantity, 400 kgs boric acid, causes an increase of the content of manganese. The smaller doses produced no changes in manganese.

5) From 12 to 14 p.p.m. Mn, in dry matter, can be regarded as a limiting value for the occurrence or non-occurrence of the grey speck disease.

The K:Ca relation and its influence on grey speck disease. —We made many observations in the field, which proved the fundamental significance of the potassium supply. Our plants did not fail in a soil with a high content of potassium. On this fact we based the assumption that a high potassium supply suppresses the solubility and the availability of calcium. This means, according to SAMUEL and PIPER, that the K:Ca relation would keep the occurrence of the grey spot disease under control. This hypothesis became of a practical importance, when, in spite of the war, our potassium supplies remained sufficient. In field experiments, fertilising with large quantities of potassium 300, 600 and 1200 kgs K_2O/ha produced a definitely favourable effect. To prove this, an experiment was undertaken under extreme conditions. The soil used was a mixture of marshy soil with sphagnum peat, the dressing per pot consisted of 1.5 g of phosphoric acid, 0.7 g of nitrogen and 1.0 g of potash (see PLATE 3).

The supplementary dressings were applied in three series:

I) 6 g of potassium hydrate with 0 g, 15 g, 30 g and 45 g of quicklime per pot respectively

II) 12 g of potassium hydrate with 0 g, 15 g, 30 g and 45 g of quicklime per pot respectively

III) 18 g of potassium hydrate with 0 g, 15 g, 30 g and 45 g of quicklime per pot respectively

The following table illustrates the results obtained with oats.

TABLE 2: *The influence of increasing amounts of potassium hydrate and quicklime on the development and yield of grain from oats* (5 points = top development):—

POTASSIUM HYDRATE g/pot	QUICKLIME IN g PER POT				
	0	15	30	45	
0	55.7	—	—	—	grains
	5				development
	0.9				K_2O/CaO
6	52.1	18.0	44.2	48.8	grains
	4	3	4–5	5	development
	5.7	4.5	4.8	5.0	K_2O/CaO
12	33.1	55.0	57.0	51.6	grains
	3	4–5	5	5	development
	8.6	7.3	8.1	7.3	K_2O/CaO
18	6.5	55.4	54.4	48.6	grains
	2	5	5	5	development
	13	11.3	10.6	10	K_2O/CaO

The principal results of this test may be summarised as follows:

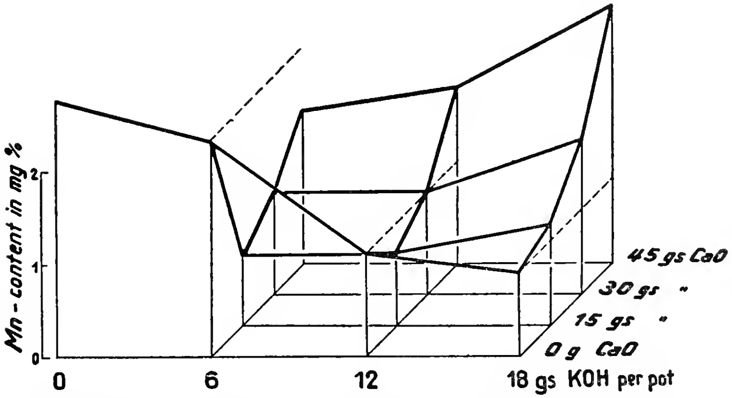
1) The grey speck disease can also be produced on a sound, marshy soil by heavy fertilising with potassium hydrate.

2) Where the disease is produced by moderate doses of potassium or calcium hydrate, it can be reduced or suppressed by an increase of one or the other of the hydrates.

3) The occurrence or non-occurrence of the disease seems to be controlled, in principle, only by the OH-ions concentration, the K:Ca-relation is of no, or only of secondary, importance.

4) The plant analysis confirms LUNDEGARDH's findings, according to which the manganese content of plants in acid soils, on one hand, and in very alkaline soils, on the other, is higher than in neutral or slightly alkaline soils.

Summary:—An unfavourable concentration of OH-ions, and not a higher fixation of boron, is the immediate cause of disturbances in plant growth after excessive use of lime in acid soils. In these soils, boron does not react in the normal way as a nutrient, but rather as a medicine; it strengthens the roots against the hydrating effect of the excessive concentration of OH-ions. Soils retaining a detrimental excess can be leached by watering. The grey speck disease of oats can be produced in an originally acid, marshy soil by adding calcium carbonate and also by calcium or potassium hydrate. When the dressing with these hydrates is very heavy the soil shows a strong alkaline reaction and the disease does not occur. It can also be prevented by manganese. Small quantities of boron favour, while large doses suppress, the grey speck disease. The K:Ca relation has no appreciable effect on this disease. The manganese content of oat straw reveals that the availability of manganese is greater in acid and very alkaline soils than in neutral and slightly alkaline soils.



TEXTFIGURE 3.—Influence of increasing quantities of potassium and calcium hydrate on the manganese content of oat straw.

References

GERRETSEN, F. C., 1937: Manganese deficiency of oats and its relation to soil bacteria (Annals of Bot. N.S. 1:207-230).

GISIGER, L., 1944: Kalk und Kali (Landw. Jahrbuch d. Schweiz 58:515-589).

HUDIG, J., 1911: Ueber eine eigentümliche Bodenkrankheit (Landwirtsch. Jahrbücher 40:613-644).

LUNDEGÅRDH, H., 1932: Die Nährstoffaufnahme der Pflanze (Jena: G. Fischer).

MASCHHAUPT, J. G., 1934: Das Rätsel der Dörrfleckenkrankheit (Zeitschr. für Pfl.Ern., Düngung u. Bkde. 13:313-320).

NOACK, K., 1936: Festrede auf dem Reichstreffen der deutschen Chemiker in München (Zeitschr. f. angewandte Chemie 49:673-681).

PALLMANN, H., 1931: Ueber Hydratation und Quellung (Vierteljahresschrift der Naturforsch. Gesellschaft Zürich 76:16-41).

POPP, M. und Mitarb., 1934: Das Rätsel der Dörrfleckenkrankheit (Zeitschrift für Pfl.Ern., Düngung u. Bkde. 13:66-73).

SAMUEL und PIPER, cit. nach LUNDEGÅRDH.

SCHMUCKER, TH., 1932: Bor als biologisch entscheidendes Element (Naturwissenschaften 20:839).

SCHROPP und ARENZ, 1940: Versuche mit Bor zu einigen Gramineen (Bodenkunde u. Pflanzenernährung 16:169-209).

TRUNINGER, E., 1940: Borgehalt des Mergels als Ursache der verschiedenen Düngwirkung von gemahlenem Kalkstein und Mergel (Landw. Jahrbuch der Schweiz 54:689-705).

TRUNINGER, E., 1927: Beobachtungen über den Einfluss einer Düngung mit kohlensaurem Kalk auf saurem Bodèn (Landw. Jahrbuch der Schweiz 41:765-786).

Points from Discussion of Paper by Dr. L. Gisiger

Professor Burström:—

Have you made any observations on the hydration and osmotic properties of the root tips in your material? I believe that you really have to study these properties on your own plants.

Professor Wallace:—

Professor GISIGER's data show higher manganese intake at low and high *p*H values with minimum values at intermediate *p*H points.

In this connection the results of QUASTEL and his co-workers showing that manganese is biologically oxidised by soil organisms over the range *p*H 6.0-7.9 may be of significance.

Dr. Löhnis:—

Do the mustard plants in the soil without B added, the roots of which might be hydrated, show any symptoms of boron deficiency in the foliage? Mustard is very susceptible to boron deficiency.

Dr. Gisiger:—

We did not have the possibility to study the hydration and osmotic effect of boron and OH-ions in plants. Boron deficiency in mustard foliage is often observed by the curled-in foliage. Mustard plants, damaged by overliming, seem to flower normally, but the seed production is very much reduced or even nil.

CRITERIA OF ESSENTIALITY OF INORGANIC MICRONUTRIENTS FOR PLANTS

With Special Reference to Molybdenum

by DANIEL I. ARNON, Ph.D.,

Division of Plant Nutrition, University of California, Berkeley, California

It is now generally accepted that the inorganic requirements of higher plants are no longer fully represented by the classical list of ten elements. In the past few decades manganese, boron, copper, and zinc and, very recently, molybdenum have been recognized as additional essential nutrients for plant growth. Chemically different as these five elements are, they are characterized as plant nutrients by the common feature of being required in minute amounts. The methods and circumstances surrounding their discovery give rise to the question whether other elements need not be regarded as indispensable to plant life. The question of the completeness of the list of essential elements is important from the standpoint of plant physiology as well as agricultural practice. It is clear, however, that an answer to this question must be preceded by the recognition of certain well defined criteria of essentiality.

Suggestions for considering a given element essential generally emanate from two sources, agriculture and biochemistry. In agricultural practice it is sometimes observed that the addition of a given element to the soil results in improved plant growth. Interesting and even important as such observations may be in terms of a particular soil-crop system, they seldom permit of unequivocal interpretation, when a micronutrient is involved. For example, in the investigation of the little-leaf disease of fruit trees in California a large application of iron sulphate to the soil resulted in a marked recovery. The favourable response from adding iron sulphate was not obtained in a second trial. Further experimentation disclosed that the particular lot of iron sulphate which was used successfully contained a large zinc impurity and that zinc deficiency was responsible for the little-leaf disease (1).

Other factors also contribute to the complexity of soil-plant interrelations. A chemical analysis of a soil offers no reliable guide to its supplying power for a nutrient. In a California zinc-deficient soil, quantities of this element were found sufficient to meet plant requirements for many centuries. The zinc was, however, held so tenaciously by the soil as to be unavailable to the plant. The significance of the unavailability of elements present in the soil is also illustrated by the kind of negative evidence sometimes obtained in soil trials with micronutrients. The addition of several hundred pounds of zinc sulphate per acre failed to produce a favourable response in zinc-deficient trees owing to the fixation of the added zinc in the soil. There is also the problem of soil microflora. For example, the application of small amounts of molybdenum to the soil may benefit the plant indirectly by favouring the nitrogen-fixing bacteria (5, 6).

The inconclusiveness of soil experiments with regard to the essentiality of micronutrients is in sharp contrast to the decisiveness, whenever available, of biochemical evidence on the specific function of a micronutrient element. This has been forthcoming from the studies of enzyme systems and is best illustrated in the case of copper which was found to be the prosthetic group of several oxidases such as polyphenol and ascorbic acid. The demonstration of the essential rôle of these enzyme systems in plant metabolism constitutes also conclusive evidence of the indispensability of copper. It is by no means implied that the discovery of one or more enzyme systems with copper as the prosthetic group exhausts the function of copper in the green plant. What can be asserted is that if copper is required for at least one essential enzyme system, copper is indispensable to plant life for that reason alone, regardless of other functions it may also perform.

The history of micronutrients reveals, however, that for every element, the biochemical elucidation of its function is either as yet largely unavailable or, when it was forthcoming, it invariably followed rather than preceded the acceptance of the micronutrient as essential. Important physiological advances and agricultural application of our knowledge of the indispensability of boron, manganese, copper and zinc have occurred either in advance or in absence of any knowledge of their functions within the plant. It seems reasonable, therefore, to expect that, in the future as in the past, the plant physiologist will be called upon to assess and elaborate on the evidence from the field, by conducting critical growth experiments intended to determine the essential status of a given element. Because of the complexity of the plant-soil system it is inescapable that such experiments will be carried out in artificial culture media. In this undertaking, the formulation of definite criteria of essentiality can be of distinct aid. In our laboratory this subject was linked with the discovery of the indispensability of molybdenum for the growth of higher plants (2). A recounting of this investigation will serve to illustrate our present point of view.

In a comparison of ammonium with nitrate as sources of nitrogen, a preliminary experiment indicated that molybdenum, chromium and nickel improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen (3). This suggested that the list of micronutrients, then confined to boron, manganese, zinc and copper was incomplete. This possibility was tested by arranging a number of elements in groups and by observing how the addition of a given group affected the growth of plants in culture solution (4). Three supplementary solutions, each containing different elements in minute quantity, were prepared. One solution, designated A4, furnished the recognized four micronutrients, boron, manganese, copper and zinc. The basic culture solution, supplemented with the A4 solution, therefore furnished the plant with a seemingly complete list of essential elements. Another supplementary solution, designated B7, contained the following seven elements: molybdenum, vanadium, chromium, nickel, cobalt, tungsten and titanium—a somewhat arbitrary grouping based on the consideration that each of these could assume various valency levels and hence, conceivably, participate in oxidation-reduction processes within the plant cell. The already mentioned findings on the rôle of metals in the nitrogen nutrition of barley (3) suggested this particular grouping. The third supplementary solution, designated C13, supplied thirteen elements: aluminum, arsenic, cadmium, strontium, mercury, lead, lithium, rubidium, bromine, iodine, fluorine, selenium and beryllium. Sodium and chlorine, though not singled out, were provided from several sources in these solutions.

In experiments with lettuce and asparagus (4) a marked improvement in growth was observed from supplying, in addition to A4, the B7 solution. The further addition of thirteen more elements supplied by the C13 solution produced no measurable effect on either the lettuce or the asparagus plants. The results obtained with lettuce are given in TABLE 3.

Of the seven elements contained in the B7 solution only molybdenum and vanadium had been associated with biological phenomena in plants, especially with nitrogen fixation by *Azotobacter* (5, 6). Mention was already made that under special conditions of nitrogen nutrition (3) chromium and nickel were associated with favourable responses on the growth of barley. It was undertaken therefore to formulate definite criteria of essentiality by means of which the status of each of the seven elements comprising the B7 group could be tested.

TABLE 3 (*from* ARNON (4)): *Effect of adding different groups of micronutrients on the growth of lettuce plants* in culture solution:—*

MICROELEMENTS ADDED	SHOOTS	ROOTS
None	71.4	14.5
A4	105.7	22.0
A4 + B7	1068.3	188.6
A4 + B7 + C13	984.4	196.2

* Average fresh weight of plants in grams.

The following criteria were set up: an element is not considered essential unless (*a*) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (*b*) such deficiency is specific to the element in question and can be prevented or corrected only by supplying this element; and (*c*) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium.

The criterion of the foremost physiological significance is the requirement of an inorganic element for the successful completion of the life cycle of a plant. This is, of course, different from merely demonstrating a favourable effect on growth. The experimental procedure involved in putting this criterion to the test must be based on removing the element in question from the nutrient medium of the plant. This, however, is beset with difficulties. First, it is impossible to remove *completely* an element that may be contained in the seed. Second, the same obstacle applies to the nutrient medium. Regardless of how effective purification procedures are, they cannot be regarded as having removed the last atom of a contaminant originally present in the water and nutrient salts, or one that is derived from the container in which the plants are grown, or one gaining access to the nutrient medium in the course of an experiment.

Experimentally the problem resolves itself into selecting a species which has a high requirement for a given micronutrient and using purification procedures capable of reducing to a minimum the level of contamination in the nutrient medium. Different species vary greatly in their requirement for a given micronutrient. Beans, for example, have a far greater requirement for boron than barley. Alfalfa is capable of absorbing enough zinc from a medium in which corn shows acute deficiency symptoms. The extent to which it is necessary to purify the culture medium in order to produce deficiency symptoms may be reduced through selection of plants having a high requirement for an element.

In our experiments the water culture technique was used and tomato was selected as the principal test plant. This plant is characterized by a relatively small seed in relation to the emerged plant, thus rendering it likely that seed reserves would prove inadequate for the requirements of the growing plant which is, in addition, capable of successive vegetative and fruiting cycles. The indeterminate type of growth is desirable when an experimentally produced deficiency is later to be corrected by adding the missing element.

In purification of the nutrient medium stress was laid first on the preparation of nutrient media of reproducible degree of purity and, second, on determining and expressing the level of remaining contamination in quantitative terms (8). Pyrex glassware was adopted for containers as the best material as far as freedom from metal impurities was concerned (it is not suitable, however, for boron work). Molar stock solutions of the various nutrient salts were individually purified by precipitation and adsorption of impurities at high temperatures and slightly alkaline reactions in the presence of calcium and phosphate ions. Distilled water was purified by redistillation, using a Pyrex glass condenser. The purified water and chemical stock solutions were stored in Pyrex containers.

After purification the purity of water of the various molar stock solutions of nutrient salts was determined by means of an adaptation of the diphenylthiocarbazone (dithizone) test (8).

This test permitted the detection of 0.0005 mg and, with special care, 0.0001 mg of the *combined* content of a group of metals: zinc, copper, nickel, cobalt, lead, mercury, cadmium, thallium and bismuth. The purification procedure reduced the metal content of the nutrient salts from 0.5-5.0 to less than 0.01 parts per million, and the redistillation reduced the metal content of distilled water from 0.010-0.100 to less than 0.001 p.p.m. Each lot of water and stock solution of chemicals was tested and, if found pure, approved for future use in the preparation or replenishing of nutrient solutions. The purified chemicals and water could thus be added independently of each other, to give nutrient solutions of equal and reproducible purity. It was possible, using this technique, to obtain consistent and reproducible responses in plants from adding minute amounts of metals to the nutrient medium (for example, 1 part of zinc in 200,000,000 parts of culture solution, which amounted to 0.001 mg of zinc to a plant).

When tomato plants were grown from the seedling stage in nutrient media purified in this manner and supplied with a complete nutrient solution including the four micronutrients B, Mn, Zn and Cu, characteristic deficiency symptoms became apparent in a few weeks (2). The lower leaves developed a distinct mottling, different from any other deficiency symptom previously noted in the tomato. In latter stages, necrosis at the margins and a characteristic involution of the laminae accompanied by abscission of blossoms were noted. Thus it was found that by the first criterion of essentiality, the completion of the life cycle, the nutrient medium was deficient in some essential element. Complete recovery was obtained upon adding the B7 solution. A breakdown of this group of seven elements disclosed that molybdenum was the needed micronutrient. The development of these deficiency symptoms was prevented by adding 1 part of molybdenum as molybdic acid to 100,000,000 parts of nutrient solution.

After experimentally producing a characteristic deficiency syndrome and demonstrating that it could be prevented or cured by the addition of molybdenum, the next step was to show that this effect was peculiar to Mo and that other elements could not be substituted. This was done by supplying the cultures with the six other metals in the B7 solution and with the thirteen elements in the C13 group. The deficiency symptoms persisted unless Mo was provided. Neither was there any additional improvement in growth when the application of Mo to molybdenum-deficient cultures was accompanied by the other elements in the B7 and C13 solutions.

To test the results by the last criterion, that of the direct effect of an element on the plant as distinguished from its possible influence on the root environment, molybdenum-deficient plants were sprayed with a dilute solution of molybdic acid (1 p.p.m. Mo) so as to bring about absorption only through the aerial parts of the plant. Recovery and resumption of normal growth with the disappearance of the molybdenum-deficiency symptoms took place. This provided the last link in the chain of evidence for the indispensability of molybdenum for the tomato plant.

The results with tomato were subsequently repeated with mustard and lettuce. The essentiality of molybdenum was soon confirmed by PIPER (9) for oats and by HOAGLAND (19) for Myrobalan plum. Molybdenum was recognized as an essential element and included with the A4 solution which was redesignated A5, whereas the B7 was changed to B6. The addition of A5 to a basic nutrient solution gave for the species tested the same results as a further addition of B6 and C13.

Does the inclusion of molybdenum among the essential elements complete their list? An unequivocal answer to this question cannot be given, notwithstanding the fact that a number of different species of plants have been grown successfully in rigidly purified nutrient solutions which supplied only boron, manganese, copper, zinc and molybdenum. It is certain that, despite all caution, minute impurities of other elements persisted in the nutrient medium as well as in the seed. It would seem best to attempt to answer this question in a quantitative rather than a qualitative manner: to determine analytically whenever possible the upper limit of impurity for a given element that may be contained in the nutrient medium and to measure a growth response with and without a further addition of the element in question. This can be illustrated as follows. It was found with the dithizone test that when the combined zinc, copper, lead, cadmium and mercury content of a nutrient solution was less than 0.0001 mg per plant, severe deficiency symptoms occurred in the tomato. Recovery was brought about by adding 0.002 mg Cu and 0.002 mg Zn, but no further improvement was produced by supplying 0.0005 mg each of lead, cadmium and mercury. These results, while confirming the indispensability of zinc and copper in amounts greater than those found in the nutrient medium, were interpreted as permitting no final conclusion as to the rôle of cadmium, lead and mercury. The possibility that these or other elements studied in a similar manner may be required in amounts smaller than the incidental impurities which could not be removed from the culture solution by the present technique cannot be *a priori* excluded.

If these views are accepted there can be no objection to regarding almost every element in the periodic table, and particularly those most frequently encountered in plant tissues, as susceptible of being shown at some time to be essential for plants. What can be asserted definitely is that if an element now regarded as dispensable for a given plant should at some future time be found essential, it will be shown to be required in exceedingly small amounts—within the limits of contamination still encompassed by the refined methods now used for purifying the nutrient medium.

This quantitative approach to the problem of essentiality of micronutrients is regarded not as a mere theoretical generalization but as a point of view conducive to a search for more refined analytical methods and procedures for growing plants which would make it possible to investigate the rôle of a number of new elements in plant nutrition.

References

1. Hoagland, D. R., 1944: Inorganic Plant Nutrition (Waltham, Mass.: Chronica Botanica Co.).
2. Arnon, D. I. and Stout, P. R., 1939: Plant Physiol. 14:599.
3. Arnon, D. I., 1937: Soil Science 44:91.
4. Arnon, D. I., 1938: Amer. J. Bot. 25:322.
5. Burk, D. and Horner, C. H., 1935: Third Intern. Soil Sci. Congr. Trans. A. 1:152.
6. Bortels, H., 1937: Arch. Mikrob. 8, 1:13.
7. Arnon, D. I. and Stout, P. R., 1939: Plant Physiol. 14:371.
8. Stout, P. R. and Arnon, D. I., 1939: Amer. J. Bot. 26:144.
9. Piper, C. S., 1940: Austr. Inst. Agri. Sci. 6:162.
10. Hoagland, D. R., 1940: Proc. Amer. Soc. Hort. Sci. 38:8.

Points from Discussion of Paper by Prof. Arnon

Dr. Brenchley:—

If Professor ARNON has proved that any micro-nutrient is essential for any one or more plants, does he consider that that indicates that the element concerned is essential for all plants?

Prof. Wallace:—

In trace element cultures the risk of contamination of trace elements arises during the later stages of experiments due to accumulation of elements from the use of the large quantities of water which are necessary. This may result in deficiency conditions ceasing to exist in a given technique.

Prof. Němec:—

Have attempts been made to determine whether it is necessary that every cell in the plant gets at least one molecule of every microelement? If it should be so, then it could be possible to assume that the molecule is a constituent of the hypothetical sensitive point of the cell. The necessity that every cell must get at least one molecule of a trace element would give the lower limit for further trace elements.

A MEMORANDUM
REGARDING NOMENCLATURE

by Daniel I. Arnon, Ph.D.
Division of Plant Nutrition, University of California, Berkeley, California

In the English-speaking countries elements required by plants in minute quantity are often called "trace," "minor" or "rare" elements. While these terms have acquired a more or less definite meaning among plant physiologists, there has been a recent tendency among workers in the field of soil and fertilizers to extend these terms to cover all inorganic elements derived by the plant from the soil, except for the three "major" fertilizer elements, nitrogen, phosphorus and potassium.

In recent years sentiment has been crystallizing in America to designate elements required by plants in minute quantity by the term "inorganic micronutrients" or "micronutrients" for short, when the context precludes confusion with organic micronutrients. The term micronutrients describes manganese, copper, boron, zinc and molybdenum. It is suggested that, disregarding historical reasons, it should also include iron, which is required by plants in small amounts. The analogous term "macronutrients" would then describe the six inorganic nutrients derived from soil and absorbed by plants in large amounts: potassium, calcium, magnesium, nitrogen, phosphorus and sulphur.

The objections to the terms "rare," "trace" and "minor" elements can be stated as follows. They are not "rare" either in the plant or in the soil. They are not "minor" in the sense that a deficiency of them would be of less consequence to the plant than a deficiency of a "major" element. They are not "trace" elements in the sense that their presence can be detected but their content is too small to measure. On the contrary, the current trend in research in this field is notable for the quantitative determination of these elements within the plant as well as in the nutrient medium.

It is submitted that the adoption of the term "micronutrients" in the English-speaking countries would further clarity of thought and implication, and be a distinct aid in teaching. This proposal is limited to English terminology and does not bear on well serving analogues in other languages.

IMPORTANCE OF COPPER AND MOLYBDENUM IN THE NUTRITION OF HIGHER PLANTS AND MICRO-ORGANISMS

by E. G. Mulder, Ph.D.
Landbouwproefstation, Eemskanaal, Groningen, Netherlands

Copper:—Copper deficiency in cultivated plants may occur on sandy and peaty soils in Europe as well as in the U.S.A. and Australia. In Holland and other countries of Western Europe the deficiency symptoms are known as reclamation disease.

Since the discovery of Hudig and Meyer in 1925 (4) that reclamation disease can be prevented for a number of years by adding one dressing of 50-100 kg $CuSO_4$ per ha, many farmers in the affected areas of Holland have treated their land with copper sulphate. As a result of this the pronounced symptoms of the disease are seen rather sporadically in Holland nowadays. The mild cases, however, which are recognized less easily, still occur frequently.

To prove that the reclamation disease was due to a lack of available copper in the soil, three series of experiments were carried out:

1) The symptoms of copper deficiency in various species of plants growing in nutrient solutions were compared with those of the reclamation disease. These symptoms appeared to be quite similar. This was not only so in the case of pronounced cases in which dead, white tips appeared at the youngest leaves while no ears emerged, but also of the mild cases in which the ears emerged normally, but grain production was reduced (Plates 4, 5 and 6).

Plant species less susceptible to the reclamation disease (potatoes, rye) required considerably less copper than highly susceptible plants such as wheat, barley and oats.

2) Copper determinations according to the carbamate method (5) were carried out on plants grown in normal and diseased soils. Grain and straw of ripened plants, and leaf and stem material of young plants were analysed. In the latter instance the

plants were harvested when the first disease symptoms were observed. TABLES 4 and 5 show some of the results.

3) In a number of normal and diseased soils the plant-available copper was estimated by a microbiological assay method. This assay is based on the fact that the fungus *Aspergillus niger* requires small amounts of copper for the formation of normal black spores. In a nutrient solution purified from copper by charcoal treatment *Aspergillus* develops a white sterile mycelium, with 0.2 µg of Cu in 40 ml of nutrient solution yellow spores are formed, with 0.4 µg the colour of the spores is yellowish-brown, with 1.0 µg grey-brown and with 1.5 µg grey-black. By contrast 2.5 µg and higher amounts of copper induce normal black spores. Thus, the color of the *Aspergillus* spores provides a measure of the amount of available copper in a nutrient medium.

TABLE 4: *Copper content, mg per kg dry weight, of wheat (grain), grown on different soils:—*

SOIL	TREATMENT	REMARKS	Cu, mg/kg dry weight
Sandy soil	Control	Slightly diseased plants	0.9
Sandy soil	50 mg of $CuSO_4$ per 2 kg of soil	Normal plants	3.0
Peaty soil	Control	Slightly diseased plants	0.7
Peaty soil	50 mg of $CuSO_4$ per 2 kg of soil	Normal plants	1.3
Peaty soil	Control	Slightly diseased plants	0.9
Peaty soil	100 kg of $CuSO_4$ per ha	Normal plants	1.6
Peaty soil	Control	Normal plants	1.2
Clay soil Groningen	Control	Normal plants	6.0
Clay soil Zeeland	Control	Normal plants	2.5

For the estimation of the available copper in soils, one gram of air-dried soil is added to 40 ml of a purified nutrient solution in an Erlenmeyer flask. This medium is inoculated with a suspension of *Aspergillus* spores and after four days incubation at 30°C the colour of the mycelia is compared with the colour scale of a series of standard cultures to which copper in increasing amounts from 0-2.5 µg per culture has been added.

By comparing the results of the *Aspergillus* assay with those of pot and field experiments with cereals it was found that soils containing 0.6 µg of available copper or less, per gram of air-dried soil, produced cereals with pronounced symptoms of the reclamation disease. With 0.6-1.5 µg slight symptoms of the disease were seen whereas with 2 µg and more available copper per gram of soil a normal plant growth was generally obtained.

From the results of these three series of experiments it was concluded that the reclamation disease is brought about by a lack of available copper in the soil.

Plants growing in copper-deficient soils had to be supplied with amounts of copper more than a hundred times higher than those required by cultures in nutrient solutions. By using a special technique it was shown that these differences were due to a fixation of copper by the black humus present in many copper-deficient soils. In this experiment oat plants were grown with part of their roots in a copper-deficient soil and with the other part in a nutrient solution. When the copper was added to the solution, very small amounts resulted in normal plant growth, but much larger quantities were required if added to the soil.

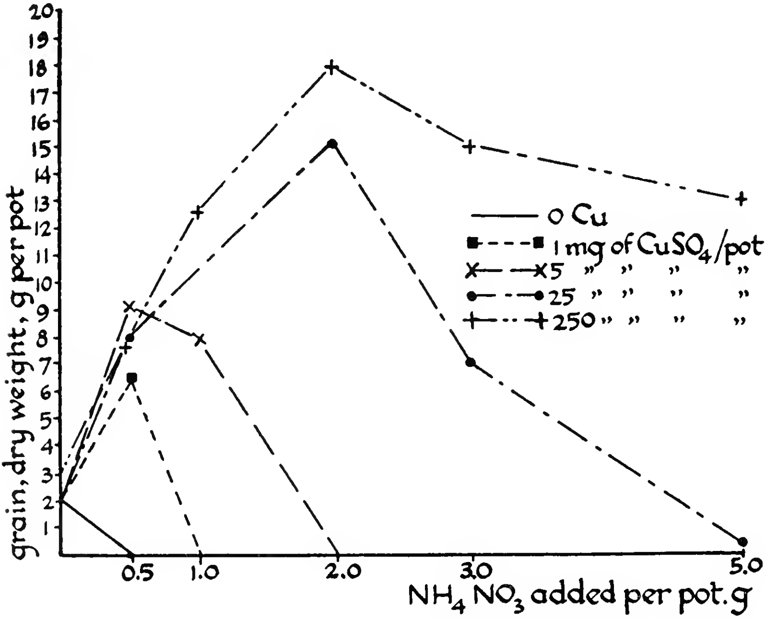
TABLE 5: *Copper content of five weeks old oat plants (stems and leaves)*:—

SOIL NO.	CONDITION OF PLANTS IN PREVIOUS POT EXPERIMENTS	Cu, mg/kg dry weight
1	Pronounced disease symptoms	1.9
2	Somewhat less pronounced disease symptoms	3.5
1	Provided with 50 kg/ha $CuSO_4$, normal plants	8.1
3	Pronounced disease symptoms	2.4
4	Slightly diseased	5.0
4	Provided with 50 kg/ha $CuSO_4$, normal plants	8.5
5	Slightly diseased	5.7
5	Provided with 50 kg/ha $CuSO_4$, normal plants	8.0

In using the *Aspergillus* assay it was shown that copper fixed by the black humus was not available to the fungus.

In further experiments with *Aspergillus niger,* as well as with cereals in nutrient solutions, it was shown that copper precipitated by hydrogen sulphide producing bacteria was unavailable to both the fungus and the higher plants. As copper sulphide can be used as a copper source by *Aspergillus* and cereals the copper precipitated by H_2S-producing bacteria apparently was present in a compound other than CuS.

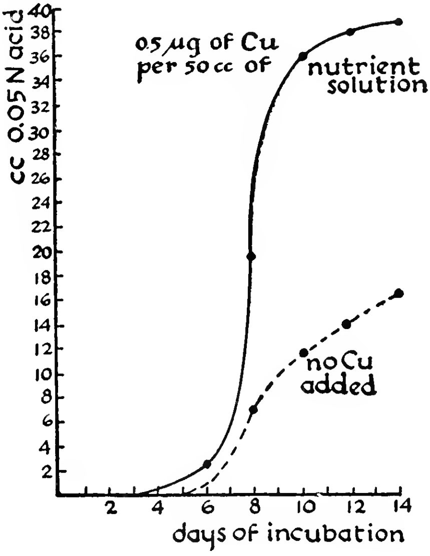
In pot experiments with wheat growing in a copper-deficient soil a relation between the copper and nitrogen nutrition of this plant was found. Without an addition of copper, normal though small plants were obtained when no nitrogen was added to the soil. With the addition of increasing amounts of nitrogen increasing amounts of copper had to be given in order to obtain normal plant growth (Figure 4).



Textfigure 4.—Effect of increasing amounts of copper and nitrogen on wheat grown in a copper-deficient soil.

In experiments with microorganisms the essentiality of copper for a number of biochemical reactions was shown. The oxidation of ethyl alcohol to acetic acid by *Acetobacter aceti* was much stimulated by minute amounts of copper as is seen in Figure 5. The formation of the black pigment in the spores of *Aspergillus niger* and of cultures of *Azotobacter chroococcum* requires copper. The same is true of the oxidation of manganous compounds to manganese dioxide by fungi. In these reactions copper apparently has the function of an oxidation catalyst.

Molybdenum:—In the experiments on copper with *Aspergillus niger* the nutrient solution was purified by shaking with charcoal. In a second series of experiments the copper impurity of the nutrient salt was removed by recrystallizing the salts. In a medium prepared with these salts and provided with copper the growth of *Aspergillus* was very poor. Obviously the medium purified with charcoal, though free from copper, contained some substance which was removed by recrystallization of the nutrient salts and the sugar. As STEINBERG (7) in 1936 had shown that *Aspergillus* niger requires small amounts of molybdenum when cultivated in a medium with nitrate as the sole source of nitrogen, it was suggested that molybdenum deficiency was the cause of the poor growth of *Aspergillus*. A third series of experiments was started with recrystallized salts, this time with a trace of molybdenum added to some of the cultures. In this medium a normal develop-



TEXTFIGURE 5.—Effect of copper on the production of acetic acid by *Acetobacter aceti*.

ment of *Aspergillus* took place, thus showing, in agreement with STEINBERG, that molybdenum is an essential element for *Aspergillus niger* when growing in a nutrient solution with nitrate nitrogen. Increasing amounts of molybdenum added to the nutrient solution resulted in a progressive increase in weight of the mycelia and in a more abundant spore formation (TABLE 6). The growth-rate curve as well as the different sporulation were used for the estimation of very small amounts of molybdenum in various materials.

When ammonium compounds were used as a nitrogen source the response to molybdenum was much smaller. It was shown that the difference in response to molybdenum between nitrate and ammonium cultures was due to a much higher requirement of molybdenum when the fungus was grown in a solution with nitrate nitrogen (6). Probably molybdenum is required as a catalyst in nitrate reduction.

TABLE 6: *Effect of increasing amounts of molybdenum on the yield of Aspergillus niger in a medium with nitrate-N:—*

$Na_2MoO_4 \cdot 2H_2O$ per 50 cc of nutrient sol µg	Yield* (dry weight) g	Sporulation	Appearance of mycelium
0	0.165	0	Entirely mucous
0.001	0.253	0	Entirely mucous
0.0025	0.294	0	Partially mucous
0.005	0.450	0	Partially mucous
0.010	0.558	0	Partially mucous
0.025	0.797	Scant	Normal
0.050	0.868	Normal	Normal
0.100	0.900	Normal	Normal

* Average of duplicate values.

The importance of molybdenum in nitrate reduction was shown in experiments with denitrifying bacteria and higher plants. In the denitrification experiments four strains of bacteria were used. Two of these strains did not show any growth in a molybdenum-free solution; the other two brought about insignificant denitrification. With 5 µg of Na_2MoO_4 added per bottle all strains, however, showed intensive denitrification. Some of the results are presented in TABLE 7.

In experiments with tomato, barley and oat plants the importance of molybdenum for the normal growth of these plants was shown. In agreement with the results obtained by ARNON and STOUT (2) a very poor growth of tomato plants was obtained in the absence of molybdenum (PLATE 7). In order to show the effect of molybdenum on nitrate reduction in barley and tomato, these plants were grown in nutrient solutions without molybdenum and with a moderate supply of nitrogen. When the plants had grown for some weeks in this medium slight symptoms of molybdenum deficiency, as well as of nitrogen deficiency, were observed. Abundant potassium nitrate was then added to the cultures and in addition some of them were given sodium molybdate. Some days later the plants were harvested and nitrate, as well as organic nitrogen fractions, were determined in leaves, stems and roots. In the absence of molybdenum practically no nitrate reduction had taken place (TABLE 8).

TABLE 7: *Effect of molybdenum on denitrification (incubation time 10 days)*:—

BACTERIAL STRAIN	$Na_2MoO_4 \cdot 2H_2O$ per bottle (50cc) μg	After 10 days incubation at 25° C. NO_3–N disappeared* mg
W 1	0	1.4
W 1	5	9.3
S 1	0	4.5
S 1	5	13.1
S 2	0	0.0
S 2	5	10.2
W 2	0	0.0
W 2	5	8.0

* Initial nitrate concentration: 13.1 mg N per bottle (50 cc).

The nitrate content in leaves and stems appeared to be high, whereas the organic nitrogen fractions did practically not increase. In the plants with molybdenum added an intensive nitrate reduction and formation of organic nitrogen compounds had taken place. With barley similar results were obtained. Apparently molybdenum has the function of a catalyst in nitrate reduction in microorganisms as well as in higher plants.

The Importance of Molybdenum in the Fixation of Gaseous Nitrogen:—BORTELS (3) in 1930 was the first to show that molybdenum is indispensable for the nitrogen fixation of the free-living nitrogen-fixing bacterium *Azotobacter chroococcum.* The question whether this element is required for the nitrogen fixation of this bacterium only or is needed for growth in a medium with combined nitrogen as well has been discussed by various authors. Although some of these authors contended that molybdenum is essential for the nitrogen fixation only, most of them observed a stimulating effect of this element when *Azotobacter* was grown in a nitrate medium.

In an experiment with *Azotobacter chroococcum* the effect of increasing amounts of molybdenum was determined in nutrient solutions with nitrate, ammonium sulfate and without combined nitrogen, respectively. It appeared that molybdenum was essential for growth in gaseous N_2 and in a nitrate medium. In a medium with $(NH_4)_2SO_4$ no response to molybdenum was observed. With nitrate-N about ten times smaller amounts of molybdenum were required than in the absence of combined nitrogen when the bacterium had to fix the nitrogen of the air. This result indicates that the molybdenum requiring processes involved in N_2 and nitrate assimilation are not similar.

TABLE 8: *Effect of molybdenum on the nitrate reduction in tomato plants:—*

LEAVES

$Na_2MoO_4 \cdot 2H_2O$ added µg	Fresh weight	Protein N mg	Sol. org. N mg	NO_3–N mg
0*	11.30†	15.5	3.85	0.00
0*	11.72†	14.7	4.58	0.00
0**	6.68 ± 0.41	15.8 ± 0.58	5.42 ± 0.28	4.19 ± 0.48
200*	6.10 ± 0.26	19.1 ± 0.93	5.65 ± 0.26	0.61 ± 0.18
		STEMS		
0*	——	8.09	4.09	0.00
0*	——	7.41	3.93	0.00
0**	7.94 ± 0.82	6.74 ± 0.80	3.79 ± 0.42	3.53 ± 0.36
200*	8.61 ± 0.71	8.63 ± 1.16	6.20 ± 0.66	2.70 ± 0.30
		ROOTS		
0*	2.75	6.23	2.52	0.00
0*	2.75	——	——	——
0**	3.34 ± 0.88	5.26 ± 0.57	2.62 ± 0.35	1.41 ± 0.46
200*	3.61 ± 0.15	4.92 ± 0.41	3.12 ± 0.55	1.32 ± 0.18

* Plants harvested before adding the nitrate.
† Fresh weight of leaves and stem.
** Average values of six separately analysed plants.

In culture solution experiments with pea plants it was shown that molybdenum is essential for the fixation of gaseous nitrogen by the nodules. In these experiments the pea plants were first grown in a solution to which combined nitrogen had been added. Nodules were formed abundantly in both series; without molybdenum the colour of these nodules was yellow-brown in contrast to the nearly pink colour in the presence of this element. Nitrogen fixation, however, was quite inadequate in the absence of molybdenum. The leaves of the pea plants became yellow-green due to nitrogen deficiency and the plants died in an early stage. Plants with molybdenum added, harvested when colour differences between the two series were shown, had more than 50% higher N-content in their tissues than those without molybdenum (TABLE 9).

TABLE 9: *Effect of molybdenum on the nitrogen fixation by the nodules of pea plants:—*

$Na_2MoO_4 \cdot 2H_2O$ added	Leaves and stems			Roots		
	Dry weight	Nitrogen	Nitrogen	Dry weight	Nitrogen	Nitrogen
μg	g	mg	%	g	mg	%
0	4.15	84.9	2.05	1.78	40.4	2.27
0	4.07	79.0	1.94	1.50	32.9	2.19
200	3.22	108.6	3.37	1.35	42.9	3.18
200	4.20	159.9	3.81	1.60	51.9	3.22

The fact that molybdenum has the function of a catalyst in nitrogen fixation by the nodules of legumes is in good agreement with the relatively high content of molybdenum found in these nodules.

In Australia and New Zealand responses to molybdenum applications have been obtained by ANDERSON, THOMAS and OERTEL (1) with alfalfa and subterranean clover growing on acid ironstone soils. Apparently molybdenum stimulated the nitrogen fixation in these plants. Although it is improbable that the occurrence of molybdenum deficiency is limited to these Australian soils, so far there is no evidence that this deficiency occurs in other countries.

Addendum:—It was recently shown, by the author, that molybdenum deficiency in clover may be found in Holland on many peat soils containing iron as well as on acid, sandy soils (Landbouwk. Tijdschr. 62:311, 1950; Trans. 4. Int. Congr. Soil Sci. 2:124, 1950). Similar reports came from England (particularly concerning cauliflower) and from the U.S.A. (Ann. Rev. Plant Physiol. 1:1, 1950).

References

1. Anderson, A. J., Thomas, M. P. and Oertel, A. C., 1946: Plant responses to molybdenum as a fertilizer (Counc. Sci. Inst. Res. Commonw. Australia Bull. 198).

2. Arnon, D. I. and Stout, P. R., 1939: Molybdenum as an essential element for higher plants (Plant Physiol. 14:599-602).

3. Bortels, H., 1930: Molybdän als Katalysator bei der biologischen Stickstoffbindung (Arch. Mikrobiol. 1:333-342).

4. Hudig, J. and Meyer, C., 1925 and 1926: De ontginningsziekte en haar bestrijding (De Veldbode).

5. Mulder, E. G., 1938: Over de beteekenis van koper voor de groei van planten en microorganismen (Thesis, Wageningen).

6. Mulder, E. G., 1948: Importance of molybdenum in the nitrogen metabolism of microorganisms and higher plants (Plant and Soil 1:94-119).

7. Steinberg, R. A., 1936: Relation of accessory growth substances to heavy metals including molybdenum, in the nutrition of *Aspergillus niger* (J. Agr. Research 52:439-448).

Points from Discussion of Paper by Dr. Mulder

Prof. Burström:—

1) You have demonstrated a relation between nitrate and molybdenum very convincingly, but in your experiments with *Azotobacter* you have given the amount of organic nitrogen per culture. I suppose that you have growth responses parallelling the amounts of utilized nitrogen. Why can you not conclude that there is a direct relation between molybdenum and nitrate assimilation?

2) Have you been able to find nitrite or other presumed intermediates in your experiments with or without molybdenum?

Dr. Mulder:—

1) The amount of organic nitrogen in the cells of *Azotobacter* may be used to measure growth. If nitrate is being added, as a source of nitrogen, we observe a direct relation between nitrate assimilation and molybdenum.

2) No tests for such compounds have been carried out.

Prof. Arnon:—

Your results with higher plants suggest that Mo is involved in NO_3 reduction whereas those with *Azotobacter* indicate that Mo is needed for nitrogen fixation. Now if we assume that *Azotobacter* fixes atmospheric nitrogen by reduction *i.e.* combination with hydrogen rather than oxidation *i.e.* combination with O_2, then it would appear that Mo is not performing the same function in the two classes of organisms.

In this connection, did I understand that the test for nitrate reduction in the higher plants involved the measurement of *organic* nitrogen? If so, then would it not be justifiable to interpret the results as suggesting that Mo[1] may be necessary for the formation of organic compounds of nitrogen?

Dr. Erkama:—

In a preliminary culture experiment at Helsinki Biochemical Institute we have observed that the presence of molybdenum in the nutrient solution somewhat prevents the poisonous effect of copper on peas.

In the experiment the nutritive salts were freed from heavy metals by dithizone treatment. The peas were inoculated with *Rhizobium* strains and grown in sterilized N-free nutrient solution. The same basal nutrition was used with varying amounts of copper. An addition of molybdenum (1 mg $Na_2MoO_4.2H_2O$ per litre) increased the yield and, on the whole, also the fresh weight of the root nodules, as indicated by the following figures.

COPPER NUTRITION GAMMA PER LITRE		DRY WEIGHT OF SPROUT	FRESH WEIGHT OF NODULES PER PLANT
		g	mg
Cu 0	without Mo	0.464	256
	with Mo	0.614	209
Cu 10	without Mo	0.542	160
	with Mo	0.598	166
Cu 100	without Mo	0.479	23
	with Mo	0.568	113
Cu 1000	without Mo	0.196	7
	with Mo	0.278	31

Dr. ERKAMA's answer to Prof. ARNON concerning the mechanism of the symbiotic nitrogen fixation: According to Prof. VIRTANEN it is to be expected from the energetic point of view that the first steps in nitrogen fixation are oxidation-reduction processes provided that hydroxylamine is formed as an intermediate product. If, however, nitrogen fixation leads to ammonia without the forma-

---

[1] Though molybdenum deficient plants were low in organic nitrogen after treatment with nitrate (TABLE 8), this has been probably a result of the poor nitrate reduction. Since this Symposium was held, many investigations on molybdenum and copper were made in our laboratory and I am drawing the attention of the reader to these by the addendum on page 49. (*Author's Note.*)

tion of hydroxylamine the reaction is probably a reduction process (*cf.* Biol. Rev. 22:239, 1947).

Dr. Hewitt:—

There are three points that arise in discussion of the results described by Dr. MULDER with regard to the effects of molybdenum deficiency in plants and microorganisms. In the first place it is interesting to hear that Dr. MULDER found that accumulation of nitrate occurs in plants when molybdenum is withheld, as a similar result has been obtained with molybdenum deficient plants grown in sand culture at Long Ashton. In these experiments it was found that considerable nitrate accumulation occurred in tomato, mustard and cauliflower when molybdenum was lacking, but not in savoy cabbage, although all the plants mentioned showed marked pathological effects of molybdenum deficiency, which were not those caused by nitrogen deficiency. It thus seems that the influence of molybdenum in nitrate reduction may differ according to species and also that molybdenum may have more than one function in plants.

In the second place the relative effects of molybdenum on the metabolism of the nitrogen fixing *Azotobacter* and of denitrifying bacteria in the presence of different nitrogen compounds requires comment. It is reasonable to assume that the reduction of nitrate prior to its final assimilation proceeds through a number of steps and that the fixation of atmospheric nitrogen also involves several steps from a lower initial energy level before either ultimate assimilation or entry at some link in the general chain of reactions involved in nitrate reduction. The fact that the amount of molybdenum needed for fixation of atmospheric nitrogen is about ten times that required for reduction of a corresponding amount of nitrogen as nitrate by the same organism may imply that molybdenum is involved in two distinct reactions and not in one only that is common to both processes. Further studies on the assimilation of nitrogen from likely intermediate compounds would be of value in the elucidation of this problem.

Finally the apparent unimportance of molybdenum for *Azotobacter* when nitrogen is supplied as the ammonium ion raises the question of its essentiality in the terms of the criteria proposed earlier by Dr. ARNON, since the substitution of one metabolite by another may apparently eliminate the need for this element in the normal development of the organism.

ON THE EFFECT OF COPPER AND MANGANESE ON THE IRON STATUS OF HIGHER PLANTS

by JORMA ERKAMA, Ph.D.
Biochemical Institute of the University, Helsinki, Finland

The parallelism of different physiological processes has become more and more obvious in recent developments of biochemistry. This point of view is very important also when interpreting the rôle of trace elements in the physiology of plants. The characteristic properties of an element only become clear if studied in conjunction with other metabolic factors. Contrasts and coincidences, as well as interactions and antagonisms, between various elements will reveal many details about their physiological properties which would never have been discovered if the elements had been examined on their own.

TABLE 10: *Variations in copper, iron, and manganese content of samples analyzed:—*

	NUMBER OF SAMPLES	Content in dry matter			MEAN DEVIATION	
		Mean	Maximum	Minimum		
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	per cent
Copper	69	10.5	31.7	5.3	± 3.8	±36
Iron	69	152.9	710	22	± 94.8	±62
Manganese	68	237.5	1760	4	±235.6	±99

Taking both their structure and their biological effects into consideration, copper and manganese—with a third important element, iron—form a physiological unit. By studying these three heavy metals, at the same time, we will obtain useful new ideas about their physiological functions (6).

Some Aspects of the Occurrence of Copper, Manganese, and Iron in Plants:—It is clear from analytical data that external factors have more effect on the manganese content of a plant than on its copper or iron content. The manganese content of plants also varies more than that of the other heavy metals. This is shown in TABLE 10 which gives a survey of variations in copper, iron, and manganese content of a number of plant samples.

If we compare the copper, iron, and manganese content of ash with the ash content of a plant, we see that the uptake of heavy metals is apparently the reverse of the uptake of other ash components. The copper or iron content of ash seems to be fairly independent of the relative ash content of plants. Between the manganese content of ash and the ash content of plants there exists, on the other hand, a distinct negative correlation, as we will see from TABLE 11. Most of the plants analyzed there had been grown in the same soil. The ratio Cu:Fe seems to be more constant than the ratio Mn:Fe. The smaller the ash content of a plant, we note, the more manganese it contains.

TABLE 11: *Relation of the ash content of plant to the heavy metal content of ash in some plant leaves:—*

PLANT	ASH IN 100 g DRY MATTER	CONTENT IN 100 g ASH			Cu:Fe	Mn:Fe
		Cu	Fe	Mn		
	mg	mg	mg	mg		
Sedum telephium	19.0	10	92	101	0.114	1.10
Taraxacum officinale	17.2	10	148	46	0.069	0.31
Rumex acetosa	14.6	12	232	115	0.051	0.50
Sambucus racemosa	13.2	7	198	286	0.033	1.43
Trientalis europaea	9.7	11	170	308	0.066	1.81
Majanthemum bifolium	9.2	10	174	500	0.056	2.88
Rubus chamaemorus	8.8	6	130	387	0.048	2.99
Linnaea borealis	8.5	11	280	205	0.037	0.74
Convallaria majalis	8.5	13	115	142	0.112	1.23
Rubus saxatilis	7.6	7	200	474	0.036	2.28
Oxalis acetosella	7.6	9	175	825	0.051	4.72
Fragaria vesca	7.6	10	145	737	0.040	3.02
Rubus idaeus	6.6	12	358	892	0.034	2.50
Vaccinium myrtillus	4.3	24	247	1722	0.096	7.00
Vaccinium vitis idaea	3.6	24	131	1198	0.181	9.15

Copper has been found strongly bound in protoplasm. Manganese, on the contrary, seems to be easy to dialyze from the cell

(12, 17). If we determine the copper and manganese content on a protein basis, we find a negative correlation, in the tissue, between the protein content and manganese content. The copper content, on the other hand, seems to be fairly constant.

Iron in plant cells (1, 7, 9, 13, 15, 18, 19, 21, 23) occurs chiefly in undissolved form. Most of it is bound in chloroplasts or in the cytoplasm and mainly in the form of ferric iron. This "inactive" or "residual" iron, is hardly dialyzable and seems to be ineffective in chlorophyll production. On the other hand, the fraction with a direct relation to chloroplasts, called "active" iron, is more easily soluble in its ferrous form. According to LIEBICH (15) 82% of the total iron in green spinach leaves is found in chloroplasts, 5% in cytoplasm, and 13% in water solution.

TABLE 12: *Sterile water culture experiments with peas:—*

TRACE ELEMENT NUTRITION	DRY WEIGHT PER PLANT Sprout	Root	CONTENT IN DRY MATTER OF SPROUTS Cu	Mn	Fe
	g	g	p.p.m.	p.p.m.	p.p.m.
A-Z, complete	3.967	0.262	7.5	71	274
A-Z, without Cu	2.848	0.310	4.8	75	218
A-Z, without Mn	0.811	0.153	11.7	32	762
Cu only	1.139	0.143	13.8	33	309
Mn only	2.532	0.186	6.5	104	227

Effect of Copper and Manganese on Iron Uptake:—In sterile water culture experiments with peas, a synergism between the copper and iron content of plants and an antagonism between their manganese and iron content is obtained. The "A-Z solution" of HOAGLAND was used with the nitrate solution. In all series iron was given in the form of ferric citrate. We will see from TABLE 12 that the plants without copper nutrition contain less iron per unit dry weight than the plants with copper. On the other hand, the plants without manganese showed most iron.

The effect of copper deficiency on the heavy metal content of plants is shown very distinctly in an experiment where a pea plant was grown from a copper-deficient seed in a culture solution without copper. Seeds, deficient in copper, were obtained from plants which had developed in a culture solution with a low copper content. Such copper-deficient seeds germinated so poorly that only one plant became available for our analysis. TABLE 13 illustrates some of its characteristics.

In addition to a low iron content, TABLE 13 reports on the exceedingly high content of manganese which shows us an antagonism between copper and manganese.

TABLE 14 gives the effect of additional manganese nutrition on the heavy metal content of peas grown in a nutrient solution deficient in manganese. Manganese was added 35 days after sowing, and the plants were analyzed when 60 days old.

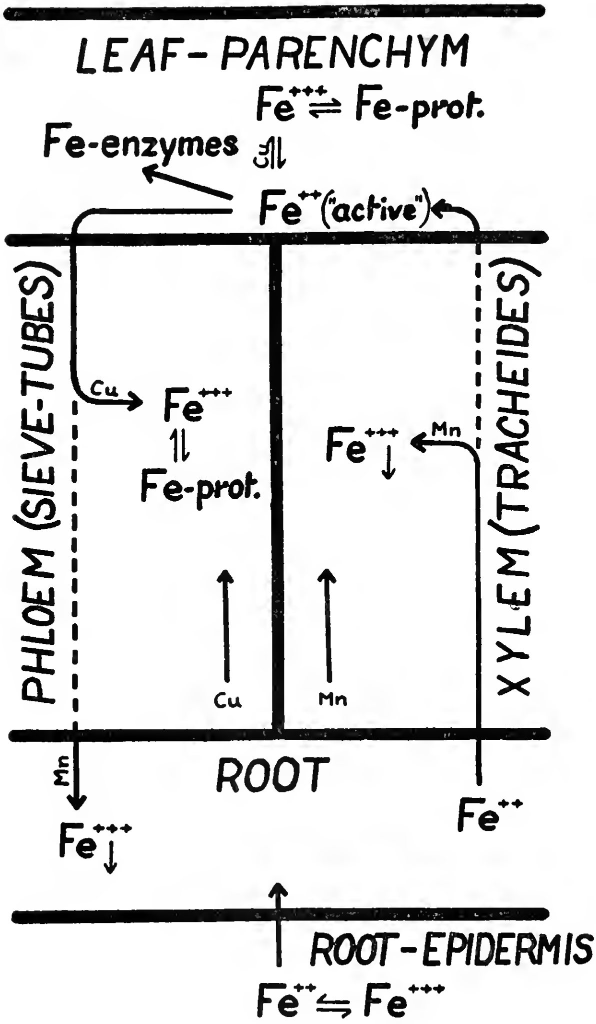
Addition of manganese caused a pronounced decrease of iron content in the green parts of our plants, especially in the chlorotic upper leaves. The decrease of iron content in leaves is probably not affected by the increase in the dry matter after the addition of manganese, because the iron content of leaves, after manganese addition, was lower than in normal plants. The increase of iron in roots apparently results from precipitation, caused by the oxidizing action of manganese.

TABLE 13: *Copper-deficient peas from copper-deficient seed:—*

LENGTH OF SPROUT	DRY WEIGHT	ASH	CONTENT IN DRY MATTER Cu	Mn	Fe
cm	g	per cent	p.p.m.	p.p.m.	p.p.m.
96	1.031	16.6	3.0	174	143

Discussion:—Most factors which have an effect on the uptake and availability of iron regulate its degree of oxidation. Both ferrous and ferric iron are present in the soil, as well as in the plant. The relation between these two forms depends on the metabolism of the plant, and particularly on oxidation-reduction conditions. Thus, the problem of the influence of copper and manganese on the iron status of plants should also be discussed in connection with oxidation-reduction phenomena.

As early as 1920, MAQUENNE and DEMOUSSY (16) gave attention to the oxidizing effect of copper salts on ferrous ions in nutrient solutions and to their preventive action on iron uptake. Later, many other investigators, *e.g.* DENSCH and HUNNIUS (5), CHAPMAN *et al.* (3), and WILLIS (24), reported similar observations.



TEXTFIGURE 6.—Diagram showing some possible functions of copper and manganese in the iron metabolism of plants.

Manganese is also able to reduce the absorption of iron from a nutrient solution. The investigations of JOHNSON (11), HOPKINS (8), SCHOLZ (22), and PEARSE (20) showed that manganese oxidizes iron in culture solution to the ferric state decreasing its uptake. According to KRIEL (14), as quoted by BENNET (1), an excess of manganese in culture solution produces chlorosis by reducing iron absorption.

TABLE 14: *Effect of manganese addition on the heavy metal content of manganese-deficient peas:—*

MANGANESE GIVEN PER LITRE OF NUTRIENT SOLUTION	CONTENT IN KG DRY MATTER								
	Roots			Leaves 1–8			Leaves 9–18		
	Cu	Mn	Fe	Cu	Mn	Fe	Cu	Mn	Fe
γ	mg	mg	mg	mg	mg	mg	mg	mg	mg
5	9.5	38	735	9.5	33	405	10.1	36	440
5 + 100	9.3	111	870	10.8	57	274	5.3	86	188

What is the explanation of the action of copper and manganese within the plant itself as well as on the iron content of the plant?

KLIMAN (13) suggests that the plant takes iron, chiefly, if not wholly, as ferrous iron. In my own experiments, iron was given in the form of ferric citrate. It is, therefore, most probable that the reactions now observed between heavy metals are to be attributed mainly to oxidation-reduction reactions in the plant itself.

TABLE 15: *Relations between copper, manganese, and iron in plants:—*

	COPPER		MANGANESE	
	Deficiency	Excess	Deficiency	Excess
Vacuolar sap	—	Fe-deficiency	Fe-excess	$Fe^{++} \rightarrow Fe^{+++}$
Protoplasm (Chloroplasts)	Fe-deficiency	Fe-excess	Fe-excess	Fe-deficiency

TABLE 15 and FIGURE 6 illustrate some possible relations between copper, manganese, and iron in plants.

Copper, as we have seen, accumulates particularly in the protoplasm. DELF (4) found that the movement of copper takes place almost exclusively in phloem, the copper being transferred, from cell to cell, by an exchange between surfaces (10). If we consider that copper in protoplasm, as well as in the soil, oxidizes iron to an insoluble ferric state, copper, we may say, eliminates ferrous iron from water solution.

Manganese, which is assumed, for a good part, to enter and to move in solution, catalyzes, according to Chapman (2) and Pearse (20), the oxidation of ferrous iron to insoluble ferric compounds in vacuolar sap and tracheids. This results in a lack of iron in protoplasm. Consequently, manganese has the same effect on iron in vacuolar sap as copper in protoplasm. Copper in plants results in iron deficiency in plant sap as well as in an iron excess in the protoplasm. Manganese in plants, on the contrary, causes an excess of ferric iron in plant sap and an iron deficiency in the protoplasm.

References

1. Bennet, J. P., 1945: Iron in leaves (Soil Sci. 60:91-105).
2. Chapman, G. W., 1931: The relation of iron and manganese to chlorosis in plants (New Phytologist 30:266-283).
3. Chapman, H. D., Liebig, G. F. and Vanselow, A. P., 1939: Some nutritional relationship as revealed by a study of mineral deficiency and excess symptoms on citrus (Soil Sci. Soc. Am., Proc. 4:196-200).
4. Delf, E. M., 1946: Translocation of copper in plants (Nature 157:666-668).
5. Densch, A. and Hunnius, T., 1924: Versuche mit Kupfersulfat (Z. Pflanzenernähr. Düngung A 3:369-386).
6. Erkama, J., 1947: Über die Rolle von Kupfer und Mangan im Leben der höheren Pflanzen (Ann. Acad. Sci. Fennicae A II, 25:1-105).
7. Fischer, F. G. and Hultzsch, K., 1938: Über die Bindung des Eisens in Eiweisstoffen (Biochem. Z. 299:104-122).
8. Hopkins, E. F., 1930: The necessity and function of manganese in the growth of *Chorella* sp. (Science 72:609-610).
9. Jacobson, G., 1945: Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content (Plant Physiol. 20:233-245).
10. Jenny, H. and Overstreet, R., 1939: Surface migration of ions and contact exchange (J. Phys. Chem. 43:1185-1186).
11. Johnson, M. O., 1917: Manganese as cause of the depression of the assimilation of iron by pineapple plants (J. Ind. Eng. Chem. 9:47-49).
12. Kisser, J. and Lettmayr, K., 1934: Untersuchungen über die Auswaschbarkeit der von Samen absorbierten Salze und ihre Bedeutung für die Samenstimulation (Z. Pflanzenernähr. Düngung A 34:172-181).

13. Kliman, S., 1937: The importance of ferrous iron on plants and soils (Soil Sci. Soc. Am., Proc. 2:385-392).

14. Kriel, H. T., 1941: A study of the effect of excess manganese on iron and chlorophyll content and growth of tomato plants (Unpublished thesis, Univ. of California, Berkeley. Ref. by Bennet, 1945).

15. Liebich, H., 1941: Quantitativ-chemische Untersuchungen über das Eisen in den Chloroplasten und übrigen Zellbestandteilen von *Spinacia oleracea* (Z. Botan. 37:129-157).

16. Maquenne, L. and Demoussy, E., 1920: Sur la toxicité du fer et les propriétés antitoxiques du cuivre vis-à-vis des sels ferreux (Compt. rend. 171:218-222).

17. Neish, A. C., 1939: Studies on chloroplasts, II (Biochem. J. 33:300-308).

18. Olsen, C., 1935: Iron absorption and chlorosis in green plants (Compt. rend. trav. lab. Carlsberg 21:15-52).

19. Oserkowsky, J., 1933: Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves (Plant Physiol. 8:449-468).

20. Pearse, H. L., 1944: Iron and manganese in plant nutrition (Farming S. Africa 19:688-694).

21. Rogers, C. H. and Shive, J. W., 1932: Factors affecting the distribution of iron in plants (Plant Physiol. 7:227-252).

22. Scholz, W., 1934: Über die Chlorose des Leins in ihrer Beziehung zum Eisen und Mangan (Z. Pflanzenernähr. Düngung Bodenk. 34:296-311).

23. Thorne, D. W. and Wallace, A., 1944: Some factors affecting chlorosis on high lime soils, I. Ferrous and ferric iron (Soil Sci. 57:299-312).

24. Willis, L. G., 1936: Evidence of the significance of oxidation reduction equilibrium in soil fertility problem (Soil Sci. Soc. Am., Proc. 1:291-297).

Points from Discussion of Paper by Dr. Erkama

Prof. Seekles:—

Did you notice the *p*H value of the soil in the experiments on the absorption of Mn, Cu and Fe in plants? *p*H has a very considerable influence on the absorption of Mn.

Dr. Erkama:—

Almost all the plants here analyzed had grown in the same soil. The soil was acid, and its *p*H varied between 4.5 and 5.4.

Prof. Arnon:—

In discussing the reciprocal effects of one micro-nutrient metal on another two phases can be distinguished. First the effect of one on the *absorption* of another and second, the interrelation of two or more metals *within* the cell. As for the latter it would be useful to interpret the observed effects, wherever applicable in terms of enzyme systems known to be functioning in the plant cell. For example, can the "antagonistic" effects of Mn and Fe be possibly due to the displacement of Fe by Mn when in high concentration in the porphyrins involved in the cytochrome or catalase system?

Prof. Burström:—

You have found an inverse relationship to exist between the contents of Mn, Fe and Cu. As far as I am aware it was shown more than ten years ago that such an inverse relationship generally exists between all cations, alkali—earth, alkali and heavy metals, according to their simple physical properties. Thus I do not quite believe that you are entitled, from only the concentrations of the heavy metals, to conclude as to the redox-conditions.

Prof. BURSTRÖM's answer to Prof. ARNON concerning heavy metals: I think that Dr. ARNON is right; the heavy metals, especially Fe and Mn, are so similar in all respects that they must compete in every reaction and on every point, where they meet in the cell.

Dr. Erkama:—

My conclusions of the oxidation-reduction conditions in plant are hypothetical. On the basis of the analytical data we can, however, notice regular relations between some elements, which very probably determine these conditions.

Dr. Hewitt:—

It is necessary to be cautious in interpreting the effects of copper or manganese or of other metals on oxidation reduction reactions in which the equilibrium between ferrous and ferric iron compounds is involved. The need for such caution is evident when the oxidation-reduction potentials (E_0 values) of the following reactions are considered:—

$$Cu^{+} = Cu^{++} + e^{-} \qquad E_0 = -0.17V.$$
$$Mn^{++} = Mn^{+++} + e^{-} \qquad E_0 = -1.51V.$$
$$Co^{++} = Co^{+++} + e^{-} \qquad E_0 = -1.84V.$$
$$Fe^{++} = Fe^{+++} + e^{-} \qquad E_0 = -0.77V.$$

Now it has been found experimentally in sand culture experiments at Long Ashton that symptoms of iron deficiency can be induced in sugar beet by the addition of Cu^{++}, Mn^{++} or Co^{++} ions and by other ions also. The severity of induced chlorosis varies widely, however, and is in the order of Co^{++}, $>$ Cu^{++}, $>>$ Mn^{++} which bears no relation to the E_0 values shown. Furthermore, it is unlikely that free Co^{+++} ions could exist in living plants, and in order for any ion to cause oxidation by loss of an electron that ion must first be oxidised to the higher valency state. Thus oxidation reduction potentials of reactions involving simple ions cannot account for experimental observations.

It is probable that many metals exist in plants as complex compounds and the following examples show that the E_0 values for reactions with complex ions may differ greatly from those with the simple ions.

Thus for $Co(Cn^1)_6{}^{4-} = Co(Cn)_6{}^{3-} + e^-$, $E_0 = +0.83V$, and in the $Fe^{++} \rightarrow Fe^{+++}$ change where the iron is present as a complex with o. phenanthrolene $E_0 = -1.14.V$. It is thus evident that it is not possible to formulate the action of metals such as copper or manganese in oxidation-reduction reactions particularly in relation to the ferrous-ferric iron equilibrium until detailed knowledge is available as to the nature of the particular metallic compounds involved, and of their respective oxidation-reduction potentials.

Addendum:—Recent experiments (ERKAMA, 1949: Acta Chem. Scand. 3:850-857) have shown that the effect of copper on the iron uptake of peas, in sterile solution cultures, is always correlated with the effect of copper on the oxidation-reduction potential of the culture solution. There is reason to believe that the conditions in the plant sap, as regards redox-relationships, are somewhat similar to those in the culture solution. On the other hand, the interpretation of the oxidation-reduction systems, by the measured redox-potential, has no significance. With regards to E_0 values, it should be emphasized that oxidation-reduction equations are based on the assumption that the systems are in a state of equilibrium. However, a living cell is never in equilibrium in respect to all its redox-systems.

INJURY THROUGH EXCESS OF MANGANESE

by M. P. Löhnis, Ph.D.
Laboratorium voor Microbiologie der Landbouwhogeschool,
Wageningen, Netherlands

The investigations, about which I will report, were started during the years of the German occupation of the Netherlands. For a long time, literature from the Allied countries was unavailable, and I was unaware of the results obtained in other places. The chemical analysis of the samples obtained in the summer of 1947 has not yet been completed, and the full results will be published later.

When the Netherlands were invaded by the German army, in May 1940, the need for an increased production of food crops was felt. In this connection, the experimental plots of the Laboratory for Microbiology at the Wageningen Agricultural College were planted with beans (*Phaseolus vulgaris*) a crop which had never before been grown there. The plots are 41 m^2 each and are well separated. They were originally established for the study of a variety of problems in the microflora of the soil. In 1922 three ranges of six plots each were dressed with different amounts of limestone (60-30-15-10-5-0 kg). After 1922 two further plots were fertilized with chilean nitrate and two with ammonium sulfate, as the only sources of nitrogen. In the later years of the war, nitrate being unavailable, all plots had to be fertilized, however, with ammonium sulfate. Except for nitrogen, all plots have been fertilized and planted in the same manner since 1922.

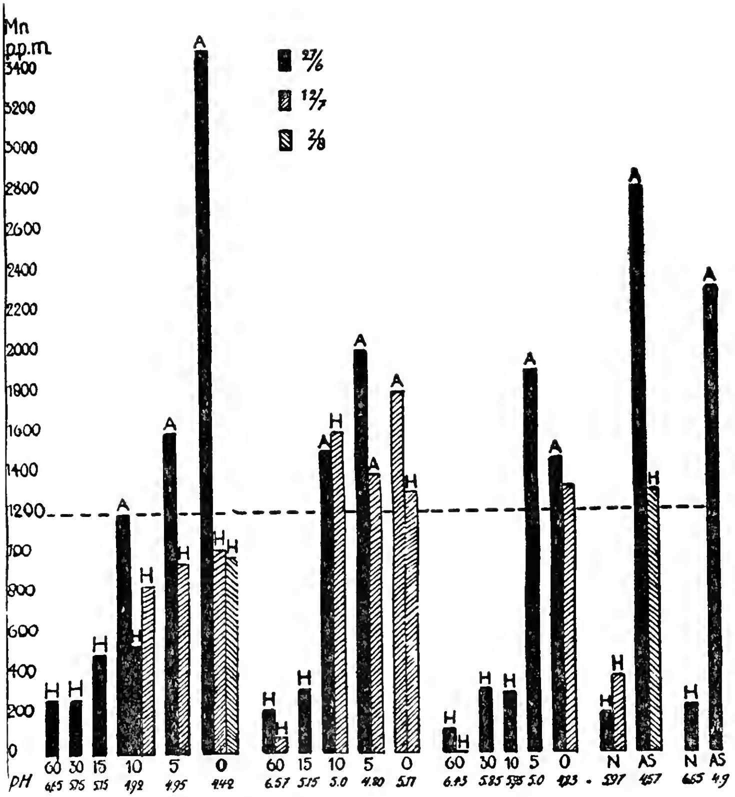
From 1940 to the present, half of each plot has been planted with beans and half with potatoes, the crops in the two sections alternating yearly. In some years, a single row has been sown with another crop. The first crop of *Phaseolus vulgaris* consisted of kidney beans raised for the ripe seed. Later the variety "Dubbele Prinsesseboon" has been chiefly grown. This is a French dwarf bean, the unripe pods of which are used as a vegetable, while the ripe, white seed, called "witte bonen," is dried and used in the same way as brown beans.

On the more acid plots, an injury to the foliage was noted early in the summer of 1940; in all following years these symptoms occurred regularly in the same plots. In young leaves, a strong marginal chlorosis, extending into the intervenal areas, occurs. When older, the leaves become somewhat crinkled and the intervenal areas show small whitish blotches. Finally, in the latter, necrotic spots occur. Petioles and the lower parts of the stem show small, brownish-purple spots. Severely injured plants remain stunted and produce hardly any flowers or seed. Less injured ones often recover, later in the season, but owing to the initial injury the final yield is much smaller than usual.

In the following years, a French dwarf bean, the variety "Dubbele Prinsesseboon," was noted to be even more susceptible to this injury. The chlorosis in the young leaves was most conspicuous and the canary-yellow plants may be recognized in the fields from quite a distance. Purple spots on the petioles do not occur. This variety has been studied most closely by us.

Accidentally the cause of this injury became clear. In a variety of beans ("van Tol's bruine boon") the occurrence of an internal necrosis of the seed had been noted on certain soils. As the phenomenon is very much like "marsh spot" in peas, caused by a deficiency in manganese, I grew "van Tol's bruine boon" in solution cultures, deficient in manganese, in order to ascertain whether the injury might be induced by the same cause. In order to learn also what symptoms would be induced in beans by an excess of manganese, I prepared some cultures with large amounts of $MnSO_4$. Quite unexpectedly, the symptoms induced in this way appeared to be quite similar to those I had noted in the fields. The same pattern of chlorosis, mottling and crinkling of the leaves occurred and the specific purple spots appeared on the petioles.

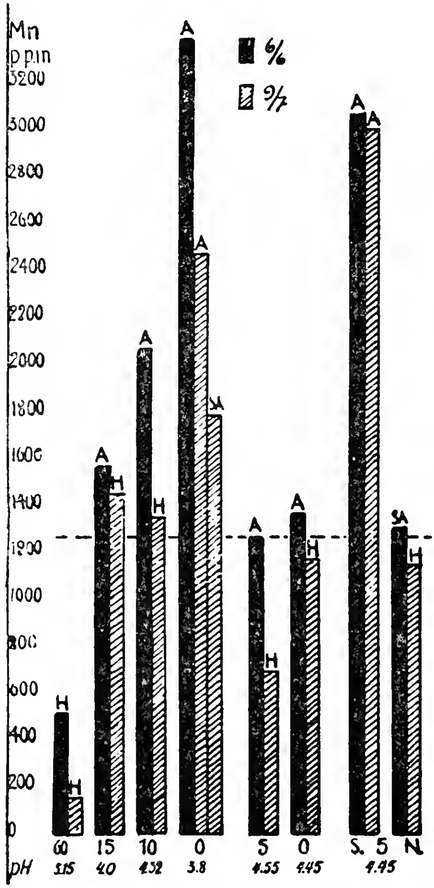
These observations induced us to compare the manganese content of healthy and affected foliage. In sampling healthy and affected foliage always the youngest, full-grown leaves were gathered. The air-dry material was analyzed for manganese (method of MARSHALL as amended by STEENBJERG (3)). The first analysis was made with material collected in 1943 from a number of varieties grown in the various fields. The affected leaves contained much more manganese than the healthy foliage (2).



Textfigure 7.—Manganese content of foliage of *Phaseolus vulgaris* (Dubbele Prinsesseboon) grown on plots with various amounts of limestone (1946).

In all graphs the upper figures, at the bottom, indicate the amounts of limestone in kg which has been supplied to the plot concerned. N indicates a plot permanently fertilized with nitrate, AS with ammonium sulfate. The lower figures give the *p*H of the individual plots. The *p*H for all plots was estimated in 1946, on July 24, and in 1947, on July 17. As there are three ranges of limed plots and two of those supplied with nitrogen, a plot occurring on various graphs can be identified only by the *p*H figure. The letters at the top of the columns mean: H—healthy, A—affected, and S.A.—slightly affected. Sometimes, when healthy, as well as affected material, has been gathered on a single plot, this has been represented by two columns with the same date.

The variety "Dubbele Prinsesseboon" was chiefly studied in 1946. Samples were taken on June 27, when symptoms on the young plants were severe, and on July 12, when recovery had set in on the less acid plots. Plants sometimes recovered to such an extent that mainly the oldest leaves continued to show injury. The results of the chemical analysis are summarized in FIGURE 7.



TEXTFIGURE 8.—Manganese content of foliage of *Phaseolus vulgaris* (Dubbele Prinsesseboon) grown on plots with various amounts of limestone (1947).

When the symptoms were most conspicuous (June 27) a very distinct difference was found between the content of manganese in healthy and affected plants. All samples with a content of at least 1210 p.p.m. Mn, in dry matter, were affected, all those with a lower content (max. 536 p.p.m.) were healthy. The level of 1210 is shown with a dotted line in all figures with the results of 1946 observations.

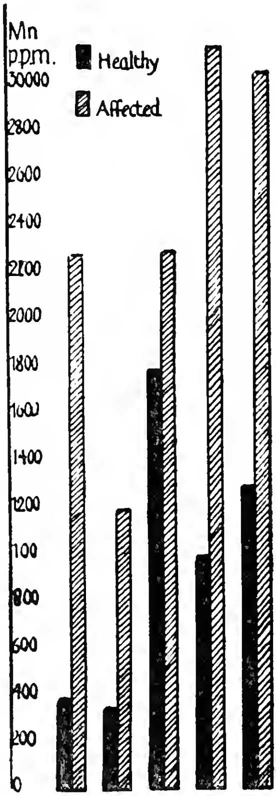
The samples collected on July 12 were taken to see whether the partial or total recovery of the beans would coincide with a lower content of Mn if compared with affected material. In most cases, indeed, the content was lower. Several times, however, the content surpassed 1210 p.p.m. (plot 10 kg, *p*H 5.0, 1598 p.p.m. Mn), while the foliage did not show any symptoms. More mature beans appear to tolerate a higher content of manganese without showing any injury.

In 1947 samples were taken on June 6, when the plants, being very yellow and poorly developed (PLATE 8*a*), were severely affected, and on July 9, when many plants showed recovery. Only part of the samples has been analyzed as yet and the results are given in FIGURE 8. The lowest content of Mn found in affected foliage was 1285 p.p.m., near the threshold value found in 1946. The border line at the 1285 p.p.m. level has been shown in all figures relating to 1947. On July 9, recovery had progressed markedly. The Mn content of all samples was lower than on July 6. The last columns represent the values for a single plot, the southern part (S) of which was strongly, while the northern part (N) was only slightly affected. The plants on the South side hardly differed in content from that found on June 6.

FIGURE 9 shows the amounts found for a number of years in material from healthy and affected plants grown on the same fields, often from special sections. In all cases studied there exists a very definite difference in Mn content and the occurrence of injury coincides always with a high manganese content.

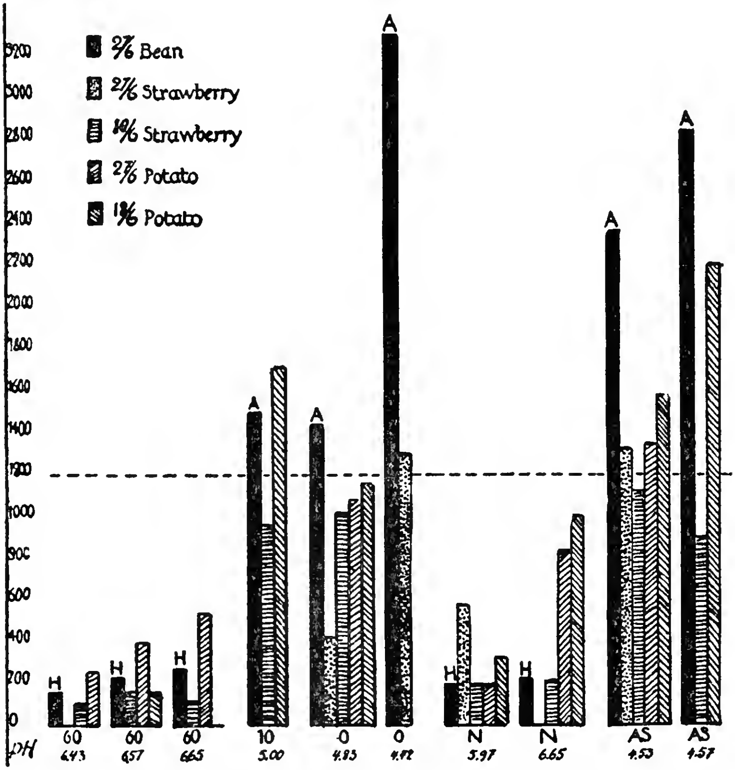
During the years this phenomenon has been studied in beans, potatoes were grown on one half of each plot and in some years the whole or half of a row has been planted with other crops as strawberries, oats, tobacco, cauliflower. None of these crops showed any symptoms of injury. As far as their foliage has been analyzed for manganese, the results are shown in FIGURES 10 and 11. Strawberries have a much lower Mn content than beans. They rarely surpass the "danger line" of 1210 p.p.m. for beans.

Their tolerance for excess manganese in the soil may well be caused by a lower intake. Potatoes will generally exceed beans in Mn content. For higher amounts, however, their content will not reach that of the beans, though it will distinctly surpass the "danger line" for beans. Potatoes, apparently, tolerate a much higher content of manganese, than beans will stand, without injury.



Textfigure 9.—Manganese content of foliage of healthy and affected beans grown on the same field.

Figure 11 presents a few values for oats, the content in manganese of which in the most acid plots exceeds only slightly the value found in the most strongly limed plots. The tolerance of oats for injury by excess of manganese appears to be due to their low intake. This may also explain the susceptibility of oats for the "grey speck disease" caused by a manganese deficiency.



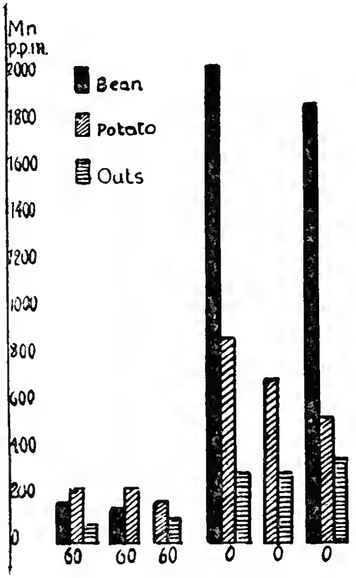
Textfigure 10.—Manganese content of foliage of a number of crops grown on the same plot (1946).

Will soils, varying in causing injury to beans, vary also in content of plant available manganese? To answer this question I determined the exchangeable manganese in soil samples following the method developed by Heintze (1):—

5 g Soil are mixed for one hour with 100 ml N $Ca(NO_3)_2$ and then filtered off. Manganese is analyzed in the filtrate.

The filtrate was always prepared a few days after the soil samples had been taken.

Figure 12 gives the results obtained in 1946. The low Mn content found in the foliage coincides fairly well with a low content of exchangeable Mn, although the plots 10 kg, *p*H 5.75, and 15 kg, *p*H 5.15, form exceptions. The higher amounts in the plants, however, do not coincide with higher content of exchangeable manganese.

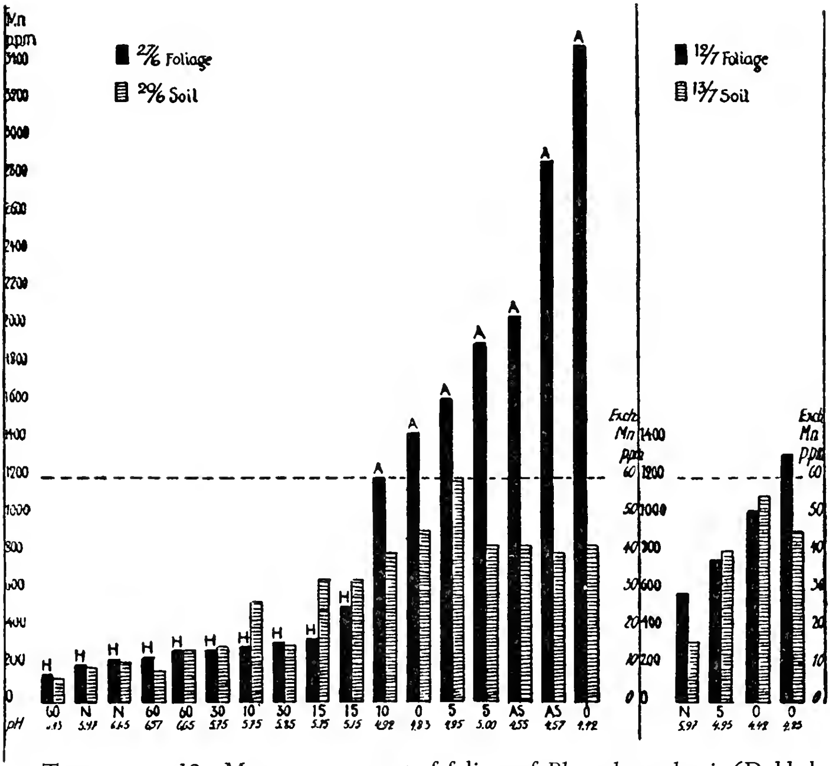


Textfigure 11.—Manganese content of foliage of three crops grown on the same plot (1944).

In Figure 13 the amounts, as far as they have been estimated in soil sampled in 1947, are presented. The lower amounts in soil sampled on May 22 (ranging from 22-54.1 p.p.m.) are markedly higher than those found in 1946 (3.8-55.5). The amounts found in samples taken on July 7, ranging from 31.1-111, exceed the former figures considerably.

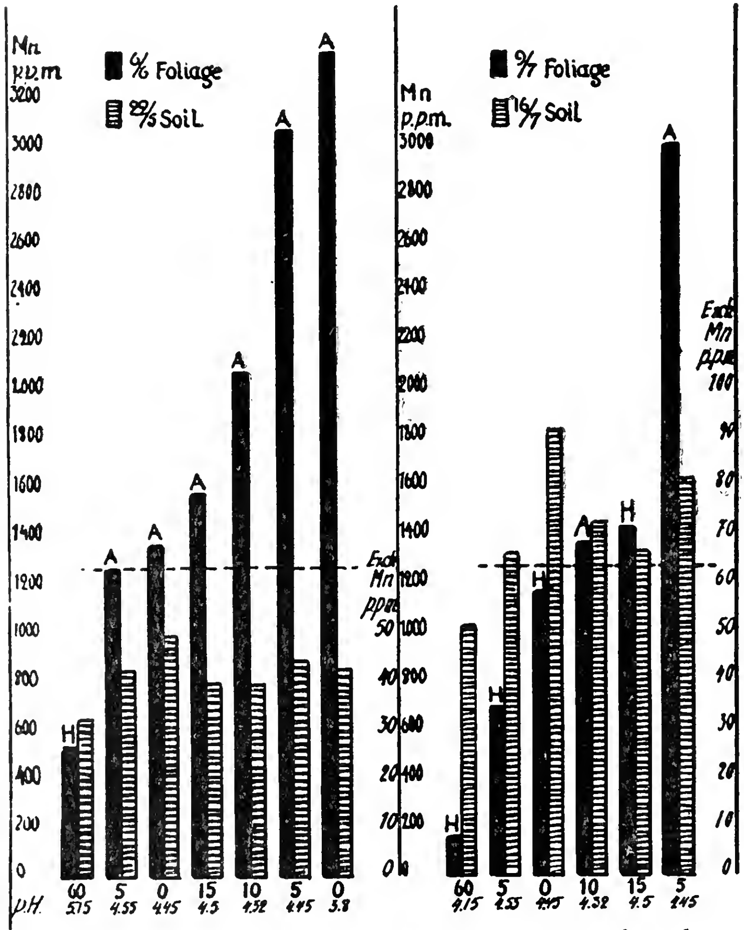
In Figure 14 all amounts of exchangeable manganese determined in samples taken in 1946 and 1947 are given. The variation, on one and the same plot, tested at various times, is very striking. The higher content of exchangeable manganese in the soil, however, does not result in a higher content in the foliage, as the figures for the latter ranged in 1946 from 100-3510, in 1947 from 167-3300 p.p.m.

The summer of 1947 was exceedingly dry and hot. The fact that no increase in the intake of manganese occurred may possibly be ascribed to a high concentration of other ions in the soil solution which may have interfered with the intake of manganese.



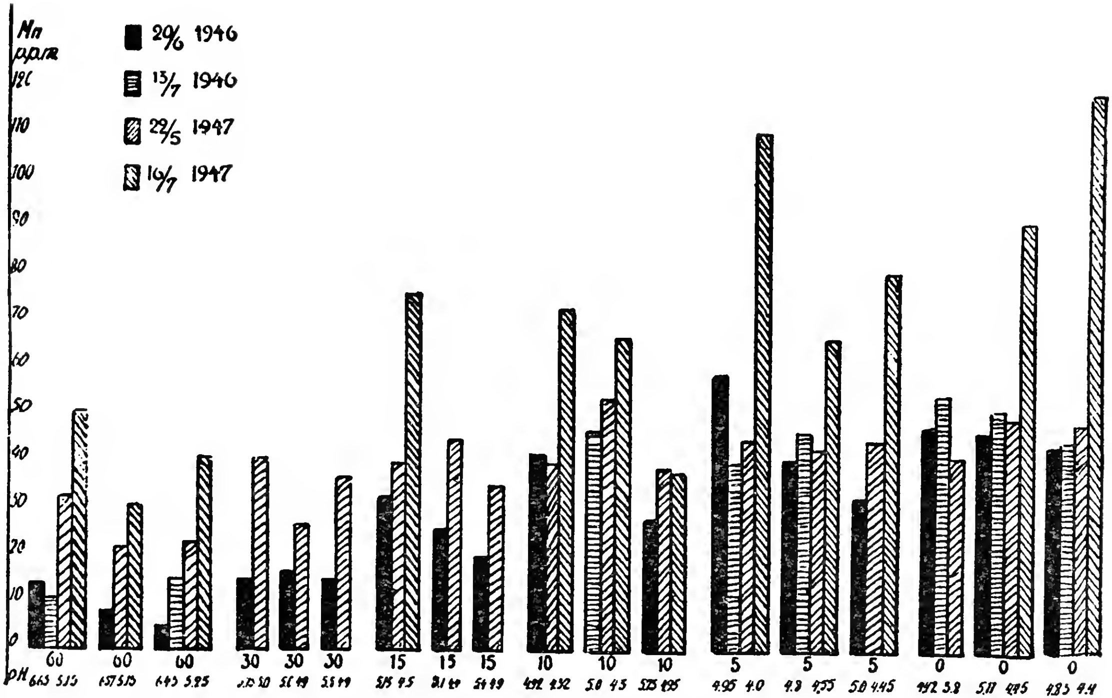
TEXTFIGURE 12.—Manganese content of foliage of *Phaseolus vulgaris* (Dubbele Prinsesseboon) and exchangeable manganese in the soil (1946).

A coincidence between injury in bean foliage and a high content of manganese has definitely been established but proof that the high content is the real cause of the injuries had not yet been rendered. For further study, beans were grown in nutrient solutions varying in $MnSO_4$ content. Symptoms identical with those in the field could be induced by 25 or 37.5 mg/L (Plate 8*b*). Water colours, which I made, also show this very nicely.



Textfigure 13.—Manganese content of foliage of *Phaseolus vulgaris* (Dubbele Prinsesseboon) and exchangeable manganese in the soil (1947).

In my opinion, the deciding experiment was the one in which beans were grown in glass dishes (Neubauer dishes) with soil (400 g soil) from a toxic plot. Some of the dishes received



TEXTFIGURE 14.—Exchangeable manganese in the soil of the same plots at different times.

$CaCO_3$ (0.8 g) and half of this number, in addition, $MnSO_4$ (250 mg). In the untreated soil, young bean plants showed the same symptoms as the plants grown in the plot from which the soil came. In the soil, with $CaCO_3$ added, healthy, normal plants developed. When $MnSO_4$ had been added plants developed typical symptoms (PLATE 9). Acidity, as such, cannot be the causal agent. The addition of 250 mg $MnSO_4$ has been large enough, it appears, to keep part of the manganese in an unoxidized soluble condition. In fact, at the end of such an experiment 86 p.p.m. Mn was still found in the soil.

In the summer of 1947 we found in vetch (*Vicia sativa*) a plant even more susceptible to excess manganese. This plant was used in our test because, being known to be highly non-susceptible to manganese deficiency, it might well take up manganese easily. In all experimental plots half a row was now planted with vetch. It proved, to such a degree, susceptible to excess of manganese that in the more acid plots I hardly succeeded in gathering samples for chemical analysis as it died off too quickly. On the more heavily limed plots, however, large and healthy plants developed. The content of manganese in these samples has not been estimated as yet.

Affected plants show a purplish discolouration of the margins of the leaflets, chlorosis of the young leaves, and stunted growth (PLATE 10). In the dry summer of 1947 the affected plants died before well developed.

Solution cultures furnished proof that the injury was actually due to excess of manganese. When the nutrient solution had been supplied with 25 or 37.5 mg $MnSO_4$, the field symptoms could be reproduced exactly.

In this case, soil cultures in Neubauer dishes, similar to those carried out with beans, were also very conclusive.

As the analysis of exchangeable manganese in the soil will furnish widely divergating results depending on the time of sampling, it can hardly serve as a sound method of testing soils suspected of excess of manganese. It seems to me that culture experiments with beans ("Prinsesseboon") or vetch, in Neubauer dishes, may give more reliable results.

My investigations have not been concluded, as yet, and could only be presented to you in this preliminary form.

Addendum:—Later experiments with lucerne showed that this plant also is very susceptible to excess of manganese. The results will be published in *Plant and Soil,* in the course of 1951.

References

1. HEINTZE, S. G., 1938: Journ. of Agric. Science 28:175.
2. LÖHNIS, M. P., 1946: Tijdschr. over Plantenziekten 52:157.
3. STEENBJERG, F., 1933: Tidsskr. for Planteavl 39:401.

Points from Discussion of Paper by Dr. Löhnis

Prof. Steenbjerg:—

Have you in Holland used a determination of the exchangeable manganese of the soil with success as a criterion of manganese deficiency disease?

Dr. Löhnis:—

It was outside the scope of this investigation but at an earlier date[1] I have tried whether the *Aspergillus* method might be made suitable for the estimation of small amounts of manganese in soil. It has appeared, however, that independently of the presence of nitrate or ammonium salt as source of nitrogen, manganese deficiency symptoms could merely be induced in very acid solution (*p*H 2.5). In less acid solution *Aspergillus* developed abundantly in the absence of any manganese intentionally added. So for estimation of manganese in soil the method appeared of no use.

Professor Wallace:—

Regarding the point made by Dr. LÖHNIS that the injurious effects of manganese toxicity of beans and non-injurious effects on oats but injurious effects on the latter under conditions of deficiency and that these may be related to ease of intake of manganese: it should be noted that of the three plants quoted by Dr. LÖHNIS, *viz.* French bean, strawberry and oat, in our experience at Long Ashton French bean is highly susceptible to both manganese toxicity and deficiency; strawberry is resistant to both; and oat is resistant to excess but susceptible to deficiency. It would thus not appear that a simple explanation based on ease of intake of Mn accords with these observations.

---

[1] Antonie van Leeuwenhoek 10:101-122, 1944.

Dr. Hewitt:—

It is particularly interesting to note that the symptoms of manganese toxicity in beans just described by Dr. Löhnis are identical with those produced with an excess of manganese in sand culture experiments at Long Ashton. This independent confirmation provides an interesting example of the reproducibility of visual symptoms and gives further evidence of the value of visual methods.

Extensive experiments in sand cultures have shown that manganese toxicity may produce a great variety of effects in different plants but certain of these symptoms however appear to be common in different groups of plants. Thus speckling of petioles is seen in beans, mustard, potato and tomato; marginal cupping is common in several brassicae; vascular necrosis may occur in tomato, potato, clover and others, and stem lesions, particularly around nodes, occur in tomato and potato. The susceptibility of crop plants also varies widely. Although beans are sensitive, red clover is much less so. Most Brassicae are also relatively sensitive including marrow-stem kale, which differs markedly from the resistant hungry-gap kale; potato, oat and sugar beet, in this order, are among the more tolerant plants.

The beneficial effect on manganese toxicity of liming the soil noted by Dr. Löhnis raises the question of the effects of calcium supply in sand culture. Increased calcium supply as calcium nitrate markedly reduces manganese toxicity effects and also the accumulation of manganese, but when calcium is supplied as calcium carbonate mixed in the sand the beneficial effect on manganese toxicity may be little or nil in spite of an adequate supply of available calcium, and this is in marked contrast with effects produced in soil.

THE ACTION OF MANGANESE ON ROOTS

by H. Burström, Fil.Dr.
Botanical Laboratory of the University, Lund, Sweden

It is my intention to give only a very short account of some recent results from experiments concerning the influence of manganese on the growth behaviour of root tips. We know, as a matter of fact, very little about the specific influence of manganese on roots as well as on the process of growth in general.

Some years ago, I studied the connection between manganese and the assimilation of nitrate in wheat roots and arrived at the conclusion that manganese promotes, probably catalyses, the nitrate reduction in this organ. In this function it can be replaced by iron to some extent only; all other cations, including iron, may, under certain circumstances, inhibit manganese action. This was assumed to depend upon some kind of competition of the cations, in the system that promotes nitrate reduction. This hypothetical function of manganese has been partly confirmed by the results of Noack's work on *Chlorella,* but it is also contradicted by Steinberg's experiments with *Aspergillus,* and especially by Mulder's investigations with various plants. These workers have found a correlation between molybdenum and nitrate assimilation. I only worked with isolated wheat roots, and our objects are so dissimilar, that our results do not necessarily exclude each other. It seems possible that the same function in different organs or plants may be fulfilled by different elements, or that the metals may replace each other to some extent.

Another way of testing the action of manganese is to study its effect on the morphological development of roots. The supply of nitrogen and the reduction of nitrate are important growth factors; on the other hand, there are, as far as I am aware, in the literature, no records of direct effects of manganese on root development, nor of any specific signs of manganese deficiency in roots at all. This cannot be correct if manganese really plays some part in nitrate assimilation. It may also be possible, by means of a morphologenetical study, to locate the action of manganese at some special point in the metabolism of the cell.

In order to approach this problem we first had to study the morphological action of nitrate. This was done by determining the rates of cell multiplication and cell elongation of the epidermis of wheat roots. The methods and some results have already been published (BURSTRÖM 1947: K. Fysiogr. Sällsk. Förhandl. Vol. 17). A reduction in the nitrate concentration below its optimum has a very characteristic effect on root growth. A decreasing supply causes, of course, a reduction in the root length, which is due to a greatly decreased rate of cell elongation. As a result the fully developed, mature cells are short, but there is at first little reduction in the number of cells. This means that the rate of cell multiplication continues, and that the optimum nitrate concentration, for this part of the growth process, must be lower than that for the cell elongation. This nitrate action is almost the reverse of that of phosphorus. A decreasing supply of phosphorus primarily affects meristematic growth but not cell stretching. I mention these circumstances in order to show that it is possible, on a large amount of material, to distinguish experimentally between these two phases of growth, thereby making a quantitative analysis of the growth process.

Experiments performed in order to illustrate the action of manganese in the same way meet, of course, with greater difficulties. We started from the assumption that if there is really some direct influence of manganese on nitrate transformation, the morphological effect must reveal itself in short time tests, even if the effect is not so prominent as when nitrate itself is withheld from the roots. In experiments of long duration, complications may arise, due to the adaptation to manganese deficiency, as assumed by NOACK, and to the indirect effect of manganese.

We experimented with attached as well as detached roots of wheat seedlings which had been grown for 4 to 6 days in manganese-free solutions under constant light and temperature conditions. In neither case did we obtain any regular or significant change in the length of the roots if manganese was supplied for up to three days, but the morphology of the roots was markedly affected by addition of manganese in amounts down to 1 p.p.m.

Attached roots, of course, reacted less than excised roots. Very clear results were obtained when all roots were removed from the seedling, except one of the first two adventitious roots, and when the shoot was cut off, at the beginning of the experiment, a little above its basis. The test plant then consisted of a single root attached to a piece of stem of a few mm length. Wholly isolated roots grow very slowly, owing to a lack of some unknown factor of a probably hormonal nature, but if the shoot basis is left on, growth continues at an almost normal rate, for some time, if carbohydrates and other nutrients are supplied externally. The roots are then rather sensitive to external conditions.

Good results were also obtained with wholly isolated roots. I must admit, that we did not succeed, in every instance, in obtaining an effect of manganese on such roots in 24 hour tests, but usually we did, and then the roots always reacted in the same manner. The first thing we observed was that the growing point was shortened if the roots obtained manganese. Observed under the microscope, we saw that the meristem is rather sharply set off from the zone of elongation, and the length of the growing point, before the start of the elongation, can be determined very accurately. This proved to be distinctly shorter (up to 20%) in the roots supplied with manganese. Further it was almost constantly found that the mature epidermal cells of these roots were significantly longer than those without manganese. The absolute length of the full-grown cells varies very much and depends upon age and nutrient status in general, but the manganese effect shows consistently. Some figures may be given:—

	−Mn	+Mn
	cell length μ	
excised roots	127 ± 2	152 ± 3
attached roots	174 ± 5	220 ± 5
	249 ± 5	315 ± 5

The last pair of figures refers to roots with unusually long cells. The differences seldom exceed 25% but are highly significant. These two observations, according to which the meristem is shortened and the cell length increased, were taken as a starting point for a detailed examination of the manganese action.

It appeared that the real rate of cell elongation was always much higher in the manganese roots and much more than was to be expected from the differences in the final cell lengths. Such a difference of 15 to 25% usually corresponded to a difference in the rate of elongation of 40 to 50%. These figures are computed graphically from the growth curves of the cells. The elongation with manganese also starts earlier and this causes the reduction found in the length of the meristem.

By some prolonged tests it was observed that the cell growth in the absence of manganese was even increased, and the cells obtained unusual lengths of more than 300µ. In one experiment, on four consecutive days, the following figures were noted for the cell length of the newly formed cells:—

Day	−Mn	+Mn
1st	204 ± 5	229 ± 5
2nd	174 ± 5	220 ± 5
3rd	242 ± 4	233 ± 4
4th	305 ± 5	259 ± 5

Without manganese the growth is irregular. In spite of the abnormally increased cell lengths in this case, the rate of elongation was always higher with manganese, but the stretching ceased earlier. Without manganese it continued for a longer time but at a slower rate. A rapid increment in net length of the excised roots does not necessarily imply a favourable progress of growth.

Summarizing, we have found, hitherto, that manganese, already during the first 24 hours, causes a more rapid start of the elongation of the cells and a more rapid elongation. It seems as if the period of cell elongation went more rapidly in the presence of manganese, but the net result as to cell and root length may be variable, depending upon known or uncontrolled factors.

This action of manganese agrees partly with the one obtained with nitrate, in as far as nitrate also enhances the rate of the cell elongation. But nitrate does not cause the elongation to start earlier, nor does it shorten the period of cell elongation, so that the picture we receive is not identical with the one outlined before. It may be that manganese has also other influences, or that nitrate is acting partly by itself, without reduction, by increasing the ion concentration of the roots. We cannot say more than that the results do not contradict our assumption that manganese regulates nitrate assimilation.

The following figures show the combined action of manganese and nitrate on excised roots. One experiment, continued for three days, gave the following result:—

Mn	–	–	+	+
NO_3	–	+	–	+
	Cell lengths in μ:—			
1st day	105	135	112	151
3rd day	c.80	105	c.80	163
	Rate of elongation rel.:—			
	x	70	x	104

Without nitrate the cells and the roots become disorganised and the figures are of little interest. But also, with the treatment without manganese cell elongation declined; it continued normally only in the presence of both nutrients. Nitrate, indeed, shows its full action only in the presence of manganese.

I do not know if manganese is indispensable for the continued growth of excised wheat roots. GLASSTONE (Am. J. Bot. 1947) has recently stated that tomato roots only need Fe and Cu but not Mn. We have not investigated this problem, in particular, in tests of long duration, but I am inclined to believe that roots need manganese for normal growth.

Another point that has not been clarified is the interaction of iron and manganese with respect to the properties of the roots which we studied. Preliminary experiments have shown, however, that the manganese action occurs, both in the absence and the presence of iron, if given in the same concentration in both cases. Iron itself causes, in the absence of manganese, a marked reduction in the length of cells, apparently owing to a more rapid cell development. This action seems to be weakened if manganese is present, as shown by an example:—

Fe	–	+	–	+
Mn	–	–	+	+
	Cell length in μ:—			
	172	149	193	198
	±3	±3	±4	±4

It is obvious that manganese and iron do not have altogether contrary actions on the cell growth, which they should have, if the opinion of SHIVE as to the interaction of iron and manganese is generally valid.

The salient point, in our opinion, is that it is possible, in short time tests, to demonstrate an action of manganese, and this must be regarded as showing a direct, positive influence on root development. We believe that this method of studying growth behaviour may prove of value for the location of the function of manganese, and we plan to continue our studies by combining observations on growth, with determinations of carbohydrate and nitrate metabolism, in the presence of manganese.

Points from Discussion of Paper by Prof. Burström

Dr. Mulder:—

1) Do you think that in different tissues and organisms different mechanisms of nitrate reduction exist? In *Aspergillus niger* I could not show any influence of manganese on nitrate reduction.

2) In an experiment with excised roots growth could be obtained only when the nitrogen was nitrate. With ammonium salts and asparagin no growth was possible. As a normal growth in this experiment occurred only when the roots were swimming on the surface of the nutrient solutions it looks as if nitrates have the effect of an improved oxygen supply of the solution.

Prof. Burström:—

1) Your experience with *Aspergillus* tallies with STEINBERG's. As all results go at present we must assume that we have several different mechanisms of nitrate assimilation. We know that there must exist several kinds of respiration, so it is theoretically possible that the same holds true of the nitrate reduction.

2) Nobody has succeeded in growing excised roots with nitrogen given as ammonium as far as I am aware. Oxygen is certainly an important factor in root cultures and you are quite right that with nitrate we introduce a new oxidizing agent in the tissues, and obtain a redox-system, which is lacking in ammonium cultures. It appears, however, that the roots need oxygen even in the presence of nitrate.

Dr. Erkama:—

1) Can we presume that these interesting reactions you have found in isolated roots, as well as the mechanism of the respiration

in roots catalyzed by manganese which you have earlier reported together with Prof. LUNDEGÅRDH, are acting in the same way in the roots of the green, assimilating plants?

2) What is your opinion of the relations between the respiration catalyzed by manganese and the respiration catalyzed by iron in the green, assimilating plants?

Prof. Burström:—

1) We do not know in what respects the metabolism of excised roots differs from that of attached roots. It must be dissimilar in part, since attached roots obtain their carbohydrates from within, whereas excised roots obtain theirs from the external solution. Attached roots have the power of performing the same reactions as excised, but we cannot evaluate their quantitative importance in complete plants. The same holds true for all reactions studied in isolated enzyme systems, a fact which is usually overlooked.

2) I have never studied the respiration in excised roots. However, if nitrate is reduced, something must be oxidized, *i.e.* carbohydrates, and it implies respiration. In physiological experiments we cannot separate the reduction from the oxidation; they are two sides of one redox-system. Neither can we decide whether a heavy metal acts on the oxidation or the reduction side of the system.

Prof. Arnon:—

In comparing the effects of NO_3 and NH_4 on the plant cells it would be helpful to regard the NO_3 not only as a source of N but also as a source of oxygen when the NO_3 is reduced in the plant. This oxidizing function of nitrate may be important since the NO_3 can be transported within the plant and reach regions where the oxidation-reduction reaction involving NO_3 can be particularly significant.

Prof. Burström:—

It cannot be denied that a certain amount of oxygen is brought into the cells with the nitrate, but the amount of reduced nitrate is small as compared with the normal respiration. Nitrate is reduced in the roots where lack of oxygen may occur, and in the leaves where oxygen deficiency is scarcely probable. Roots need oxygen even in the presence of nitrate. It is not known that higher plants can utilize nitrate as a source of oxygen for the metabolism.

Prof. Lavollay:—

Dr. Arnon has underlined appropriately the fundamental difference which exists between nitrates and ammonium salts. The first are sources of oxygen utilisable by the cell. In our experience of the physiological role of magnesium, we have now shown that the mycelium of *Aspergillus niger* deficient in magnesium synthesizes lactoflavine in important quantities, which is the index of a diminution in true oxidation. The production of lactoflavine is important when nitrogen is furnished in the form of an ammonium salt, which to the contrary, is reduced when one offers nitric nitrogen to mycelium. We see in this the proof that the oxygen of nitrates is utilised for cellular oxidations which is besides very evident.

Prof. Burström:—

Of course the oxygen of the nitrate is consumed in some kind of oxidation, when nitrate is reduced. Thus nitrate reduction must increase the respiration. This was shown in 1920.

ZINC DEFICIENCY OF FRUIT TREES IN EUROPE

by D. Mulder, Ph.D.,
Wilhelminadorp, Zeeland, Netherlands

In 1940 Husz described, for the first time in Europe, the rosette disease of apples growing in Hungary. He was able to cure the deficiency by an application of zinc sulphate.

In 1943 I was confronted with an unknown disease of "Golden Delicious" apples in Holland. Because leaf injection with iron sulphate improved the colour we first thought that we had to do with a special form of iron shortage in that variety. Grafting experiments showed that the symptoms were not the result of a virus disease.

In 1944 it was shown that trees which had been transplanted to another soil became again healthy. In 1945 the same disease was found on other varieties and the resemblance with the symptoms described, as a result of zinc deficiency, in the United States was noted.

In 1947 spraying experiments with zinc sulphate showed that the symptoms are indeed due to a deficiency of zinc, at least that they can be cured by application of zinc to the trees. Both the spraying of 5% zinc sulphate, in winter, and of 1.5% zinc sulphate with 0.75% lime, in summer, had good results.

The information, from other regions of Europe, about the occurrence of this deficiency disease is still very scarce.

Mr. Conrad (Maag, Zurich) told me that Dr. S. Blumer has noticed zinc deficiency symptoms in apples in Switzerland.

During a visit to Denmark I found symptoms of zinc deficiency in apples. They are attributed to the high phosphorus content of the soil by the Phytopathological Service in Denmark (zinc phosphate is highly insoluble).

The symptoms of the disease in "Golden Delicious" apples are:

1) on the leaves:
   a) a yellow mottling of the leaves sometimes according to a distinct pattern.
   b) an undulation of the leaf margin.
   c) the leaf becomes very narrow with a length of only one-third of the normal length.
2) on the shoots:
   a) shortening of the distance between the leaf insertions near the end of the shoot, with a rosetting effect.
   b) late ripening of the end of the shoot and delay in the formation of the terminal bud.
   c) insufficient formation of wood with the result that the twigs remain weak and thin, as if there were some wood disease.
   d) outgrowth of many side branches near the end of the shoot in the second year resulting in a witches' broom appearance.
3) on the fruit, no special characteristics, but the fruits remain very small.
4) on the tree as a whole:
   an erratic spread of diseased branches amidst quite healthy parts of the tree.
5) throughout the year:
   in less severe cases one will notice the varying degree of severity of symptoms in the course of one season on one shoot. A shoot, *e.g.*, may start growing well, develop clear symptoms in early summer, to show, in late summer, again a healthy top with green leaves.

In other apple varieties there is much less growth in cases of zinc deficiency than we find in "Golden Delicious." The stunted growth is more obvious and the effect of rosetting more marked.

The disease results in very poor foliage at the end of a long, weak branch. This indicates the rather sudden appearance of zinc deficiency symptoms after years of normal growth. In Holland we have seen these symptoms particularly on the variety "Perzikrode Zomerappel" and also on such varieties as Keswick Codlin, Cox, Glory of Holland, etc.

Pears may be affected too. They show stunted growth, small leaves and intervenal chlorosis. It has been proved that the disease occurs also on the cherry.

There is little to be said at present regarding the soil conditions causing this disease. There is no reason to assume that an excess of phosphorus is the reason for the disease as observed in Denmark. It occurs mostly on light sandy soils, which are rather high in lime content.

In Holland, hitherto, the disease has only been noted in the South on some islands of the province of Zeeland.

INVESTIGATIONS ON MICROELEMENTS FROM A PRACTICAL POINT OF VIEW

by F. Steenbjerg, Dr.Agr.,
Kgl. Veterinaer- & Landbohøjskole, Copenhagen, Denmark

During the past fifteen years chemical investigations on the relation of manganese and copper in the soil and in plants have been made in Denmark. In recent years, it has, moreover, been shown by Bendixen and Pedersen that a cobalt deficiency disease occurs in young cattle (heifers) on the sandy soils of Jutland. As far back as about 1910, and before that time, comprehensive investigations on the visual symptoms and etiology of those plant diseases which we now know to be caused by lack of microelements were started at the State Plant Pathological Laboratory at Lyngby.

Both iron and manganese deficiency very often occur in Danish agriculture and horticulture. While manganese deficiency is generally known and causes losses on nearly all soils in Denmark copper deficiency is known particularly on the sandy soils of Jutland where it causes severe losses.

As early as 1932 it had been shown that a determination of the exchangeable manganese of the sandy Danish soils, which are rather uniform in physical condition, gave some measure of the power of the soil to supply the plants with manganese. The amount of exchangeable manganese in the soil is regulated by the *p*H value and by the conditions of oxidation and reduction of the soil. At the same time it was shown that manganese is absorbed in a different degree in acid and alkaline soils. Moreover alkaline soils, rich in humus, seem to fix the manganese in a non-exchangeable state with a very considerable energy.

For a determination of the exchangeable manganese a one molar solution of magnesium nitrate is used as the displacing solution. The sodium, potassium and ammonium ions give smaller values for the exchangeable manganese than, *e.g.*, calcium and magnesium ions, especially in alkaline soils. The term "exchangeable" evidently means little unless the replacing ion is defined.

The early investigators stated that lack of exchangeable manganese in the soil was a most important factor in causing manganese deficiency in agricultural crops. The values for the computed total amounts of exchangeable manganese led to conclusions which are in accordance with agricultural practice, as well as with field experiments with manganous sulphate and experiences as to the external causes of manganese deficiency in crops. The results of the chemical investigations have further been confirmed by pot-experiments in which different reducing agents (and different amounts of water) were added to manganese deficient soil.

Observations in the field have shown that a content below 2.5 parts of manganous ion, that is exchangeable manganese, per 1 kg of air dried soil very often causes attacks of manganese deficiency.

At present it seems only to be proved that the manganese in the soils exists in two distinct groups or classes: (*1*) the bivalent ion existing in the soil solution or as exchangeable or non-exchangeable ion, dependent on the displacing ion used, and (2) the insoluble higher oxides of manganese. These forms are in dynamic equilibrium with one another, the equilibrium being dependent on the *p*H and the redoxpotential of the soil. In spite of the very interesting work done recently by HEINTZE, MANN and co-workers I shall use the terms "manganic oxides," and "higher oxides" so as to include all formulae from MnO_4 to MnO_2.

What are the reactivities of the higher oxides? It seems to me that this is one of the most important questions of the future. It has been known for many years that certain soils contain a highly active form of manganic oxide which is very quickly reduced by quinol, liberating Mn^{++} ($Mn(OH)_2$). According to LEEPER we have a less active form, which reacts with hyposulphite at *p*H 7 but not with quinol.

But do the plants absorb manganese from those manganic oxides of the soil? In our experience the higher oxides are of importance. During our investigations we have found that the limit for the exchangeable manganese value, below which manganese deficiency occurs, is different and usually lower when we move from sandy soils to heavy loams. This may mean that the plants on the heavier soils are able to absorb some manganese from the manganic oxides which are reduced during the growth of the plants. There is the possibility that the manganic oxides are stored as more active higher oxides in loams or clay soils than in the light sandy soil. There is perhaps, so to say, no "aging" of the manganic oxides in heavy soils.

In a series of field experiments performed mostly on sandy soils some years ago in which increasing amounts of manganese sulphate were added we found that the amount of exchangeable manganese in the manganese fertilized plots had decreased to the same order of magnitude as in the untreated plots within a month after the addition of $MnSO_4$ (also laboratory experiments have shown this fact) and still the effect was good the first year; moreover, the residual effect was not zero in the following years. This seems to show that manganic oxides are reactive and available the first, second and third years. But the availability decreases progressively from the first to the third year.

TABLE 16: *Experiments with manganese sulphate, 50 kgs per ha:—*

CROP	MANGANESE VALUE	DATE OF TOPDRESSING	YIELD 100 kgs per ha without manganese		INCREASE IN YIELD 100 kgs per ha	
			Grain	Straw	Grain	Straw
Oats	0,8	31/5	32,7	38,8	1,5	0,9
—	0,3	25/4	27,3	41,0	4,7	−1,0
—	0,3	3/6	27,3	41,0	3,4	−4,0
—	0,7	25/5	18,3	23,7	5,4	7,5
—	3,2	—	21,3	31,9	0,1	3,4
—	0,4	20/5	31,0	50,7	4,0	3,3
—	0,7	25/5	29,3	43,3	7,4	6,5
—	0,8	21/5	20,7	29,1	4,3	2,9
—	0,5	5/6	39,3	48,7	4,2	6,1
—	2,0	31/5	32,8	42,5	0,9	1,3
—	1,4	31/5	31,5	41,8	5,5	6,5
—	0,5	22/5	35,0	43,4	4,6	3,7
—	—	—	13,3	30,0	4,7	4,7
Dredge corn	0,0	2/6	11,7	36,0	1,3	0,3
—	—	16/5	28,1	57,9	2,4	2,6
—	—	—	21,0	42,5	3,2	2,5
—	—	31/5	32,0	43,7	3,3	3,5
17 experiments 1946			26,6	40,4	3,6	3,0

The amount of exchangeable manganese is affected by drying. It is increased when soil samples are air-dried, and also when dried soil is stored. Moreover the amount of exchangeable manganese is higher in the spring and summer, and lower in the autumn and winter.

Last year 17 field experiments were performed in Jutland by the Agricultural Associations and the results of the experiments are in fairly good agreement with the manganese values found as shown in TABLE 16.

It is worth remembering that the apparent disagreement, which always occurs in such experiments, between the increase in yield and the manganese value cannot be contributed to the chemical method only. If both the chemical method and the field method were without errors and correct, there would still exist a disagreement between the results obtained, because the two methods in reality determine quite different things, *sc.*, the contents in the soil of the plant nutrient in question and the reaction of the plant towards an addition of manganese at a certain level of all the other plant nutrients.

Copper deficiency, which is the other deficiency of importance, like manganese deficiency, occurs in Denmark chiefly in Jutland and more especially in the northern, western and middle parts of this peninsula. The available copper in the soil, as determined in Denmark, comprises only a certain salt or acid soluble fraction of the copper of the soil. These definitions do not presuppose the extracted copper being combined in the soil in a sharply defined manner. It may be fixed in many different ways. It has been shown here, as for manganese, that all the special soil conditions and climatic conditions which as a matter of experience have been connected with the occurrence and severity of the copper deficiency of crops also act in such a way on the available copper that their influence on the crop may be explained by their action on the available copper of the soil.

We have now fairly good data respecting the available and total amount of copper in Danish soils, the amount of copper (as well as of manganese and partly of boron) in farmyard manure, in liquid manure and in different plant materials. It is impossible for me to go into details on these points. There are, on this occasion, only three facts about which I want to say a few words.

First, a copper value of the soil is used at present in Denmark which gives fairly good results. Secondly, it has been found that the influence of the preceding crop with respect to the amount of available copper in the soil is complex. It has been shown that grass undoubtedly is the crop which causes the highest increase of the available copper of the soil, and it seems reasonable to assume that grassland has the same effect with respect to various other microelements. A vegetation of grass and a forest-vegetation, so to say, extract copper and other microelements from the deeper soil horizons and transfer them to the surface soil where they may be used by the crops following.

Thirdly, one of the most important indirect causes of white tip disease may be the humus in some soils; the ability of humus to combine with cupric ions seems to be so great that copper is made unavailable to the plant. Where it is possible over a considerable period of time, for some years, to decrease the humus of the soil substantially and hence its copper absorbing capacity this may be useful in controlling the deficiency.

TABLE 17: *Copper analyses, percentage Cu:—*

MINERAL OR COPPER FERTILIZER	FORMULA	DEGREE OF FINENESS	COPPER CONTENT AFTER FORMULA	COPPER CONTENT DETERMINED BY LOW'S METHOD
Copper pyrite	$CuFeS_2$	II	34,6	18,0
Copper pyrite	$CuFeS_2$	I	34,6	20,5
Bornite with	Cu_5FeS_4	II	63,3	58,9
copper glance	Cu_2S		79,9	
Bornite with	Cu_5FeS_4	I	63,3	61,6
copper glance	Cu_2S		79,9	
Malachite	$CuCO_3 \cdot Cu(OH)_2$	II	57,5	51,4
Malachite	$CuCO_3 \cdot Cu(OH)_2$	I	57,5	49,5
Cuprite	Cu_2O	*	88,8	
Copper sulphate	$CuSO_4 \cdot 5H_2O$		25,5	25,5

* No analysis of degree of fineness of this mineral was made. The degree of fineness was evaluated and was of the size which we called I.

Different copper minerals have been used to supply the element. During the years 1937 to 1946 pot experiments were made to investigate the question whether plants are able to utilize metallic copper (finely ground), copper oxides and copper sulphides, substances which as far as the oxides and sulphides are concerned have been added to the soil partly as minerals and partly as chemicals. Malachite was also tried. The following copper minerals were used: copper pyrite (chalcopyrite), bornite with an admixture of copper glance (chalcocite), red copper (cuprite) and malachite (*see* Table 17).

Field experiments with one of the minerals (chalcopyrite) have been made for several years now in Jutland with good results as compared with copper sulphate.

If the copper minerals are compared on the basis of the same degree of fineness per unit of weight, malachite and bornite with an admixture of copper glance, with respect to fertilizing value, are nearly alike and nearly equal copper sulphate in their fertilizing effect. Chalcopyrite must be ground very fine if its effect has to reach that of malachite and bornite-copper glance.

Table 18:—

	g Copper added	Surface of the mineral, m^2	Percentage copper content	Relative "copper-surface"	Increase in yield
Copper pyrite I	0,56	0,067	20	0,013	45,1
———	2,79	0,333		0,055	90,6
Bornite II	0,52	0,001	59	0,001	3,1
———	2,59	0,003		0,002	17,1
Bornite I	0,52	0,014	62	0,009	72,6
———	2,58	0,069		0,043	89,4
Malachite I	0,47	0,051	50	0,026	91,5
———	2,34	0,258		0,130	95,8
Malachite II	2,50	0,004	51	0,002	51,0

Assuming that the area of the active "copper surface" for the different copper minerals is proportional to the percentage by weight of copper in the mineral it is found that the curves showing the increases in yield for the different minerals fall together as shown in Figure 15, and Table 18. At any rate the deviations have not been really great in these investigations.* Of course it is not to be expected that the surface tension, the solubility of the different minerals at the same relative copper surface is quite the same because the crystallographic structure may be of some importance. Moreover an occlusion of the copper is possible if the copper mineral contains rather large amounts of other minerals as impurities as did copper pyrite.

According to the results of these investigations the main reason

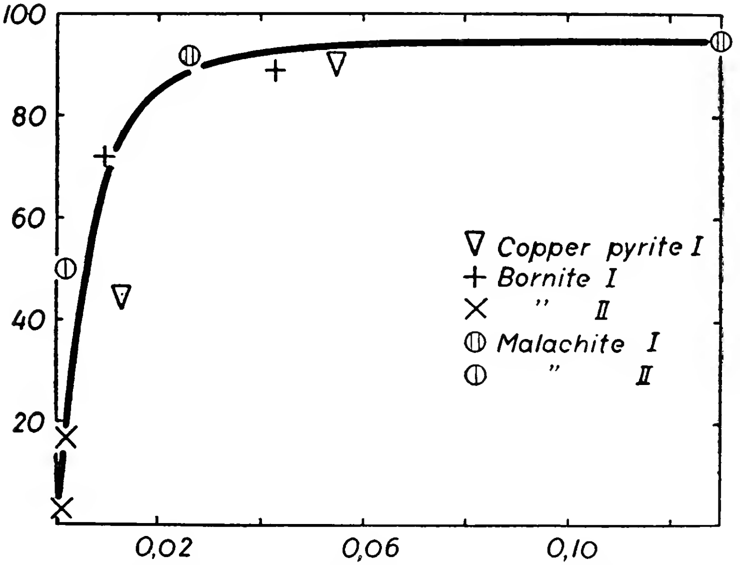
---

* The considerable excess of copper compounds of hydrolysis, left over in the soil, in increasing amounts, from year to year, may well contribute to this result.

for the different fertilizing values of the copper minerals, if the minerals are added in increasing amounts at the same degree of fineness, seems to be the varying copper content which causes a very different "copper surface." So there are three factors of importance when the value of copper minerals as fertilizers has to be evaluated:

1) The degree of fineness
2) The "copper surface"
3) The price per kg of copper

These three factors may be combined in many different ways in order to give the cheapest copper effect.



TEXTFIGURE 15.—*Abscissa:* Relative copper surface.—*Ordinate:* Increase in dry matter yield.

Finally a few words about chemical analyses of plants for microelements and the use of these analyses as indicators of deficiencies of microelements.

The relationship between the percentage of a plant nutrient in the dry matter and the amount added to the soil of the plant nutrient in question is expressed by a curve which first falls and then rises as the content of the plant nutrient increases in the soil. When the experimental results are shown in a system of co-ordinates where the ordinate expresses the amount of dry matter while the abscissa gives the amount of plant nutrient absorbed, the experimental results present an S-shaped curve, the point of inflexion of which corresponds with the minimum of the other curve showing the relation between relative content and the amount given to the soil of the microelement in question or the dry matter produced.

We have observed such curves when the soils in which the plants grow pass from very deficient to less deficient and then to a rich condition of the microelement in question. This has been found both at maturity and at other stages of growth, and for barley both in the leaves and in the grain. These facts can be explained especially by the variations in the content of carbohydrates at different levels of manuring.

These observations may explain why, when sampling crops for chemical analyses of microelements, it is often found that the sample which represents a crop seemingly most deficient in, for instance copper, has the highest or about the same percentage of this nutrient in the dry matter as a crop less deficient or not deficient.

There is, moreover, the possibility that the relative content of other plant nutrients may vary simultaneously in the same way, and this further complicates the problem of the use of chemical plant analyses alone as indicators of a deficiency of a given microelement. This problem is at present being investigated. It seems necessary to use chemical plant analyses with caution and some reservation as a diagnostic criterion of the deficiency of soils and crops in plant nutrients (both macro- and microelements). They should not be used alone, but always in connection with other methods of observation.

References

BENDIXEN, H. C. and PEDERSEN, J. G. A., 1946: Continued investigations into cobalt deficiency disease acobaltosis in cattle (Summary) (Yearbook of The Royal Veterinary and Agricultural College, Copenhagen, 80).

DION, H. G., MANN, P. J. G. and HEINTZE, S. G., 1947: The "easily reducible" manganese of soils (Journal of Agricultural Science 37:17).

HEINTZE, S. G., 1946: Manganese deficiency in peas and other crops in relation to the availability of soil manganese (Journal of Agricultural Science 36:227).

HEINTZE, S. G. and MANN, P. J. G., 1947: Soluble complexes of manganic manganese (Journal of Agricultural Science 37:23).

LEEPER, G. W., 1947: The forms and reactions of manganese in the soil (Soil Science 63:23).

STEENBJERG, F., 1933-5: Investigations concerning the manganese content in Danish soils, I, II, & III (Summary) (Tidsskrift for Planteavl 39:401; 40:337; 40:797).

STEENBJERG, F., 1940: Copper in soils and cultivated plants, with special reference to white-tip disease (Summary) (Tidsskrift for Planteavl 45:259).

STEENBJERG, F., 1943: Copper in soils and cultivated plants, II. Investigations on the fertilizing value of copper minerals (Summary) (Tidsskrift for Planteavl 47:557).

STEENBJERG, F., 1950: On the relative contents of plant nutrients in crops (Trans. Fourth Internat. Cong. Soil Sci. 1:254).

Points from Discussion of Paper by Prof. Steenbjerg

Prof. Wallace:—

1) Has Prof. STEENBJERG any data for soluble copper in instances of varying levels of copper deficiency similar to those for total copper shown in his curves?

2) In our experience at Long Ashton with total iron content we should not expect a curve similiar to Prof. STEENBJERG's curve for copper. In fact, when iron deficiency is induced by a heavy metal like zinc we have found large accumulations of iron in the iron deficient plants.

3) In our experience grass has increased the availability of iron to fruit trees but not of manganese and the availability of the latter seems to have been decreased for crops following immediately on the ploughing in of grass swards. This last point seems

to be the opposite of Prof. STEENBJERG's experience with copper.

4) Manganese deficiency is common in England on calcareous alluvial soils with high water tables. On such sites the deficiency is usually most pronounced on areas intermediate between waterlogged levels and drier levels, *i.e.* at points where waterlogging conditions fluctuate. The treatment of marsh spot in peas by spraying at blossoming time is an exception to the rule that early treatment of trace element deficiencies is most effective.

The results of Miss HEINTZE (Rothamsted) on the intensification of marsh spot symptoms by nitrogenous manuring raises the question as to whether the symptom is specific or not for Mn deficiency.

Thus at Long Ashton we have found that "hollow stem" of cauliflower is not specific for boron deficiency and has been observed even under conditions of boron toxicity.

Dr. Mulder:—

In the polders of Holland that were flooded during the war manganese deficiency of agricultural crops occurs much more frequently after the flooding than before. These results are in contrast with those of earlier observations that manganese deficiency on drained soils occurs more severely above the drain pipes where the oxidation conditions are better, than between the pipes.

Prof. Steenbjerg:—

It seems to me too that this is rather a difficult problem. It would be expected that the manganese of the soil would be more available after the flooding. If the *p*H value of the soil is the same before and after the flooding it seems to me there is the possibility that a part of the most reactive manganic oxides have been washed out during the flooding. Finally large amounts of sodium ions have been adsorbed by the soil during the flooding; this means that a cation—sodium ion—has been added in large amounts, a cation which to a certain extent is able to substitute potassium in plant nutrition so this may mean that the relative manganese deficiency has been increased.

Dr. Erkama:—

In determining the optimal content of trace elements in the culture medium it is often not realised that the optimal content may vary considerably at different stages of growth.

In my own experiments on pea in water cultures, even a slight amount of copper was proved to be poisonous at the early stage of growth. Although the copper requirement may be great at the start, as shown, for instance, by the experiments on oats by B. Rademacher,[1] the normal seed of pea contains an adequate reserve of copper which completely fulfils the copper requirement of the plant for a comparatively long period. Thus the pea grows at first more rapidly without copper than with it. Later, however, the plant not given copper was left behind, especially in regard to the generative growth, by the plant supplied with copper. My experiments do not, however, reveal whether the copper nutrition given at a later stage of growth is less for pea as appeared to be the case with oats in the experiments by Rademacher.

The experiments carried out for the elucidation of the optimal time of fertilization for different species of plants, in different soils and with different substances, are of great significance even in regard to the trace elements.

Prof. Arnon:—

I should like to take advantage of the presence of so many experts from the field of practical work of manganese to enquire whether there was any indication that light increases the requirement for Mn as reported for grey speck of oat by Samuel and Piper in Australia.

Dr. Löhnis:—

Question to Miss Heintze. Is there any larger development of the pea plants after injection with nitrogen which might induce a lower content of Mn when compared with iron-injected plants?

Miss Heintze:—

Contrasted plots on manganese deficient soils often show only small differences in total, exchangeable or readily reducible manganese. Significant differences were however found in the mineralisable nitrogen. Evidence that manganese deficiency may be connected with the nitrogen metabolism of soil and plant has been provided by results from injection experiments in peas in which increased nitrogen content caused marsh spot in peas.

---

[1] Über die Veränderungen des Kupfergehaltes, den Verlauf der Kupferaufnahme und den Kupferentzug beim Hafer (Bodenk. u. Pflanzenernähr. 64:80-108, 1939).

ON THE ROLE OF TRACE ELEMENTS IN THE AGRICULTURE OF FINLAND

by E. A. JAMALAINEN, Phil.Dr.,
Agricultural Research Centre, Tikkurila, Finland

Trace elements and related problems have been investigated in Finland from the points of view of phytopathology and agricultural chemistry. A short account of the results of these investigations may be of interest. It should be noted that the work on boron and copper has been more thorough than that on other trace elements.

Boron:—Investigations were started in Finland after the Department of Plant Pathology of the Agricultural Research Centre had proved that it was possible to control brown heart in swedes by boron. At the same time, experiments were conducted which showed the necessity of boron for controlling internal cork in apples and heart rot in sugar beets. Without giving a detailed account of these investigations (*cf.* JAMALAINEN 1935, 1936*a*, 1936*c*, and 1942), attention will be given in this connection to the occurrence of boron deficiency diseases in different plants which again will show us the importance of boron in the agriculture of Finland.

Sugar beet (Beta vulgaris v. *rapa* f. *crassa*).—The cultivation of sugar beets in Finland is limited to the southwestern and southern parts of the country. In the region of the sugar refinery for southwestern Finland, in Salo, heart rot has been found, to some extent, on soils which had been heavily limed. The disease did not appear over extensive areas, but only in small spots. As a result it did not cause any considerable loss in yield. However, if the soil has been excessively limed the disease can cause notable damage.

In eastern Finland, at the Carelian District Plant Breeding Experiment Station at Antrea, which is situated near the present frontier of the territory ceded to USSR in 1944, thorough experiments with sugar beet were carried out from 1930 to 1940. The purpose of the investigations was to demonstrate the possibilities of sugar beet cultivation in eastern Finland. The tests at this Experiment Station showed low yields. The more lime was given to the soil, the less was the growth of the roots. Similar results were also achieved, at other places, by various liming experiments. Observations showed that the low yields were due to heart rot which in many cases affected the plants severely. In 1937-39 special fertilizing and liming experiments were carried out at the Carelian Experiment Station in order to investigate the effect of boron on yield. Some results of the experiments, which have not yet been published before, are given below. The experiments were carried out by the chiefs of the Experiment Station, before 1939 by Mr. O. Saarnenheimo, M.A., and in 1939 by Mr. V. Laurila, M.A.

Table 19: *A liming and fertilizing experiment conducted in 1937 on light clay:—*

Plots[1]	Without boron		5 kgH_3BO_3 per hectare	
	Relative number of the yield of roots[2]	Diseased roots, weight %	Relative number of the yield of roots[2]	Diseased roots, weight %
0	100	48.8	118	5.9
6000 $CaCO_3$	91	91.1	123	10.3
900 Pkf + 500 K_{40}	136	65.2	146	14.1
500 K_{40} + 750 Nks	101	52.4	119	1.2
900 Pkf + 750 Nks	115	88.8	141	8.7
900 Pkf + 500 K_{40} + 750 Nks	110	85.9	153	11.0
900 Pkf + 500 K_{40} + 750 Nks + 6000 $CaCO_3$	84	100.0	149	5.0
1350 Pkf + 750 K_{40} + 1125 Nks	124	95.2	160	11.0
1350 Pkf + 750 K_{40} + 1125 Nks + 6000 $CaCO_3$	74	100.0	147	13.6

[1] Fertilizers: kg per hectare. Pkf = superphosphate, K_{40} = potash salt, 40%, Nks = nitrate of lime.

[2] Yields of roots given by means of relative numbers as compared to the yields of O-plots without boron, of which 25.630 kg per hectare was given.

In the fertilizing and liming experiments of 1937 (Table 19) heart rot was common if no boron was given to the plants. If much lime had been given to the plots, there were no healthy roots at all. Owing to this the yield of limed plots was lower than that of the unlimed ones. The disease was also found to some extent in sample areas fertilized with boron (5 kg H_3BO_3 per ha). The experiment was continued for three years. The survey of the results (TABLE 20) and of the liming experiment in 1938-1939 (TABLE 21) indicates that the yields are low for plots which have been limed but not fertilized with boron. Attention should be drawn to the fact that the soil was fairly acid in these experiments (*p*H less than 6), even in the limed plots. The soil was evidently suffering from a lack of boron, for there were many cases of the disease in unlimed plots which had not been treated with boron, though we found relatively not as many cases as in the limed plots where the lime evidently had bound the small amounts of boron available in the soil. It may be mentioned, in this connection, that most of the Finnish fields are either slightly acid or in many cases even very acid and so are in need of ample liming (TUORILA, TERÄSVUORI & TAINIO 1939). The experiments show, indeed, that there is considerable lack of boron, at least in many places in eastern Finland.

TABLE 20: *Liming and fertilizing experiments in 1937–1939 on light clay:—*

PLOTS[1]	*p*H OF SOIL IN 1938	YIELDS OF ROOTS[2]		YIELDS OF TOPS[2]	
		Without boron	With H_3BO_3[3]	Without boron	With H_3BO_3[3]
0	5.65	100	153	100	146
6000 $CaCO_3$	5.97	96	182	85	155
900 Pkf + 500 K_{40}	—	155	220	121	169
500 K_{40} + 750 Nks	—	112	164	144	219
900 Pkf + 750 Nks	—	162	231	179	253
900 Pkf + 500 K_{40} + 750 Nks	5.20	172	259	185	284
900 Pkf + 500 K_{40} + 750 Nks + 6000 $CaCO_3$	5.63	160	272	131	300
1350 Pkf + 750 K_{40} + 1125 Nks	5.19	195	286	173	334
1350 Pkf + 750 K_{40} + 1125 Nks + 6000 $CaCO_3$	5.50	152	289	116	340

[1] Fertilizing of plots in different years the same as in Table 19.

[2] The average yields of three years given as relative numbers, as compared to the yields of O-plots without boron, of which 13.230 kg per hectare was given to roots and 16.720 kg per hectare to tops.

[3] Boric acid was used in the experiment 1937 5 kg per hectare, and in 1938–1939 8 kg per hectare.

Swede (*Brassica napus* v. *napobrassica*).—Brown heart of swede is common in the central and southern parts of Finland. It is found on different mineral soils as well as on peaty soils, and it is most severe in fields which have been continuously and amply fertilized with mineral fertilizers. Both in swedes and in sugar beets the boron deficiency diseases are found on acid soils. In most cases the *p*H figure of the soil was under 6. The lowest *p*H of the soil where the disease was found was 4.34. The fields in Finland are, as I already mentioned, on the whole acid, so that only a small number of the brown heart cases investigated were found on soils with a *p*H of over 6.

TABLE 21: *Liming experiments in 1938–1939 on light clay:—*

PLOTS	*p*H OF SOIL IN 1938	YIELDS OF ROOTS[2]		YIELDS OF TOPS[2]	
		Without boron	With H_3BO_3[3]	Without boron	With H_3BO_3[3]
0	—	100	142	100	154
Check[1]	5.17	149	183	165	261
—— + 2000 $CaCO_3$	5.45	151	190	175	262
—— + 4000 $CaCO_3$	5.54	127	190	152	268
—— + 8000 $CaCO_3$	5.94	113	197	100	284

[1] 900 kg of superphosphate, 500 kg of potash salt, 40%, and 750 kg nitrate of lime per hectare.

[2] The average yields of two years given in relative numbers, as compared to the yields of O-plots without boron, of which 10.470 kg per hectare was given to roots, 13.460 kg per hectare to tops.

[3] In 1938 8 kg boric acid per hectare was used, in 1939 12 kg.

Virulence of brown heart varies considerably in different years, even in one and the same place. In some years, most of the swedes grown will be affected while in other years only a few cases are found. It cannot always be attributed to climatic conditions.

Apple (*Pyrus malus*).—Internal cork in apples is, together with heart rot and brown heart in swedes, the most common boron deficiency disease in Finland. At the Department of Plant Pathology Laboratory the disease has been under close observation since 1930. It is found everywhere where apple trees are grown in Finland. Usually only a few trees in orchards are affected by this disease, but in some years it can be rather common. In 1947, for instance, the disease was more common than usual which was obviously due to the unusually dry summer.

Boric fertilizers began to be widely used in Finland for preventing internal cork of apples and other deficiency diseases before the recent wars. During the war years boron was not available, and even in 1947 we still did not have enough boron containing fertilizers for our crops.

Clover (*Trifolium pratense*).—The effect of boron on the development of clover was investigated in 1946 in a number of fertilizing experiments. The results were positive—in three experiments out of five, boron considerably increased the yields (450-1.100 kg per hectare). These experiments suggest that clover needs considerable boron in Finland.

Of other plants found to be affected by boron deficiency, in Finland, the following may be mentioned: Cracked Stem of celery (*Apium graveolens*), Heart Rot of red beet (*Beta vulgaris* v. *rapa* f. *rubra*), Brown Heart of turnips (*Brassica campestris* v. *rapa*), and a boron deficiency disease in white mustard (*Sinapis alba*).

Copper:—The question of copper has been fairly thoroughly investigated at the Department of Agricultural Chemistry and Physics of our Agricultural Research Centre. Mr. TAINIO, M.A., gave an account of these investigations in 1946.

Experiments with copper have been carried out since 1939, but to a large extent only during recent years. For this purpose 160 field experiments were carried out in different parts of the country with different plants and on a variety of soils. Copper sulfate and domestic copper slag were used as copper fertilizers in these experiments. Copper slag is a residue produced after the refining of copper. It contains about 0.6% of copper. In addition to this, copper slag contains small amounts of other trace elements, but according to present experience it may well be regarded as a copper fertilizer. As the percentage of copper in the slag is very low, heavy dressings have been used in the experiments, 300-900 kg per hectare.

The different reaction of various soils to copper treatment has been determined in experiments with spring cereals. According to these experiments a peaty soil has the greatest need for copper. In 41% of all cases the increase in yield was definite. In sandy soil and fine sand the corresponding percentage was 34%. Clay soil seems to be least in need of copper: only 22% of the soil investigated showed definite increases in yield. In 36% of our experiments, with all plants and all soils involved, copper fertilizing definitely increased the yields. The results of the experiments, however, show considerable variation in different years.

The increases in yield observed in the experiments were noteworthy. In the years 1939-1944, copper fertilizing increased the yield of grain for spring cereals, by 400 kg per hectare in sandy soil, by 450 kg per hectare in peaty soil, and by 200 kg per hectare in clay soil. In a number of instances the increase of yield was 600-700 kg per hectare, and even exceeded 1000 kg of grain per hectare. In experiments with hay, potatoes and roots the increase was also considerable. In addition to this, copper manuring has been observed to have a beneficial effect even in the next year.

No definite difference can be observed in the effect of copper sulphate (50 kg per hectare) and of copper slag. Likewise, the differences due to different amounts of copper slag are indistinct. Copper slag has been placed on the market in Finland, and 300-600 kg per hectare has been recommended as a suitable amount to be used.

The question as to which areas of Finland require copper dressing, cannot be anwered with certainty, but results indicate that northern Ostrobothnia, some parts of central and southern Ostrobothnia (in the western part of northern Finland) as well as of central Finland are in greater need of copper than other parts of the country.

In some parts of northern Finland the "licking sickness" of cattle has been found as a result of a lack of copper in the soil. Affected animals recovered when they were given copper sulphate. Crops also suffer from lack of copper in these districts, and copper fertilizing will increase the yield of grain for spring cereals by nearly 2000 kg per hectare!

The plant diseases due to lack of copper have not been closely investigated in Finland. In some cases barley and oats were chlorotic at the tips of the leaves in the regions suffering from lack of copper. This can be regarded as a typical Reclamation Disease due to a copper deficiency. On the whole, signs of copper deficiency have not been observed whenever copper fertilizers increased the yield.

Manganese:—The work by Erkama, published in 1947, which illustrates the physiological importance of copper and manganese in plants, is likely to contribute to the investigation of the influence of manganese in the agriculture of Finland. Detailed information regarding the importance of manganese in plant cultivation and diseases caused by lack of manganese is not available for Finland, except for the Marsh Spot disease, a disease observed in peas which has occurred in only two cases (JAMALAINEN 1936*b*) in Åland (the archipelago in the southwestern part of Finland). It is evident that there is no general lack of manganese in Finnish fields which is due to the general acid reaction of the soil. This does not mean, of course, that there may not be a lack of manganese in Finland in soils which have been heavily limed.

Conclusion:—The investigations carried out so far do not, as yet, give a clear picture of the full importance of boron and copper in the practice of agriculture in Finland. They show that the lack of boron, as well as of copper, is very common in our fields. Many significant questions about the importance of trace elements in plant cultivation are still awaiting further study. There should be a thorough investigation to determine in which parts of the country and under which conditions lack of copper and boron is found. The reasons for the great variability in the occurrence of the deficiencies, not only in different localities, but in the same locality, must also be investigated. Attention will also have to be given to other trace elements, not mentioned here, and their importance in crop production.

Addendum:—Recent data about the occurrence of boron in various regions of Finland (JAMALAINEN 1949) show that boron deficiency is common in the provinces of Uusimaa, Häme and Turku, and to some extent in Pori. Additional boron is required also in the provinces of Kuopio, Mikkeli, and Kymi. In the districts, bounded by the Gulf of Bothnia, boron deficiency is less common.—Information about manganese deficiency diseases has been received from Uusimaa (oats, timothy, and red clover), Ahvenanmaa (oats), and Häme (raspberries).

References

ERKAMA, JORMA, 1947: Über die Rolle vom Kupfer und Mangan im Leben der höheren Pflanzen (Diss., Helsinki, pp. 1-105).

JAMALAINEN, E. A., 1935: Tutkimuksia lantun ruskotaudista (Referat: Untersuchungen über die "Ruskotauti"-Krankheit der Kohlrübe). (Reports of Agr. Exp. Activities of the State, Helsinki, 72:1-117).

JAMALAINEN, E. A., 1936*a*: Juurikkaiden sydän-ja kuivamädän torjunta booripitoisilla aineilla (The control of heart and dry rot in beets with substances containing boron) (Reports of Agr. Exp. Activities of the State 110:1-8).

JAMALAINEN, E. A., 1936*b*: Herneen siementen sisäinen turmeltuminen (Summary: Internal necrosis of pea seeds) (Reports of Agr. Exp. Activities of the State 79:1-8).

JAMALAINEN, E. A., 1936*c*: Boorin vaikutus kuoppataudin esiintymiseen omenissa (Summary: The effect of boron on cork disease in apple) (Reports of Agr. Exp. Activities of the State 89:1-19).

JAMALAINEN, E. A., 1942: Die Wirkung vom Bor auf Wachstum der Steckrüben in Wasser- und Sandkulturversuchen (Journ. Sci. Agr. Soc. Finland 14:29-37).

JAMALAINEN, E. A., 1949: On boron deficiency diseases and on the rôle of boron in Finnish plant cultivation (Finnish, with English summary) (Reports of Agr. Exp. Activities of the State 130:1-48).

TAINIO, AARNE, 1946: Kuparilannoituksen merkityksestä meikäläisillä viljelysmailla (Significance of copper in our cultivated soils) (Koetoiminta ja käytäntö, Experimental Work and Practice, Helsinki, 3, No. 4:34).

TUORILA, PAULI, TAINIO, AARNE and TERÄSVUORI, ARMO, 1939: Suomen viljelysmaiden kalkitustarpeesta, I osa (Referat: Über den Kalkdüngungsbedarf der Finnischen Böden, Erster Teil) (Reports of Agr. Exp. Activities of the State 104:1-529).

SUR L'ACTIVITÉ ET L'INTERDEPENDANCE DES ALIMENTS DANS LA NUTRITION D'ASPERGILLUS NIGER

Quelques Résultats Expérimentaux

par le Professeur J. LAVOLLAY,
Conservatoire National des Arts et Métiers, Paris, France

Les expériences décrites dans ce mémoire ne concernent pas les éléments très rares, mais des éléments indispensables à la physiologie des plantes, qui comme le magnésium sont à la limite des éléments dits catalytiques. De plus, ces expériences n'ont pas été réalisées avec des plantes cultivées; la cellule végétale prise comme réactif est celle d'*Aspergillus niger,* un végétal non chlorophyllien qui a cependant ouvert déjà bien des horizons sur l'alimentation des plantes supérieures. Des travaux qui vont être résumés se dégage une méthode de recherche qui semble générale et qui doit pouvoir être appliquée à l'étude d'éléments très rares comme le molybdène ou d'autres encore.

Le premier point que je voudrais rappeler est que la masse de la récolte d'*Aspergillus niger,* considérée à l'état sec, peut s'exprimer par une loi mathématique simple lorsque l'on fait varier un par un les éléments du milieu de culture.[2]

Les expériences qui nous ont permis d'établir ce fait étaient réalisées de la manière suivante. La condition fondamentale était de laisser développer le mycélium sur un milieu de composition constante.

Le milieu de culture (Raulin) occupait un grand volume (2 l) dans un cristallisoir en verre. Un cylindre de verre limitait

---

[1] Les expériences citées dans ce travail sont empruntées aux recherches poursuivies depuis 1937 au Laboratoire de Chimie Agricole et Biologique du Conservatoire National des Arts et Métiers, par J. LAVOLLAY et Mme F. LABOREY.

[2] Lorsque ce milieu est d'autre part correctement constitué.

une surface interne aussi petite qu'on le désirait par rapport au volume du liquide. À l'intérieur du cylindre les conidies étaient ensemencées. À l'extérieur, une couche d'huile de vaseline empêchait tout développement accidentel. Une hélice immergée permettait l'homogénéisation constante du liquide. Le milieu de culture renfermait tous les aliments utiles en proportion convenable, sauf celui qu'on étudiait lequel était introduit à diverses concentrations dans plusieurs appareils identiques placés simultanément dans la même étuve spécialement équipée.

La disproportion ainsi créée entre la masse des aliments et la masse du mycélium permettait de limiter les variations de composition du milieu qui restaient très minimes ($\leqq 4\%$).

Définition du Coefficient d'Action d'un Aliment:—Les poids des récoltes correspondant aux très faibles concentrations sont très petits, c'est pourquoi, dans une première série d'expériences, nous avons négligé les points correspondants en raison de l'importante erreur relative que comporte leur détermination. En exceptant donc la région basse de la courbe, nous avons vérifié, avec plusieurs éléments (Mg, K etc.) que lorsqu'on introduit un aliment minéral à des concentrations variées, on a la relation suivante entre la concentration x de l'élément et le poid P de la récolte obtenue en un temps donné, toujours le même:

$$\frac{dP}{dx} = c(A\text{-}P) \qquad \text{ou} \qquad P = A(1 - e^{-cx})$$

où A représente la récolte maxima, c une constante et e la base des logarithmes népériens.

C'est l'expression bien connue de Mitscherlich. Elle revêt cependant ici une signification différente. En effet Mitscherlich exprimait la masse de la récolte en fonction de *la quantité globale* de l'élément qui se trouvait à la disposition de la plante.

Or nous avons trouvé que la récolte est liée exclusivement à la *concentration* de l'aliment dans le milieu nutritif. Le poids de la récolte est en effet indépendant de la masse globale de l'élément qui est mise à la disposition de la moisissure; la quantité d'aliment mise à la disposition de la plante ne modifie la récolte que dans la mesure où la concentration est elle-même modifiée. Par exemple, dan certaines expériences, la masse de magnésium présente dans le milieu était suffisante pour l'édification d'un mycélium cinquante fois supérieur en poids à celui qu'on a récolté. La récolte correspondait uniquement à la *concentration*. La loi de MITSCHERLICH est donc applicable, à la condition que x représente une concentration.

Si dans l'expression

$$(1)\ P = A(1 - e^{-cx}),$$

nous posons $A = 1$, nous avons

$$(2)\ P = 1 - e^{-cx}$$

En prenant comme unité de poids, le poids maximum de la récolte, la courbe est donc entièrement définie par le coefficient c que nous appelons le *coefficient d'action* de l'élément étudié. Ce coefficient est une constante d'activité.

Si dans l'expression (2), $x = \frac{1}{c}$, nous avons

$$P = 1 - e^{-1} = 0{,}63$$

c a donc pour valeur numérique, l'inverse de la concentration x_c qui conduit à 63% du maximum

$$c = \frac{1}{x_c}$$

il est donc facile de calculer c si l'on connait x_c.

Remarquons en outre que la constante de concentration c est proportionelle à toute autre constante de concentration que l'on pourrait choisir pour définir l'activité. En effet, considérons les deux courbes correspondant à

$$P = 1 - e^{-x} \text{ où } c = 1$$

$$\text{et} \qquad P = 1 - e^{-2x} \text{ où } c = 2$$

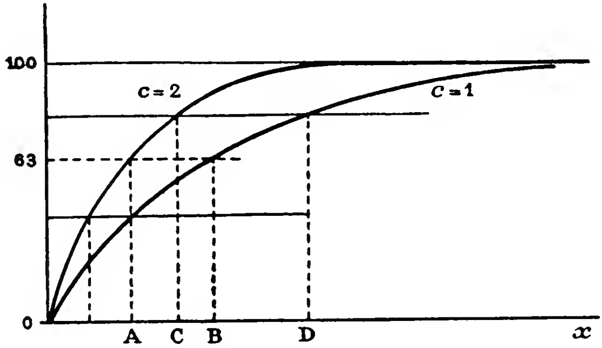
Les concentrations conduisant à 63% du maximum, sont entre elles comme les inverses des coefficients c. On a donc (FIGURE 16):

$$OB = 2\ OA \text{ et } OD = 2\ OC$$

En outre les concentrations qui mènent à 50, 80, 90, 99% du maximum sont toujours proportionnelles aux concentrations fournissant 63% de la récolte; autrement dit, elles sont doublées lorsque l'on passe de la courbe correspondant à $c = 2$ à la courbe correspondant à $c = 1$.

La concentration x_c qui conduit à 63%, ou son inverse c peut être très commodément utilisée pour chiffrer l'activité d'un élément nutritif.

Il est facile de comprendre, en raison de la forme de la fonction $P = 1 - e^{-cx}$, qu'il existe toute une gamme de concentrations pour lesquelles le maximum est approché d'aussi près qu'on le désire. On ne peut donc pas caractériser l'action de l'élément qui varie par un optimum de concentration, qui n'existe pas, dans les limites de validité de l'expression (1) et que l'on ne peut arriver à fixer par l'expérience.



— FIGURE 16 —

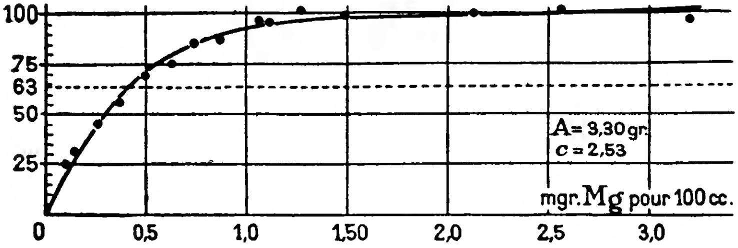
Il serait possible de mesurer cette activité par la concentration qui conduit à 50% de la récolte maxima; mais, comme il est dit plus haut, il est très commode d'avoir directement la valeur du coefficient d'action c qui figure dans l'expression de la courbe de récoltes.

Dans ce but on détermine avec précision, par l'expérience, la valeur du maximum A, puis on construit la courbe des points expérimentaux. On cherche sur le graphique quelle est la concentration qui correspond à 63% de A, c'est x_c, et $\frac{1}{x_c} = c$.

Si les concentrations sont exprimées, par exemple en mgr, c sera exprimé en mgr^{-1}.

On notera que des petites valeurs de x_c correspondent à des valeurs élevées pour c, donc à de grandes activités que sont caractéristiques des micro-aliments.

En employant cette méthode, on a trouvé pour un élément donné, *dans un milieu de composition bien déterminée,* un coefficient d'action bien défini. Par exemple, dans notre liquide nutritif, le coefficient d'action du Mg est compris entre 2 et 3 (FIGURE 17).



Courbe des récoltes en fonction de la concentration en Mg.
Les points représentent les récoltes expérimentales.
La courbe représente la fonction $P = 3{,}3(1 - e^{-2{,}53x})$.

— FIGURE 17 —

Variation du Coefficient d'Action d'un Aliment Suivant la Composition du Milieu:—Dans des milieux de composition différente, l'activité de l'élément nutritif peut être modifiée. C'est ce que je voudrais maintenant montrer.

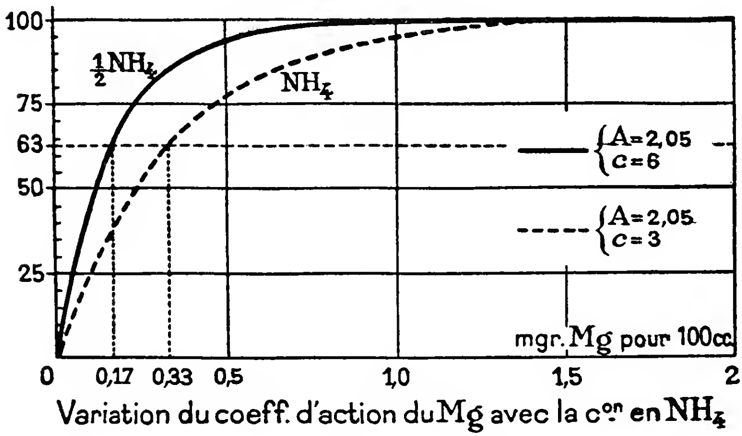
Par exemple, si l'on augmente dans le milieu de culture, la concentration d'un autre élément qui doit se présenter dans un certain rapport physiologique avec celui qu'on étudie, on conçoit que le coefficient d'action c de ce dernier puisse être modifié.

Si, au contraire le coefficient c ne varie pas, il y aura une certaine probabilité pour qu'il n'existe pas de rapport physiologique entre les deux éléments. Citons, à titre d'exemple, les variations d'activité du magnésium suivant la composition du milieu.

a) Si l'on fait varier la concentration globale du milieu en diluant le liquide au ½ avec de l'eau pure, on constate, que cette dilution a pour effet d'augmenter le coefficient d'action du magnésium.

Dans l'expérience citée, le coefficient a pour valeurs: 1,85 dans le milieu normal, et 4,75 dans le milieu à demi concentré. Ce résultat montre que c'est la concentration *relative*—par rapport aux autres constituants du milieu—qui intervient comme facteur de développement.

b) Si l'on établit des milieux où l'azote est fourni sous forme de sel d'ammonium, on constate que le magnésium présente des activités différentes suivant la teneur en azote. Par exemple, le coefficient d'action revêt la valeur 3 lorsque la concentration en NH_4 est de 1, et la valeur 6 lorsque l'NH_4 est à la concentration ½ (Figure 18).



— Figure 18 —

Il y a donc certains rapports à observer entre le magnésium et l'ensemble des autres aliments: pour obtenir un certain pourcentage de la récolte maxima il faut d'autant plus de magnésium que le milieu est plus riche en tous les autres aliments.

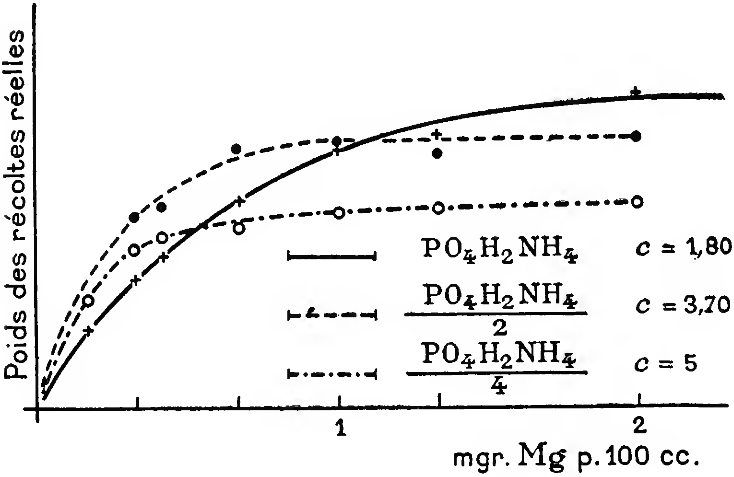
De même il faut d'autant plus de magnésium qu'il y a plus d'azote ammoniacal dans le milieu, les autres aliments étant inchangés.

c) Si l'on étudie les courbes de récoltes obtenues en faisant varier le magnésium dans des milieux où le phosphore figure à des taux différents, on constate que l'activité du magnésium dépend, dans ces milieux, de la concentration en phosphore. Plus le taux de phosphore est bas, moins est élevé le maximum de la récolte, mais plus est grand le coefficient d'action du magnésium.

Nous avons obtenu les valeurs suivantes:

teneur en *P* du milieu, p. 100 cc (mgr):	10,8	5,4	2,7	1,15
coefficient d'action de Mg (*c*):	1,8	3,7	5	50

Plus l'acide phosphorique est abondant, plus le végétal réclame de magnésium. Comme précédemment nous aboutissons donc à la notion d'un rapport physiologique.

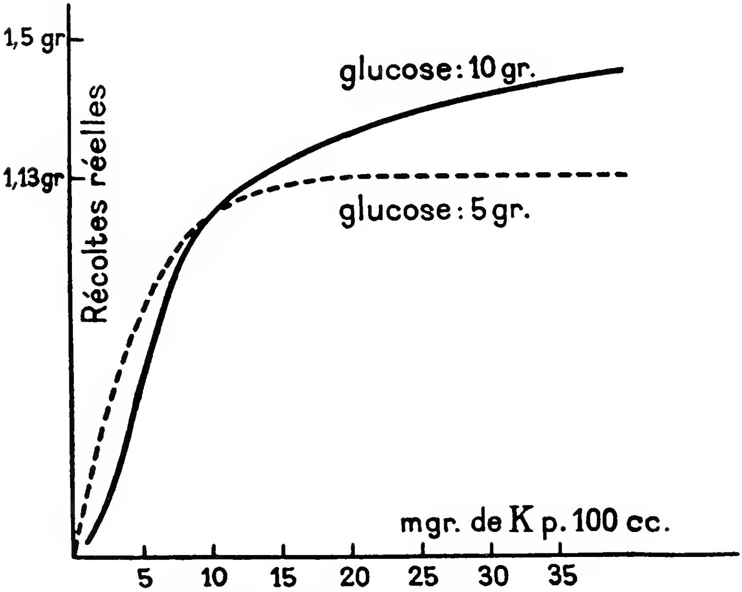


— FIGURE 19 —

L'examen des courbes des *poids réels* des récoltes est également très instructif. Il révèle en effet que les courbes correspondant à des teneurs différentes en phosphore peuvent se croiser comme sur la FIGURE 19, qui représente les courbes de récoltes en fonction de la concentration en magnésium dans des milieux différant par leur teneur en phosphore. Si l'on examine les courbes relatives aux concentrations en phosphate 1 et ¼, on constate ce fait inattendu, que l'augmentation de la proportion du phosphore accroît la récolte pour toutes les concentrations en magnésium supérieures à 0,55 mgr pour 100 cc; mais que par contre l'augmentation du phosphore, de ¼ à 1, dans les milieux renfermant moins de 0,55 mgr de magnésium se traduit par une chute de la récolte.

En d'autres termes, les courbes de récoltes révèlent que le végétal peut souffrir d'une carence magnésienne relative à la suite d'un apport de phosphore.

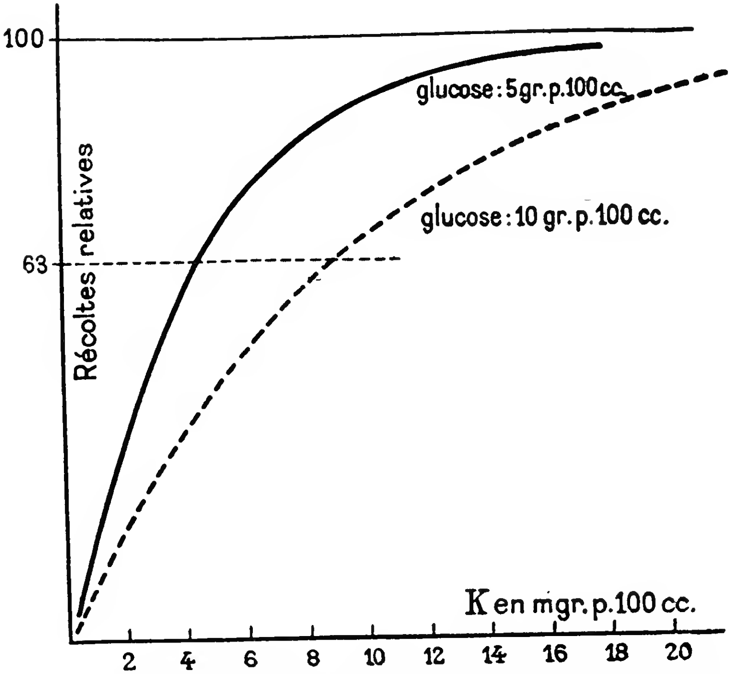
d) Si l'on prépare des milieux différant par leur concentration en potassium, et si l'on étudie les courbes de récoltes obtenues en faisant varier la concentration en magnésium de chacun de ces milieux, on constate que les besoins en magnésium sont d'autant plus considérables que la teneur du milieu en potassium est plus élevée. Cette élévation des besoins en magnésium avec la concentration d'un autre élément est donc un fait assez général, qui vient à l'appui de la notion des rapports physiologiques.



Action du K dans deux milieux différant par le glucose.

— Figure 20 —

Par contre, ce qui n'était pas prévu d'après nos connaissances antérieures, c'est que les courbes des poids réels des récoltes peuvent se croiser. Par exemple, si nous considérons les deux courbes correspondant à l'action du magnésium en présence de 5 mgr de K, et de 50 mgr de K pour 100 cc, nous remarquons que, pour toutes les concentrations en magnésium inférieures à 0,5 mgr pour 100 cc, le fait d'augmenter le taux de potassium de 5 à 50 mgr diminue la récolte; tandis que, au dessus de 0,5 mgr de magnésium, il y a, au contraire, bénéfice à augmenter le potassium.



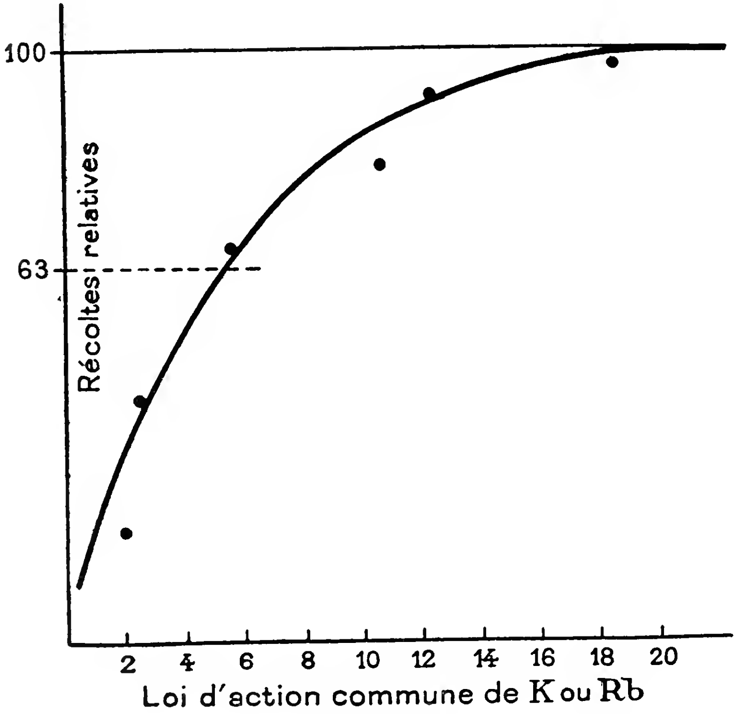
Action du K dans deux milieux différant par le glucose.

— FIGURE 21 —

En d'autres termes, le potassium ajouté au premier milieu, de manière à obtenir la deuxième courbe (addition de 45 mgr de K), a un *effet dépressif* lorsque le magnésium est à des concentrations inférieures à 0,5 mgr, et un *effet favorable* lorsque le magnésium se trouve à des concentrations supérieures. Il va de soi d'ailleurs, que la concentration en magnésium qui correspond au point d'inversion dépend de l'importance de l'apport de potassium, dans la mesure où la concentration de cet élément en est modifiée.

La notion des rapports physiologiques ne concerne d'ailleurs pas seulement que des aliments minéraux.

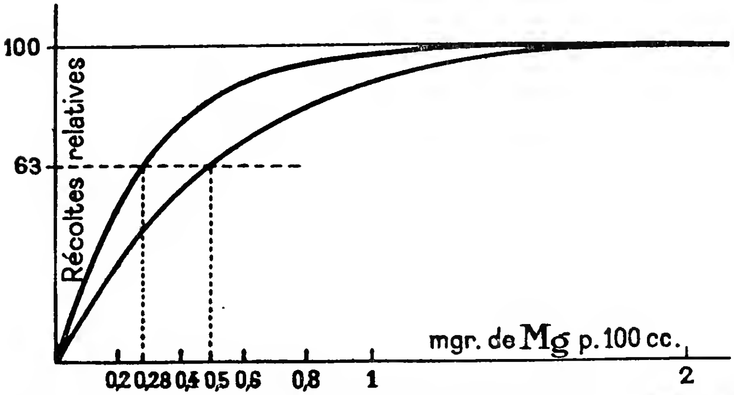
On trouve par exemple, par la même méthode, que l'activité du potassium dépend de la concentration en glucose. Les maxima sont évidemment fonction de la teneur en glucose: ils ont pour valeurs: 1,13 gr et 1,50 gr lorsque les concentrations en glucose sont respectivement de 5 gr et 10 gr pour 100 cc. Les courbes de récoltes se croisent pour une concentration en potassium voisine de 10 mgr pour 100 cc (FIGURE 20). Après avoir rapporté les récoltes à chacun des maxima, on constate que les concentrations en potassium qui conduisent à 63% des maxima, sont respectivement égales à 4,4 mgr et 8,8 mgr. Les besoins en K sont donc fonction de la concentration en l'aliment glucidique (FIGURE 21).



— FIGURE 22 —

Pour toutes les concentrations en potassium inférieures à 10 mgr, l'augmentation de 5 gr à 10 gr de la concentration en glucose a un effet dépressif; il y a au contraire avantage à augmenter de 5 à 10 gr la concentration en glucose lorsque la concentration en potassium est supérieure à 10 mgr pour 100 cc.

La méthode de travail dont il a été montré plus haut quelques applications, nous a donné d'intéressants résultats dans l'étude du rubidium. Il est déjà connu que cet élément peut remplacer en partie le potassium.



Courbes d'action du Mg en présence de deux conc.$^{\text{ons}}$ en Rb.
Rb=8,1 mgr.% c=2. Rb=4,05 mgr.% c=3,6.

— FIGURE 23 —

Par exemple pour l'*Aspergillus niger*, le remplacement de K par Rb ne conduit jamais à un maximum de récolte aussi élevé; de plus, en absence de potassium la sporulation n'apparaît pas. Cependant la récolte peut atteindre, avec le seul rubidium, un poids très appréciable.

Mais, ce qui est remarquable, c'est qu'en rapportant les poids des récoltes obtenues dans les deux milieux renfermant du potassium ou du rubidium à leurs maxima pris comme unité, on obtient exactement la même courbe d'action. Les 63% du maximum sont obtenus pour la même concentration de potassium ou de rubidium. Les coefficients d'action des deux éléments, exprimés en mgr^{-1}, sont égaux: 0,2 (FIGURE 22).

L'analogie entre le potassium et le rubidium, comme facteurs de développement, est donc assez profonde. Cette analogie est encore soulignée par la similitude des rapports qui se manifestent entre le magnésium, d'une part, et, d'autre part, l'un ou l'autre de ces éléments. En faisant varier la concentration du magnésium dans des milieux différant par leur teneur en rubidium, on trouve en effet que le taux de rubidium modifie l'activité du magnésium, dans des proportions analogues aux modifications de cette activité qui sont apportées par des variations de teneur en potassium. Par exemple, lorsque la concentration en rubidium est égale à 4,05 mgr et 8,1 mgr pour 100 cc dans des milieux exempts de potassium, le coefficient d'action du magnésium est respectivement égal à 2 et 3,6 (FIGURE 23).

Bibliographie:—LAVOLLAY: Etudes sur l'intervention du magnésium . . . (Th. Fac.Sc., Paris, 1936, Hermann).—LAVOLLAY et LABOREY: C.R.Ac.Sc. 204:1686, 1937; C.R.Ac.Sc. 208:1357, 1939; C.R.Ac.Agric. 34:787, 1948.—LABOREY, LAVOLLAY et NEUMANN: C.R.Ac.Sc. 212:624, 1941.—JAVILLIER, LAVOLLAY, LABOREY et NEUMANN: C.R.Ac.Agric. 27:398, 1941.—LABOREY: Etudes expérimentales sur les courbes de poids d'*Aspergillus* . . . (Th.Fac.Sc., Paris, 1942).—LAVOLLAY: Rapports entre le phosphore et les autres éléments . . . (Le Phosphore en Biologie, Presses Univ., p. 87, 1949).

Points from Discussion of Paper by Prof. Lavollay

Dr. Gisiger:—

Prof. LAVOLLAY has raised a question of great practical importance. We have soils in which with a minimum of nutritive elements maximum growth of plants is obtained. We have imagined that by the giving of a nutritive element in excess the harmony is destroyed and ought to be balanced by adding one or several nutritive elements. MITSCHERLICH has demonstrated to you in his 2nd law that the relations of the different nutritive elements have an influence on the coefficient of activity.

Prof. Burström:—

The relationship between external concentration and growth involves two processes: nutrient absorption and nutrient activity inside the plant. Can you decide whether the magnitude of coefficients depends more upon the second than on the first process? In most cases we have a luxury consumption of the nutrient. Do you know if your formula is valid also for a nutrient, of which there is no luxury consumption, *e.g.* nitrogen?

Dr. Lavollay:—

Oui. Le coefficient d'action du magnésium est, dans une certaine mesure, indépendant de la concentration en azote nitrique; au contraire, ce coefficient varie avec la concentration de l'azote ammoniacal.

MODE OF ACTION AND OCCURRENCE OF TRACE ELEMENTS IN PASTURES AND IN THE BLOOD AND LIVER OF FARM ANIMALS

by L. SEEKLES, Ph.D.,
Laboratory for Veterinary Biochemistry, University of Utrecht, Netherlands

About the year 1930, SJOLLEMA for the first time reported, amongst the cattle on the North East diluvial part of Holland, which is of Scandinavian origin, conditions of deficiency due to a low copper content of the pastures (1). For the southern diluvium these deficiences have not been reported, and it seems doubtful whether they occur. This diluvial soil consists mainly of slate and other material, originating from mountain areas along the river Rhine and of the Ardennes, mixed with fine material of Scandinavian origin.

During recent years, disorders in cattle frequently occurred, although there is no real shortage of trace elements, *e.g.*, copper, in the pasture (2). This may be called "conditioned deficiency" and it suggests a combined influence of the absorption and the metabolism of one or more trace elements. An interaction of trace elements with various chemical substances in the food and in the animal tissues may also play a part. The disorders are found in the Netherlands, both on the North East diluvium and on the alluvial parts, especially on peat. Presumably, they also occur on the southern diluvium but definite data are not yet available. Recently, a similar kind of hypocupremia in cattle has been reported for Great Britain by ALLCROFT (3).

In order to establish the nature of these "conditional deficiencies" it is necessary to study the basic importance of the trace elements and their function in the metabolic processes of the animals concerned. This enquiry is primarily of a scientific nature as its results will further our knowledge of the nature of the above mentioned phenomena. The enquiry also has its practical aspects as may be illustrated by the following example.

Sometimes, in a cow, a conditioned deficiency of copper is accompanied by the well known symptoms of "pica" or "licking sickness" just as those found in a case of real copper deficiency. At other times, the "pica" symptom is only shown by an irregular grazing of strip after strip, of the pastures, down to the roots. It seems as if the animals were searching for something in the very young blades of grass which they apparently miss in the older blades. With horses, in normal condition, *i.e.*, not suffering from any deficiency, strip grazing of pasture may be frequently observed. Real and conditioned deficiency of trace elements have not been found in horses in Holland.

It also happens that conditioned deficiency in cows is not accompanied by "pica" symptoms. Delayed growth of young animals occurs frequently, yet anemia, which, in this connection, is regarded by some as an important symptom, is fairly rare. It seems very questionable whether hemoglobinemia is connected with a relative or absolute deficiency of copper. Exhaustion, scouring, decreased output of milk, reduced appetite, white or greyish discolouring of the coat, shedding of hair and a rough coat may, in some years, be observed in various parts of the country. It should be pointed out that in the examination of the coat sometimes grave errors are made. Frequently, the symptoms shown by the coat, as the result from a delayed shedding of the winter coat, are attributed to a copper deficiency. Sometimes, a conditioned deficiency in copper is only shown by retarded growth of young animals and the absence of heat, whereas the condition of older animals is, on the whole, fairly sound. It may even be observed that the symptoms of hypocupremia, which in some years may be accompanied by a number of the clinical symptoms mentioned above, in another year, though existent, leave the animals in seemingly good health. This fact should make us cautious in interpreting the chemical analysis.

The great variety observed in Holland, with regard to the clinical features of a conditioned deficiency in copper, presumably also in other trace elements, renders it necessary to study the causes of this variety.

Are they mainly, or only, to be attributed to quantitative differences in the amounts of copper and other trace elements? The chemical analysis of the food, the blood and the liver of the patients does not point in this direction. Or, does a certain individual sensitiveness of the animals play a part? Although this possibility should not be excluded, the regional occurrence of the disorders, in certain years, does not make it likely. The possible influence of the weather on the chemical composition of grass, *e.g.*, the amount of precipitation and the temperature should, of course, be taken into consideration. Furthermore, farming methods, types of manuring, etc., should be considered as important factors in causing the differences found in the clinical aspects of the symptoms. During the war years, particularly, the manuring of the pastures and the feeding of the animals left much to be desired. This favours the assumption that the metabolism of copper and other trace elements in plants and animals is influenced by metabolic processes of other chemical components.

The veterinarian is generally confronted by a disease which is to be considered as the result of a number of effects caused by a (conditioned) deficiency of trace elements in the normal course of the biochemical processes involved. It is easily understood that, as a result of these effects, some general symptoms, like delayed growth and interference with other vital functions, will be observed. It goes without saying that this effect may not be regarded as being caused only by a special deficiency, *e.g.*, of copper. It should be borne in mind that disorders of quite a different nature, such as gastro-intestinal strongylosis and Johne's disease, may cause similar symptoms. A thorough clinical, parasitological and bacteriological examination should, therefore, never be omitted.

It should be emphasized that, as a rule, scouring is a prevalent clinical feature of "conditioned deficiency" of trace elements in cattle. Evidence has been obtained that in many cases an increased motility of the intestine may result in a decreased absorption of trace element and may be responsible, in this way, for the low content in trace elements which the chemical analysis shows in blood and tissues.

An increased intestinal motility may originate from a number of factors affecting the intestine, e.g., the intake of rank, young pasture grass in spring, worm infestation (gastro-intestinal strongylosis), Johne's disease and other kinds of "enteritis." A special influence of endogenous toxins, originating from gastro-intestinal autointoxication, on different organs, resulting in a mobilization of trace elements, stored in the tissues, also may not be excluded.

We observed, in any case, a significant difference between the copper content of the liver in 10 typical cases of gastro-intestinal disorders, of different origin, and in 55 normal cattle livers. The basis, respectively. In 10 cases, originating from a typical scouring area on peat soil, an average value of 16.6 mg of copper was observed, probably indicating a decrease of copper content. Five of the cases observed were calves which, as a rule, showed a higher copper content of the liver than adult animals.

The veterinarian, as a rule, will not be able to distinguish the above mentioned partial effects. This results in a certain vagueness in making the diagnosis and uncertainty in therapeutic planning.

The experience of recent years has shown that the veterinarian—and less so, naturally, the breeder or agricultural expert—is not able to arrive, as a rule, on the basis of his observations, at a definite diagnosis of (conditioned) deficiency in trace elements. To do this, with a reasonable chance of success, he needs the assistance of a chemist who will help him with the analysis of the food, the blood and the tissues of the animal.

So far, mainly the composition of pastures and the symptoms of disease in plants and in herbivorous animals have drawn our attention to the study of the functions of trace elements. This type of research will always be of the utmost value. For our knowledge of the origin of the disorders in plants and in animals, resulting from a relative deficiency or excess of trace elements, further research is required into the symptoms appearing between the absorption of the food and the moment in which pathological disorders in plants or animals begin to show. The study of the origin of these disorders is the task of the biochemist as the processes of intermediate metabolism are of a biochemical nature. Only a thorough biochemical examination of the consecutive reactions in cells, tissues and organs will lead to rational diagnostics and an effective fight against the diseases concerned.

For a number of years, in the Laboratory for Veterinary Biochemistry of the University of Utrecht research has been conducted on the occurrence of trace elements—especially copper, cobalt, manganese, molybdenum and selenium—in pasture grass, blood and liver of cows and horses. The results are based on the clinical and chemical examination of several hundreds of diseased

TABLE 22: *Action of trace elements on enzymes:*—[*]

1. Synthesis and destruction of tissue elements (mainly protein).
   A. (d-) Peptidases: Mn, Co, Fe, Zn, Mg
   (bloodserum, animal organs, plants, bacteria)
   (peptides → aminoacids)
   B. Arginase: *Mn*, Co, Ni, Ca, V
   (muscle, liver, yeast)
   (arginine → ornithine + urea)
   C. (Iso-citric acid-dehydrogenase: Mn, Mg
   (hart muscle, liver, kidney, adrenals)
   (Iso-citric acid → α-keto-β-carboxyglutaric acid)

2. Energetic processes (oxido-reductions).
   A.

Haemoglobin	*Fe*	Transference of oxygen
Haemocyanin	*Cu*	
Respiratory enzyme	*Fe*	
Cytochromes	*Fe*, Mn	
Peroxydase	*Fe*, Mn	
Catalase	*Fe*	

   B. Phosphate-transfering enzyme (yeast): Mg, Mn
   1,3-diphosphoglycerinic acid + adenosinediphosphate ⇄
   ⇄ 3-phosphoglycerinic acid + adenosinetriphosphate
   C. Enolase (yeast): *Mg*, Mn, Zn
   (2-phosphoglycerinic acid → phosphopyruvic acid)
   D. Carboxylase (yeast): *Mg*, Mn, Zn
   (pyruvic acid → acetaldehyde + CO_2).
   E. Pyruvic acid-dehydrogenase (brain): Mg, Mn
   F. Phosphatases (different organs): Mg
   G. Zymase (yeast): Mn
   H. Carbonic acid anhydrase (blood): *Zn*
   ($H_2CO_3 \rightarrow CO_2 + H_2O$).
   J. Phospholipoides-oxidizing enzyme: V
   K. Lipases: Ca, Mn.

3. Detoxication reactions.
   A. "Tyrosinase" (mushrooms): *Cu*
   (oxidation of tyrosine, p-cresol, pyrocatechol).
   B. (Poly)phenoloxydases: *Cu*
   (mushrooms, potatoes, milky juice of plants)
   (oxidation of p-cresol, pyrocatechol, tyrosine, pyrogallol, hydrochinon, p-phenylenediamine).
   C. Diaminooxydase ("Histaminase"): Co
   (mucous membrane of intestinal tract, etc.)
   (oxidation of diamines, histamine).

4. Transference of nervous stimuli.
   A. Cholinesterase: Mn, Mg, Ca, Ba
   (torpedo, bloodserum, bloodcells)
   (acetylcholine → choline + acetic acid)

---

[*] Elements in italics are bound to enzyme complexes.

animals. It was found necessary to extend our research from trace elements to other substances in order to acquire an insight into the rôle of trace elements in metabolism. Obviously, the results do not yet present a complete picture but some of the conclusions seem worth reporting.

Trace Elements as Bio-Catalyzers of Enzyme Action:—In a recent paper we showed that the ions of trace elements may act on metabolic processes in the animal body in two different ways (2). First of all they may be taken up in the molecules of enzymes, vitamins and hormones. These complexes act as bio-catalyzers in the processes of intermediate metabolism. On the other hand, they may act as activators of enzymes and in this way influence metabolic processes.

A general survey of trace elements acting on enzymes in these two different ways is given in TABLE 22.

Two facts should be noted. In many cases an optimum concentration of minor elements, with regard to the activation of enzyme action, is shown to exist. Evidence has been obtained that a surplus, as well as a deficit of those elements, lowers the tempo of enzyme action. Probably, the latter conditions may be compared with conditions of intoxication and deficiency of trace elements in the animal body. Moreover, the mode of action of trace elements, by means of enzymes, implicates some influence of the *p*H of body tissues on the action of trace elements in the animal organism (4).

TABLE 22 shows that four types of processes of a fundamental importance in normal life are subject to the action of trace elements, *viz.*, (*1*) the synthesis and destruction of tissue elements (mainly protein), (2) energetic processes (oxido-reductions), (3) detoxication of poisons of endogenous origin, (4) the regulation of nervous stimuli (2).

This opens new outlooks on the genesis and therapy of several diseases, *e.g.*, "licking sickness" (pica) in animals, ketonemia in cattle, avitaminosis and hypovitaminosis B_1 and C, autointoxication in men and animals, and perhaps even on the problem of carcinoma.

The Distribution of Copper in the Liver and the Blood of Cattle and Horses. Regulation of Copper Metabolism:—

Experiments with cattle.—The level of copper (and other elements) in the blood is often considered in connection with "deficiency." However, when doing so many problems arise. We, therefore, carried out balance trials in which copper sulfate (and salts of other trace elements) were administered orally and experiments in which copper, with different types of chemical binding, was injected intravenously. Attempts have been made to obtain a picture of the extent of saturation of body tissues before and after treatment. FIGURE 24 shows the results of a balance trial in which copper sulfate was added to normal food (hay and concentrates). This experiment was carried out with a normal heifer.

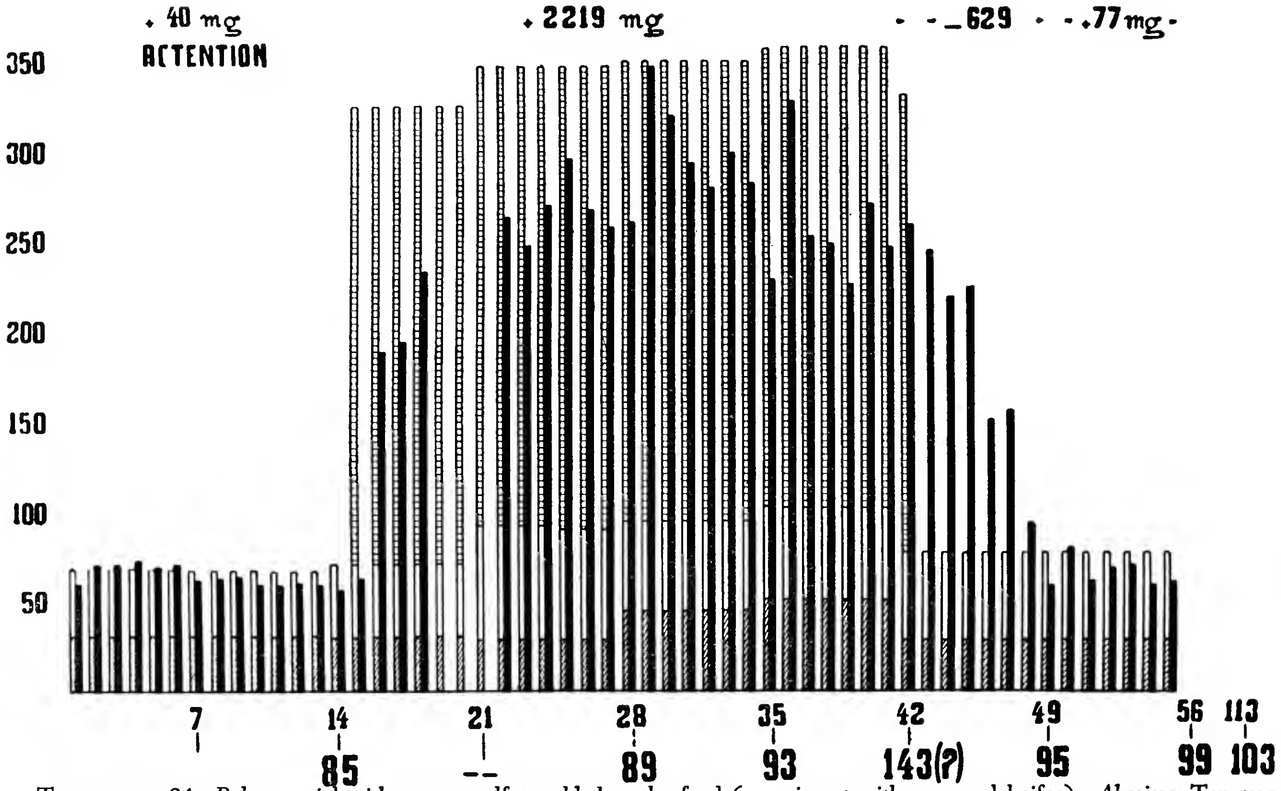
In the period previous to the administration of copper sulfate the intake of copper was shown to be almost equal to the excretion of copper in the faeces. Only traces of copper left the body through the kidneys.

As might be expected, the excretion of copper in the faeces increased during the period of copper addition as all added copper was not absorbed. The (alkaline) urine was shown to contain only traces of copper. No increase of the copper level in the blood occurred. This indicates a condition of non-saturation of the body tissues.

Over a fortnight, previous to the administration of copper sulfate, a retention of 40 mg of copper was calculated. However, it seems to be doubtful whether this is a real retention as the traces of copper leaving the body by the kidneys had not been taken into account. For the three week period of copper sulfate addition, together with the six day period following this addition, a retention of 1590 mg of copper was found, *i.e.*, about 17 per cent of the quantity of copper added. For three days no records were kept. For the next period of seven days a retention of 77 mg of copper was recorded. Apparently about 50 mg may be considered to be the real retention for this period.

To obtain an idea of the extent of saturation of the animal body with copper, determinations of copper have been carried out in 55 samples of liver originating from normal cattle aged 1½ years and older. These samples were collected in wartime. The content of copper ranged from 5 to 194 mg per kg (dry matter basis). A classification of the results is presented in TABLE 23.

We learn from this table that about 60 per cent of the livers of normal cattle had a copper content ranging from 5 to 15 mg



Textfigure 24.—Balance trial with copper sulfate added to the food (experiment with a normal heifer).—*Abscissa:* Top row, number of days; bottom row, copper in blood serum (mg %).—*Ordinate:* daily intake mg Cu.—The columns to the left show the daily intake of copper: fodder cake (*shaded*) and hay (*blank*), the black columns, at the right, show the quantity of copper leaving the body, on the same day, in the faeces.—Prepared in coöperation with R. Bijkerk.

per kg of dry matter and about 40 per cent contained more copper, up to 194 mg per kg of liver for a bull (only one case) and 91 mg for a cow.

It appears that in 60 per cent of the cases examined the extent of saturation of the liver is likely to be rather low. This may account for the relative stability of the copper content of the blood after an oral administration of copper sulfate.

Another series of experiments gives further information concerning the absorption of copper by the liver following intravenous administration. In these experiments we used radioactive copper salts (5).

Table 23: *Copper content (mg per kg of dry matter) of 55 samples of liver originating from normal female cattle (in collaboration with R. Bijkerk):—*

Classification	5–15	16–25	26–35	36–45	46–55	56–65	66–75	76–85	86–95	. . .186–195
Number of cases	34	4	3	3	1	2*	3	2	2	1†
Percentage	62	7	5	5	2	4	5	4	4	2

* One sample originated from a calf (½ years of age).

† Sample originated from a bull.

Three copper salts—copper acetate, copper glycinate and the sodium salt of copper allylthioureabenzoic acid ("Ebesal)"—were injected (calves, weight about 30 kg). The copper salts had been irradiated by a neutron generator (Ra-Be preparation) for 24 hours or more. The radioactivity of the copper acquired—measured by means of a sensitive Geiger-Müller counter—enabled us to measure the rate of absorption of the various copper salts by the liver.

The relatively slight radiation of the copper salts and the rather short half life (13h) of the radioactive copper isotope made it necessary to inject as large quantities as could be tolerated by our animals.

Table 24 presents the results.

Evidently, there is a parallelism between the "solidity" of the copper compound used for injection and the rate of its absorption by the liver.

The difference between Cu-acetate and Cu-glycinate is only slight. In both cases, copper is almost equally absorbed by compounds of the blood and transported to the tissues, including the liver. This was confirmed when we found that the lethal dose of these two salts, intravenously injected, contains, in both cases, the same amount of copper.

When we experimented with "Ebesal," the lethal dose proved to be higher and the amount of Cu taken up by the liver was greater.

These experiments, which may explain the results mentioned in FIGURE 24, seem to indicate a condition of non-saturation of the liver.

TABLE 24: *Absorption of radioactive copper by the liver following intravenous injection in calves (in collaboration with E. Havinga and R. Bijkerk):—*

	Cu-acetate	Cu-glycinate	Cu-Na-allylthioureabenzoate ("Ebesal")
Amount of Cu in the solution injected	0.73 g	0.56 g	0.78 g
Time from first injection till slaughter	3½ h	3¼ h	3¾ h
Amount of injected Cu recovered from the livers	13.7 ± 1.4% (0.10 g)	16.1 ± 1.1% (0.09 g)	24.5 ± 1.1% (0.19 g)

To conclude, we might express the opinion that injection of ionogenic compounds (Cu-acetate) or not too firmly bound complex salts (Cu-glycinate) will not make much difference in the pharmacological and therapeutical effects. Compounds which give little or no Cu-ions like "Ebesal" will behave in a different way. As they are less toxic we shall obtain a higher Cu-content of the liver (and probably a lower one for other organs) after injection.

Experiments with horses.—In normal horses, the copper content of the blood serum is somewhat higher than in cattle. Observations made in this laboratory give approximate figures of 100-130 micrograms in 100 ml. Horses in foal may show much higher copper values.

Copper glycinate, repeatedly administered by intravenous in-

TABLE 25: *Intravenous injection of copper glycinate (30 mg of Cu per injection) in horses (in collaboration with E. Havinga)*:—

	DAYS										WEEKS							
Gelding No. 1 Injection	0	1	2	4	6	7	12	13	16	19	1	2	3	4	5	6	7	8
	↓													↓	↓	↓	↓	
γ per cent of Cu in blood serum	144	87	91	—	135	—	88	96	160	131	—	—	—	100	127	139	112	120
Gelding No. 2 Injection	↓											↓	↓	↓	↓	↓	↓	
γ per cent of Cu in blood serum	82	123	—	159	—	153	—	—	—	—	153	178	97	124	110	117	181	122
Mare Injection	↓											↓	↓	↓	↓	↓	↓	
γ per cent of Cu in blood serum	121	103	—	194	—	169	—	—	—	—	169	184	122	135	111	141	155	130

Table 26: *Copper content (mg per kg of dry matter) of samples of liver originating from horses (in collaboration with R. Bijkerk)*:—

Classification		11–15	16–20	21–35	26–30	31–35	Limits of variation	Average	Number of cases
Normal	*Number*	13	16	5	2	1	13–32	18.5	37
(*c.q.*, killed by accident)	%	35	43	13	6	3			
Probably normal	*Number*	13	15	8	1	0	12–28	17.6	37
(*c.q.*, light disorders)	%	35	41	21	3	—			
Normal, in foal	*Number*	6	3	1	0	0	14–21	16.1	10
or shortly after deliverance	%	60	30	10	—	—			
Surgical patients	*Number*	3	25	4	1	0	14–29	18.8	33
(lameness, etc.)	%	9	76	12	3	—			
New born foal	—	—	—	—	—	—	223	—	1

jection in quantities of 30 mg of copper, did not result in any permanent increase of the copper content of the blood serum. The copper level of the blood serum seems to vary rather considerably. Apparently, the excess of copper injected is rapidly transferred to the tissues (TABLE 25).

In order to obtain an idea of the extent of saturation of the liver, determinations of copper were carried out in samples of liver originating from normal and nearly normal horses (TABLE 26).

The copper content of the liver of normal, adult horses shows considerably less variation than the copper values of cattle liver. Values up to 10 mg of copper per kg of liver (dry matter basis), which were observed in cattle in 51 per cent of the cases examined, did not occur in horses.

Evidence has been obtained from the examination of a small number of livers, *viz.*, 10 samples, originating from horses in foal, or shortly after deliverance, that there is a tendency towards lower copper values. In surgical cases there seems to be, however, a tendency in the opposite direction. In a new-born foal the copper content of the liver was shown to be 10 to 15 times as high as in adult horses.

Manganese in Relation to Grass Tetany and Sterility in Cattle. The Occurrence of Manganese in Pasture Grass and Hay in the Netherlands:—

Manganese in relation to grass tetany.—The observations of BLAKEMORE, NICHOLSON and STEWART, suggesting that in Lincolnshire "lactation tetany" in cattle occurs under conditions in which the manganese intake is likely to be at its maximum (6), suggested an investigation concerning the possibility of a parallelism between the occurrence of grass tetany and high manganese content of certain pastures in the Netherlands. If a correlation would be found, this would indicate a third disease among animals due to an excess of trace elements—alkali disease (Se) and the scouring of cattle on the "teart lands" of Somerset (Mo) are already known.

Just as in England, most grass tetany cases occur, in the Netherlands, when the cows are first turned out to pasture in the spring. A second, though less severe outbreak of grass tetany occurs in August and September.

This kind of grass tetany has been known in Holland for centuries. It is particularly common along the Oude Rijn river. The soil in the affected area along the river is alluvial clay, at some places mixed with sand or peat.

A relation between the intake of manganese and the occurrence of grass tetany, as suggested by BLAKEMORE *et al.*, could not be established in the Netherlands (TABLE 27).

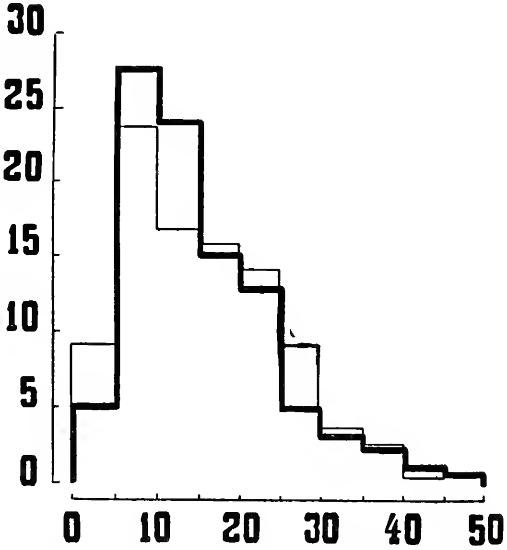
Manganese in relation to sterility.—Sterility in rodents is often considered as a result of a deficiency of manganese. In young rats, kept on a manganese deficient diet, growth seems to be normal (7). The females showed a normal cycle and produced normal young. However, the animals were unable to suckle the newborn rats. In males, kept on a manganese deficient ration, a degeneration of the testes was observed, within 100 days, causing complete sterility. According to recent experiments, rats showed the features of manganese deficiency after feeding with a ration rich in vitamin B_1 (8). The F_1 generation showed a reduced fertility and disturbed lactation, moreover, some cannibalism. In young mice, kept on a manganese deficient food, growth was stunted, ovulation discontinued and heat seldom occurred (9).

TABLE 27: *Manganese content of pasture grass in relation to the occurrence of grass tetany:—*

	MANGANESE CONTENT OF PASTURE GRASS mg p. 100 g, dry matter basis
Grass tetany in spring (40 affected farms)	4.1—19.5
Grass tetany in autumn (9 affected farms)	3.3—27.6
No grass tetany (15 farms)	5.5—17.6
"Lactation tetany" in Lincolnshire (BLAKEMORE and assoc.) (11 affected farms)	54—132
No grass tetany, in Lincolnshire (12 farms, 24 samples)	1.6—9.2

It remains to be seen whether the frequent occurrence of sterility in male and female cattle (if not due to anatomical and pathological disorders) may be connected with a relative manganese deficiency (2, 10). It has already been shown that the manganese content of pasture grass and hay, in the Netherlands, will vary considerably depending on the condition of the soil (2). Sometimes, the manganese values are as low as 2 mg in 100 g (dry matter basis). These low values have been observed on heavy clay with high *p*H values. The intake of manganese is very low in those cases. Also, it seems doubtful whether there is complete absorption of manganese in the gastro-intestinal tract.

If sterility, due to absolute or relative manganese deficiency, does occur in the Netherlands, this disorder may be expected to occur in other areas of clay soil with an alkaline reaction. These investigations are being continued.



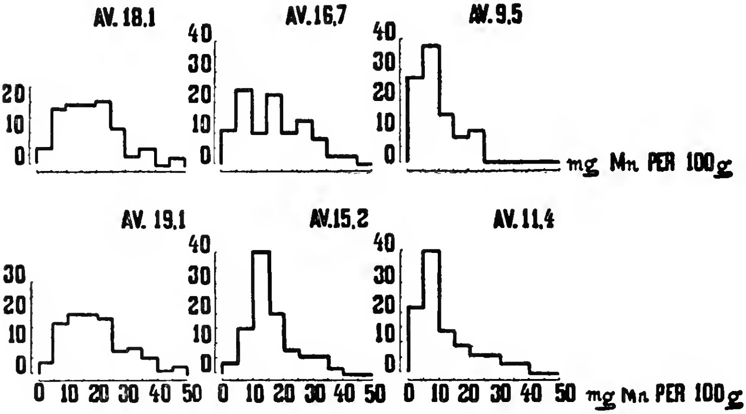
TEXTFIGURE 25.—Manganese content of pasture grass and hay without considering the type of soil, manuring or the condition of the pasture.—*Bold line:* grass (386 samples).—*Light line:* hay (259 samples).—*Abscissa:* mg Mn per 100 g (dry matter basis). —*Ordinate:* percentage of samples.—Prepared in coöperation with J. T. L. B. RAMEAU.

The occurrence of manganese in pasture grass and hay in the Netherlands.—A general survey of the occurrence of manganese in 386 samples of young pasture grass and of 259 samples of hay originating from different regions of the country and in 60 samples of hay originating from the province of Friesland is given in FIGURES 25, 26, and 27.

FIGURE 25 shows that in about 50 per cent of the grass samples examined a manganese content between 5 and 15 mg per cent (dry matter basis) was observed. About 30 per cent of the samples show values between 15 and 25 mg. Altogether 80 per cent show a manganese value up to 25 mg. The average manganese value of 386 samples of pasture grass is 15.8 mg.

The samples of hay are showing similar manganese values, in 70 per cent we find a variation between 5 and 25 per cent. The average manganese value of 259 samples of hay was 16.1 mg.

A classification of manganese values of the same samples of pasture grass and hay, dependent on the type of soil, is given in FIGURE 26.



TEXTFIGURE 26.—Manganese values of pasture grass and hay dependent on the kind of soil.—*Left:* sand.—*Centre:* peat.—*Right:* clay.—*Top row:* hay; *bottom row:* grass.—*Abscissa:* mg Mn per 100 g (dry matter basis).—*Ordinate:* percentage of samples.—Prepared in coöperation with J. T. L. B. RAMEAU.

No significant difference was shown to exist between the manganese contents of pasture grass and hay originating from either sand, clay or peat.

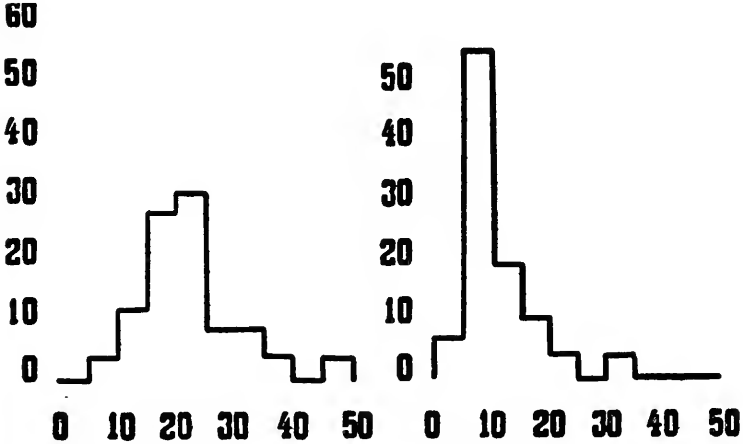
Grass originating from sandy soils, however, shows a higher manganese value than grass originating from peat. Grass originating from clay has the lowest manganese content. The average values are 19.1, 15.2, and 11.4 mg, respectively. These differences are significant.

As to hay, a higher manganese content was observed in hay from sand and peat in comparison with clay. The average values are 18.1, 16.7, and 9.5 mg, respectively. The difference between the first two figures is not significant, but the difference between these and the third is really striking.

Similar figures were found for 60 samples of hay originating from the province of Friesland (FIGURE 27).

Evidence has been obtained that the *p*H value of soils plays an important part in the absorption of manganese by pasture grass. The lower the *p*H value of the soil, the more manganese is being absorbed by the grass. This may well explain the differences observed between manganese in pasture grass and in hay originating from different soils.

After a critical survey of the results obtained in the Utrecht laboratory concerning the chemical composition, the type of soil, the manuring and the condition of the pastures, Dr. RAMEAU made a series of supplementary studies.



TEXTFIGURE 27.—Manganese in hay originating from the Province of Friesland dependent on the kind of soil.—*Left:* peat and sand.—*Right:* clay. —*Abscissa:* mg Mn per 100 g (dry matter basis).—*Ordinate:* percentage of samples.—Prepared in coöperation with J. T. L. B. RAMEAU.

As a result of the influence of the *p*H value of the soil, mentioned before, a significant difference was found in the manganese content of pasture grass (from sandy soils) of plots fertilized with lime and plots not fertilized. The addition of lime lowered the manganese content of the pasture grass. In this respect, the differences in hay were not striking.

The amount of manganese in pasture grass, originating from sandy soils not treated with lime, showed a gradual decrease from the northern to the southern provinces of the Netherlands. It is well known that in arable land, on sandy areas, the *p*H values increase accordingly. In hay the differences are hardly noteworthy.

A considerable increase in manganese in young pasture grass on clay was observed following manuring with Thomas phosphate containing 6 per cent manganese, the average figures being 8.6 and 19.0 mg, respectively. In hay the difference is small. On sand and peat the differences in manganese between young pasture grass and hay after manuring with Thomas phosphate were also small.

Evidence has further been obtained that coarse, young pasture grass from sandy soils contains less manganese than ordinary, young grass.

Supplementary Research on Copper, Cobalt, Molybdenum[*] and Selenium (*in collaboration with* J. DE WAEL):—A low content of copper in pasture grass and hay, formerly reported from the northeastern diluvial part of the Netherlands, has also been observed recently in alluvial parts. Values as low as 3-5 mg of Cu per kg (dry matter basis) are not exceptional. However, in the majority of the cases examined normal values of 10-25 mg were found. As pointed out, a low copper content of the blood serum of cattle grazing on these pastures, indicating a "conditioned deficiency," was repeatedly recorded.

Small amounts of cobalt were determined in pasture grass, hay and liver of cattle in numerous cases. In pasture grass and hay from alluvial soils figures of about 300-500γ per kg (dry matter basis) were found to be quite normal. The amount of cobalt in grass and hay originating from diluvial soils may be considerably lower, about half of the amount mentioned above or even lower. Signs of cobalt deficiency are repeatedly reported for those areas. Evidence has been obtained that the affected animals—cattle and goats—will show a favourable response to the oral administration of cobalt or a mixture of cobalt and copper. The liver of normal cattle was shown to contain about 300-400γ of cobalt per kg (dry matter basis). The liver of cattle from affected areas may show less, *e.g.* 120γ.

Selenium was found occasionally in pasture grass, hay, drink-

---

[*] Grass, from sandy soils of the North East diluvium, showed a very low molybdenum content—as a rule less than 1 mg per kg (dry matter basis), the highest values being about 2 mg. Grass from peat soils contained more molybdenum, the maximum found amounted to about 10 mg. This suggests that, in addition to the *p*H value of the soil, other factors play a rôle in the absorption of molybdenum by plants. For clover, values up to 30 mg per kg were determined.

ing water and soil from farms where cattle showed severe symptoms of shedding of hair and acetonemia. In all cases examined no excess selenium was found.

Discussion:—The great variety of clinical symptoms observed, in cases of real and conditional deficiency or in cases of an excess of trace elements, may easily be understood, if we assume a variety of action on different enzyme systems involved in the majority of processes of intermediate metabolism. The veterinarians and breeders are mostly confronted with diseases which are to be considered as a result of a number of partial effects in the normal biochemical processes in the animal body. As a result of these effects certain symptoms, like stunted growth and interference in other vital functions, will generally be observed. As a rule, these effects may not be regarded as inherent to a special deficiency or to an excess of a special trace element.

In many cases of "conditioned deficiency" of trace elements scouring is a prevalent feature. Evidence has been obtained that often increased motility of the intestine may result in a decreased absorption of trace elements and in this way may be responsible for the low content in trace elements as, *e.g.*, the small amounts of copper found in the blood and in the tissues (liver). Increased intestinal motility may originate from other factors affecting the intestine regardless of the trace elements in food, *e.g.*, the intake of proteins and other constituents of rank, young pasture grass in spring and as a result we note worm infestation (gastro-intestinal strongylosis), Johne's disease and other kinds of "enteritis."

In cattle, the copper content of the liver was shown to vary between 5 and 194 mg per kg (dry matter basis). In 62 per cent of the cases observed, the copper content did not exceed 5-15 mg indicating a low saturation of the liver. This may account for the relative stability of the copper content of the blood following a daily administration of copper sulfate by mouth to a heifer for a period of three weeks. In this balance trial about 1.6 g of copper, *i.e.*, about 17 per cent of the copper added to the food, was retained in the tissues. That the liver is of prime importance with regard to the accumulation of copper is shown by experiments in which radioactive copper salts were intravenously administered to calves.

In horses, the copper content of the blood serum is somewhat higher than in cattle. Several intravenous injections of copper glycinate were shown to cause a rather irregular variation of the copper content of the blood serum. The copper content of the liver of normal, adult horses shows considerably less variation than the copper in cattle liver, the limits observed being 13 and 32 mg per kg (dry matter basis).

A relationship between the intake of manganese and the occurrence of grass tetany in cattle in the Netherlands could not be established.

A general survey of the manganese content of 645 samples of pasture grass and hay originating from various soils results in several conclusions. Significant differences were shown to exist between the manganese values of pasture grass originating from sandy, peaty and clayish soils, the average values being 19.1, 15.2, and 11.4 mg per 100 g, dry matter basis. Presumably the *p*H value of the soil is of most importance as really alkaline soils show the lowest manganese content in pastures.

No significant difference was observed between the manganese values in hay originating from sandy and peaty soil, respectively. A significant difference was, on the other hand, shown to exist between these two kinds of hay and hay originating from clay.

The occurrence of sterility in cattle, as a result of manganese deficiency, should not be excluded. This kind of sterility may be expected to occur in clay areas with an alkaline reaction of the soil.

Summary

1) Real and conditioned deficiency of copper in cattle has been observed in the diluvial and alluvial parts of the Netherlands. So far a shortage of cobalt in cattle has been mainly observed in diluvial areas.

2) Pasture grass originating from sandy soils has more manganese than pasture grass from peat soils. Pasture grass from clay has the lowest manganese content. The manganese content of hay from clay is clearly lower than of hay originating from sand and peat. The *p*H value of soils plays an important part in the absorption of manganese by plants, the lower the *p*H, the more manganese is absorbed. Manuring of pastures on clay lands with Thomas phosphate, containing much manganese, results in a considerable rise of the manganese content of the pasture grass. Ster-

ility, due to manganese deficiency in cattle, may well be expected in clay areas with a high *p*H. No relation could be established between the occurrence of grass tetany in cattle and the manganese content of pastures.

3) Trace elements act as bio-catalyzers of enzyme action in fundamental processes of intermediate metabolism. This accounts for the great variety of clinical symptoms in diseases due to a deficiency of trace elements.

4) In many cases scouring is a prevalent symptom. Increased motility of the intestine results in a decreased absorption of trace elements and it may well be essential in originating the low content of trace elements in blood and tissues (liver). An increased intestinal motility may result from different chemical and microbiological factors affecting the intestine.

5) As a rule, cattle liver shows a low saturation with regard to copper and a wide variety in the copper content. In horse livers there is less variation of copper content and the average copper content is higher.

6) In a three weeks balance trial with a heifer, about 17 per cent of the copper sulfate added to the food was retained in the body. Radioactive copper, intravenously injected into calves, was stored in the liver to a considerable extent, *e.g.*, up to 24.5% of the quantity injected over 3¾ hours. Evidence has been obtained that copper, intravenously injected in horses, is, in the same way, rapidly transferred to the tissues.

7) So far no evidence has been obtained that selenium and molybdenum play a part in farm animal diseases in the Netherlands.

I should like to acknowledge the assistance received from my co-workers Dr. R. BIJKERK, Professor E. HAVINGA, Mr. S. T. HOFSTRA, Dr. J. T. L. B. RAMEAU, and Dr. J. DE WAEL. We are indebted to Mr. H. HOOGHOUDT and Mr. J. P. L. KRANENDONK, our technical assistants.

References

1. Sjollema, B., 1933: Biochem. Zeitschr. 267:151.
2. Seekles, L., 1946: Tijdschr. v. Diergeneesk. 71:304.
3. Allcroft, R., 1946: Nature 158:798.
4. Seekles, L., 1946: Tijdschr. v. Diergeneesk. 72:721.
5. Havinga, E. and Bijkerk, R., 1947: Rec. Trav. Chim. 66:184.
6. Blakemore, F., Nicholson, J. A. and Stewart, J., 1937: Vet. Rec. 49:415.
7. Orent, E. R. and McCollum, E. V., 1931: J. Biol. Chem. 92:651.
8. Perla, D. and Sandberg, M., 1939: Proc. Soc. Expt. Biol. Med. 41:522.
9. Kemmerer, R., Elvehjem, C. A. and Hart, E. B., 1931: J. Biol. Chem. 92:623.
10. Anon., 1946: J. Amer. Vet. Med. Ass. 99:288.

Points from Discussion of Paper by Professor Seekles

Dr. Gisiger:—

In countries where the leaves of sugar beets are sprayed with copper products against the leaf-spot disease (*Cercospora beticula*), the effect of the copper in the animal's body gains great interest. This influence becomes more important in years of low rainfall, when the copper is washed away insufficiently.

When too much copper is given, it is retained in the liver, and probably after a certain time, the liver is saturated and the copper goes into the blood and becomes harmful.

In Switzerland, during the last autumn, leaves of heads of sugar beets containing 30-40 mg of copper in 100 g dry matter were used as food. Oxen which received daily about 50 kg of this food during more than one month, did not show any disturbances.

Addendum

Since this paper was read, new data have been obtained in our laboratory that throw further light on the relations between metabolism, in normal and diseased man and animals, and the copper content of the liver. The critical interpretation of the results obtained presented many difficulties and a special method of statistical analysis had to be worked out for us by Dr. H. Freudenthal, Professor of Mathematics in the University of Utrecht. The results have been published in detail by R. Bijkerk in his doctoral thesis (Utrecht University, 1949).

The lowest and highest copper values, as given below, refer to mg of copper per kg of liver, dry matter basis. The data concerning diseased animals are followed by data relating to normal animals.

In normal man (6 cases examined), we found copper values of the liver ranging from 31.6 – 87.0. In 10 normal white rats, the figures ranged from 13.6 – 55.0, in 10 normal guinea pigs, from 59.4 – 183, and in 3 normal dogs, from 58.0 – 156. The range of variation, in all cases, is approximately the same as previously found in horses. It appears that the copper metabolism in man and these animals is better regulated than in cattle. It is interesting to note, in this connection, that the syndrome of real or conditioned copper deficiency, which often occurs in ruminants, has not been recorded in man nor in the animals we have studied.

In a sheep, which had died from the intake of a mixture of copper sulphate and lime, used as a spray for plants, we found a copper content of the liver of 1536 mg, about four times as much as the maximum copper value hitherto determined in our laboratory in the livers of normal sheep.

Significantly lower copper values were found in ten livers of pregnant horses (13.6 – 21.2), but 33 surgical cases (mainly lame horses) showed again higher copper values (14.0 – 28.8). We also examined 77 sick horses, classified, after post mortem examination, into a number of groups. They showed a greater range of variation than normal horses. It was not too easy to classify the diseased animals, and the classification followed may seem rather arbitrary – we had to make a selection based on the relative importance of the disorders found. Occasionally cases had to be included in several groups.

Significantly higher copper values of the liver have been recorded in sick horses; for gastro-intestinal disorders, 14 cases (13.2 – 43.4); for infectious diseases, 21 cases (11.2 – 38.2), and for disorders of the lungs, 11 cases (14.2 – 26.8). The first two ranges of variation are different from what might have been expected as a result of our observations in cattle suffering from gastro-intestinal troubles and infectious diseases. The cause of these differences is not known.

We classified 167 cases of sick cattle, examined in a similar way, as the sick horses. The range of variation is very considerable amongst cattle suffering from infectious diseases and particularly in cattle suffering from tuberculosis.

Animals, which showed morbid processes localized in the liver, were found to have much less copper in the liver: 5.2 – 154, in 59 cases examined. In gastro-intestinal disorders (14 cases): 5.0 – 37.8; in infectious diseases (114 cases): 5.0 – 516, and among these cases a group of 49 animals suffering from tuberculosis: 4.8 – 516.

The lowest copper content of the liver ever determined in our laboratory (3.6) was found in a two year old cow that had been killed because it was suffering from eczema solare. This unusually low content is approximately the same as found in the liver of cattle suffering from "falling disease" in Western Australia as described by H. W. BENNETTS.

AUTHOR INDEX

PLATES

PLATE 1. — Growth of mustard on limed soil (*cf.* page 24). — *a*, first cultivation; *b*, second cultivation; *c*, second cultivation after leaching. — *From left to right:* 0, 4.8, 9.6, 19.2, 28.8, 38.4, 72, 144 gs $CaCO_3$ per pot.

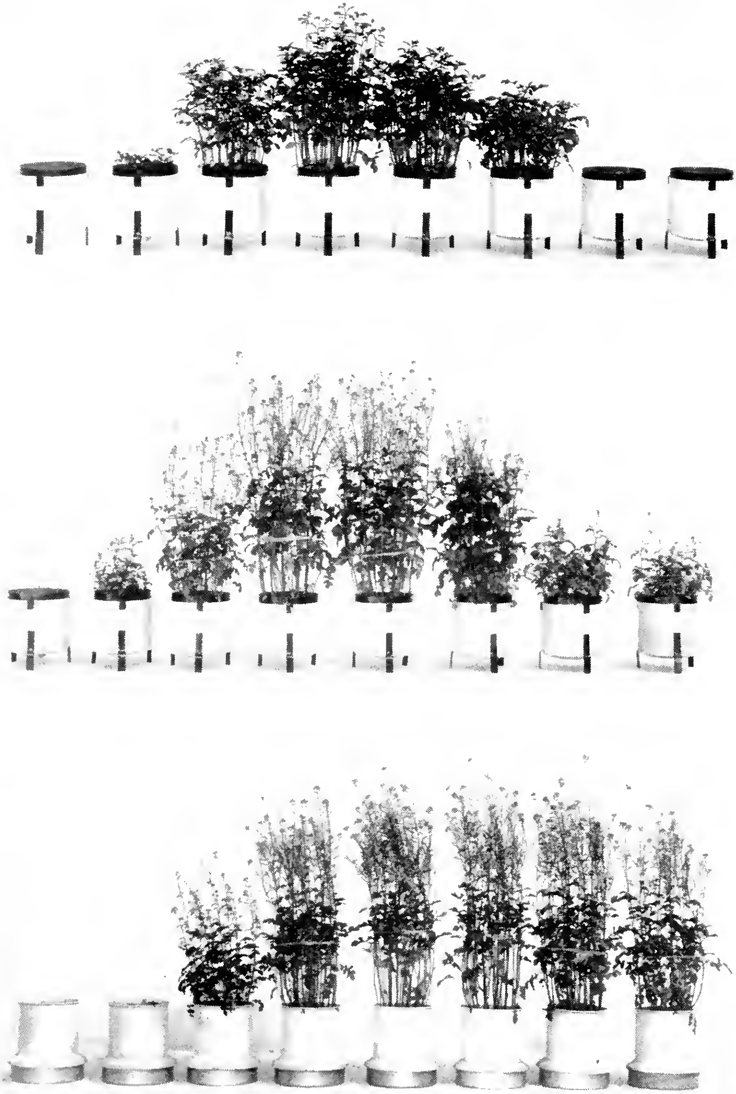


PLATE 1 *a-c*

PLATE 2. – Mustard on a soil rich in lime (*cf.* page 24). – *At left*: unleached; *at right*: leached. – *From left to right*: 1: nothing added, 2: B added, 3: P_2O_5 added.

Plate 2

PLATE 3. – Influence of increasing amounts of quicklime and potassium hydrate on grey speck disease. – The plants in the pots at the very left in each photograph received only PNK. – KOH was added to all pots, except those shown at the left in each photograph, top row: 6 g, middle row: 12 g, bottom row: 18 g. – CaO was added to all pots, except the first two from the left, in each photograph, as follows, 3: 15 g, 4: 30 g, and 5: 45 g.

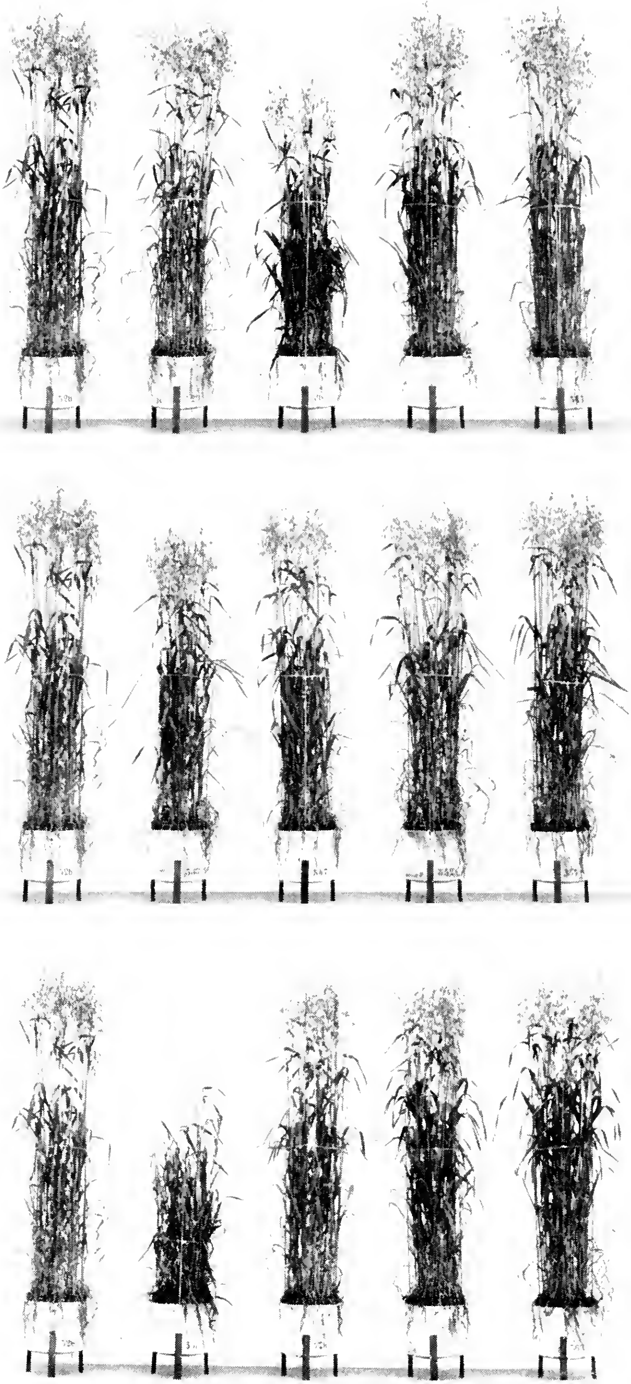


PLATE 3 *a-c*

Plate 4*a*

Plate 4*b*

PLATE 5

Plate 6*a*

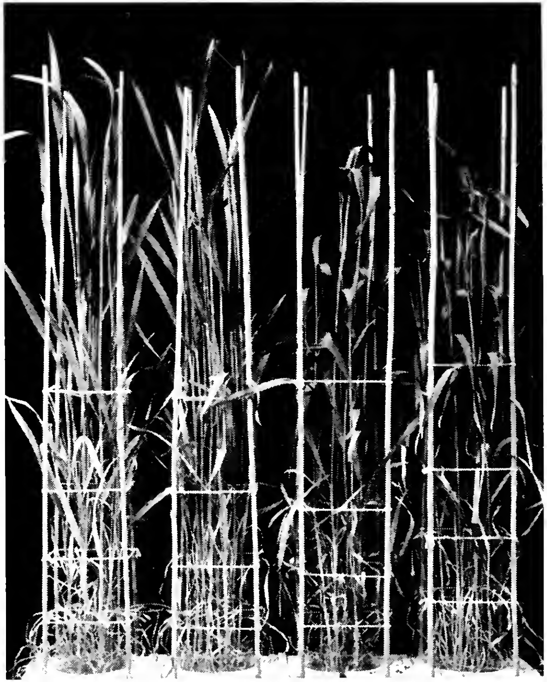


Plate 6*b*

PLATE 7

PLATE 4*a*. – Barley in a nutrient solution. – *From right to left:* 0, 2, 10, and 400 (4 × 100) μg of Cu added.

PLATE 4*b*. – Barley in a "diseased" soil. – *Right and centre:* no copper added. – Left: 6.25 mg of Cu per pot.

PLATE 5. – Oats in a nutrient solution. – *Right:* without; *Left:* with 150 μg of Cu per culture.

PLATE 6*a*. – Oats in a "diseased" soil. – *Right and centre:* no copper added. – *Left:* 6.25 mg of Cu per pot.

PLATE 6*b*. – Wheat in a "slightly diseased" soil. – *Right:* no copper added. – *Left:* 25 mg of Cu per pot.

PLATE 7. – Tomatoes in a nutrient solution. – *Right:* 100 μg of $Na_2MoO_4 \cdot 2H_2O$ per culture. – *Left:* without molybdenum.

PLATE 8*a*. – *Phaseolus vulgaris* (Prinsesseboon), field crop (1947). – *Left*: unlimed plot. – *Right*: 60 kg lime added.

PLATE 8*b*. – *Phaseolus vulgaris* (Prinsesseboon), grown in solution. – *Left*: 25 mg $MnSO_4$/L. – *Right*: 2.5 mg $MnSO_4$/L.

PLATE 9. – *Phaseolus vulgaris* (Prinsesseboon), grown in unlimed soil. – 1: no addition. – 2: + $CaCO_3$. – 3: + $CaCO_3$ + $MnSO_4$.

PLATE 10*a*. – *Vicia sativa* (Voederwikke), field crop (1947). – *Left*: unlimed plot. – *Right*: 60 kg lime added.

PLATE 10*b*. – *Vicia sativa* (Voederwikke) grown in unlimed plot.

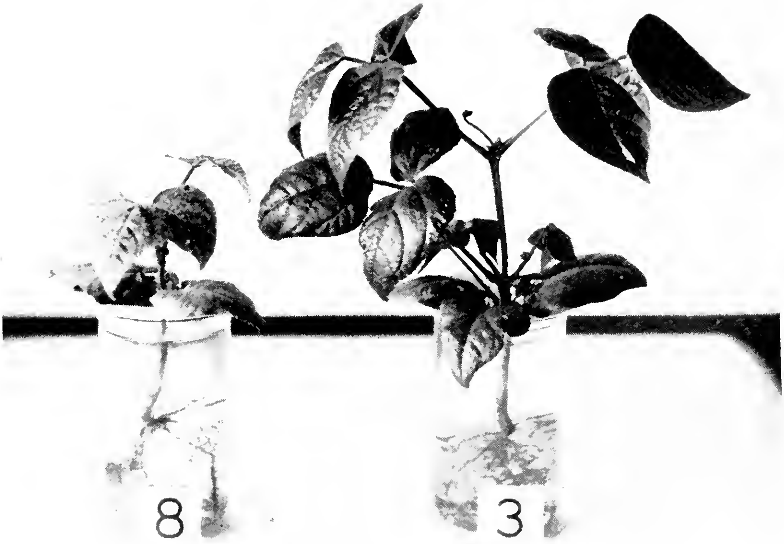


Plate 8*a*

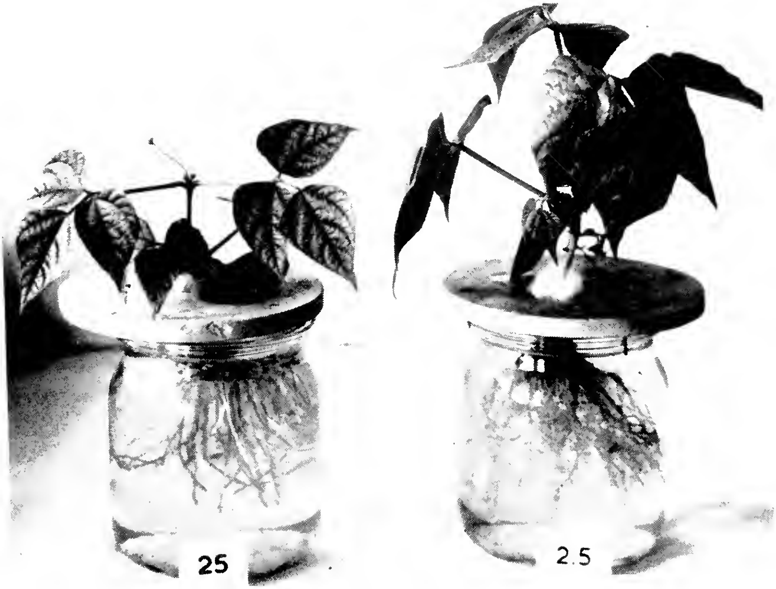


Plate 8*b*

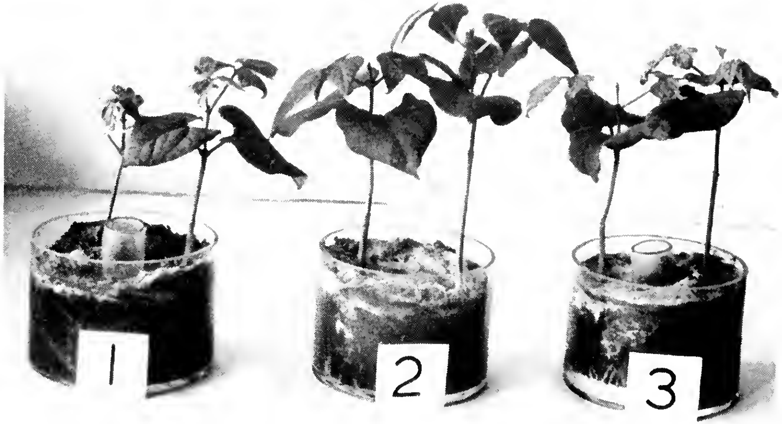


PLATE 9

Plate 10*a*

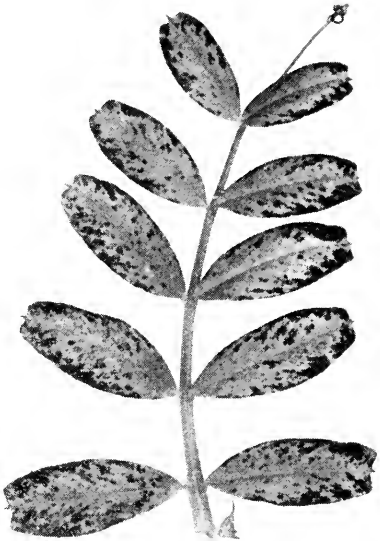


Plate 10*b*


I U
BB SS


HERBARUM INCREMENTA
MINORA ELEMENTA