DIAGNOSTIC APPROACHES TO PROBLEMS IN CROP NUTRITION

By W.R.N. NATHANAEL
Chemist, Coconut Research Institute

Historical Résumé

The sources from which plants derive their substances has been the subject of much speculation since the earliest of time and attracted students of nature long before the era of experimentation began. Like many other branches of science, perhaps nutrition too, may be traced back to *Aristotle* (B.C. 384). He was of opinion that the food of both plants and animals was composed of various combinations of what were then thought to be the four primary elements; earth, air, fire and water. Some of these speculations survived for some hundreds of years and had a profound effect on natural philosophy in its early days.

Influenced by such ideas, the earlier investigators on the growth and nutrition of plants were actually on the search for some single factor—a 'principle' or a 'spirit' of vegetation which was directly responsible for plant growth. For instance, the view (attributed to Thales—B.C. 600) that plants derived all their food and their whole substance from water alone, persisted to the early sixteen hundreds and culminated in the very beautiful and often quoted classic experiment done by *Johan Baptista Van Helmont*, a Flemish alchemist of Brussels, in 1620. In his attempt to explain plant nutrition, he planted a willow cutting weighing 5 pounds, in 200 pounds of oven dried soil contained in a pot. The surface of the soil was protected against contamination and the willow was allowed to grow for five years, nothing but rain water being supplied during that period. The plant was then removed out of the soil and it was found to weigh 169 pounds and 3 ounces. Van Helmont also recorded the dry weight of the soil remaining in the pot and found it to be 2 ounces less than its original weight of 200 pounds. On the assumption that this was an experimental loss he arrived at the irresistible conclusion that the growth made by the willow arose from water alone. Though the experiment itself was quite good Van Helmont's thesis was fallacious because he unfortunately overlooked the part played by the air (in supplying carbon dioxide) and also the mineral matter (represented by those missing two ounces) in the growth of the plant. In any case, this work of Van Helmont had no fruitful consequences and may be said to have been wholly without effect on agriculture.

About this period, a further belief persisted which however was not as old as the 'water theory' of nutrition. The adherents of this view maintained that plants fed upon decaying animal or vegetable matter in the soil, on the assumption that like all other living organisms plants also could feed *only* upon materials of like nature with themselves and not on materials of unlike nature.

Scientific proof of the incorrectness of the conclusion of the Brussels experiment and the other erroneous theories of the time however became available only at the close of the 17th century when *Woodward* in 1699 made the first water culture experiment on record. He observed that the spearmint (*mentha spicata*) grew better in impure water than in rain water and also that the growth performance of the plant was at its optimum when some garden soil was shaken up with the water. The nutrition of the plant was thus recognised for the first time to be contingent on something additional to water (now known as the *mineral nutrients*) which apparently came from the soil.

Though continued progress was no doubt made in this field until the close of the 18th century, yet even at this time plant nutrition still remained something of a mysterious and obscure phenomenon. Empiricism became so entwined with speculation that the results of scientific work were completely submerged in a maze of inaccuracies with no practical benefit to the agriculturist.

The advent of modern agricultural chemistry into the picture may be said to really date from the beginning of the 19th century when *Theodore de Saussure* of Switzerland evinced interest in problems of plant nutrition. On the basis of sound quantitative experimental evidence he formed the first clear concept as to the specific contributions of air, water and soil to the growth and nutrition of plants. He discarded the prevailing 'humus theory' (which before his time *Albrecht Thaer* of Germany had valiantly upheld), and he clearly showed that the ash of plants was derived from the soil and that the atmosphere played a vital role in plant growth.

The real turning point in the history of agricultural science came in the year 1840, when the lectures of *Justus von Liebig* before the British Association for the Advancement of Science appeared in his epochal publication *'Organic Chemistry in its Application to Agriculture and Physiology'*. When this was released, the principles of plant nutrition became finally clarified in the public mind. From the scientific point of view Liebig's work may be described as a brilliant piece of synthesis and simplification. For all time he effectively disposed of the age old mystery that plants could be nourished only by substances of like nature, and it its place he propounded his simple but brilliant hypothesis that *plants feed upon simple mineral and gaseous substances which they build into complex products*. The whole process thus became susceptible of investigation and in short, manuring appeared as a simple application of chemistry.

One of the immediate results of the masterful presentation of Liebig's work was the introduction by the Frenchman, *Jean Baptiste Boussingault*, of the method of exact field experiments which may be described as one of the first proper diagnostic techniques to be used in the elucidation of some general problems of plant nutrition. Ever afterwards, primary consideration has been given to analyses of the ash of plants in relation to the mineral content of the soils on which they are grown. The simple generalization which Liebig extracted from an apparently hopeless tangle of complexity contained the germ of highly interesting scientific problems which have indeed proved a key to further progress. By a process of progressive modification and expansion the experimental genius of later workers has built up our present advanced knowledge in this field, with its diversity of approaches to the nutritional diagnosis of crop plants. Until the development of genetics in the present century, when the attention of biologists was turned to the potentials of modifying plants by breeding, the improvement of the soil by fertilizers and soil amendments continued to be the centre of research in the field of agricultural investigation. Doubtless, our present fund of knowledge in this field constitutes one of the most important scientific foundations of modern crop production—a subject always shrouded in a reticulum of intricate problems.

Survey of Crop Nutrition Problems

Plant nutrition is not of itself a science, but its study rests on the application of other sciences to a vastly complex system. Because man is an obvious parasite on plants the subject itself is of superlative importance and one of broad general interest, even though the actual problems in this field are the direct concern of the practical farmer and the agricultural scientist. In order to give a general perspective of this field of inquiry, it would be appropriate to make a preliminary survey of some of the difficult problems which confront the plant diagnostician in relation to the stern realities of crop production.

The plant, which comprises a dynamic system also grows in a dynamic soil system and is further subject to ecological and climatic influences of its environment. Thus, the multiphase interlocking system which the crop nutritionist has to explore is that of the plant—soil—atmosphere with its innumerable interrelations and interactions. Inherent in the growing plant are all the complexities common to living organisms and added to these are the puzzling phenomena of the soil medium, and a constellation of factors associated with the atmospheric environment. The plant, the soil and the climate are so completely interdependent that it would even seem injudicious to think of one apart from the others. As the growing of crops in the field is actually the resultant of a multiplicity of factors affecting three phases, it is obviously beyond human ability to put all these factors together and predict the results with any degree of accuracy or certainty. Though empirical methods have given some advances in the past, they have now been found to be too slow and inconclusive. Exact knowledge of the factors encountered in the system plant—soil—atmosphere in all its bearings, has proved to be the only sure basis for the formulation of reliable diagnostic procedures and the attainment of positive results in crop production.

THE PLANT:—It is now generally accepted that an organism is the product of its genetic constitution and its environment. A plant passes through a number of growth stages during its development from zygote (fertilised egg) to zygote, and the broad view now taken is that (provided hereditary factors are constant), the conditions required for the satisfactory completion of its vegetative and reproductive cycles of growth must be sought (besides other things), from facts of both plant physiology and soil science.

The raw materials needed for plant growth consist of carbon dioxide, water and the so-called mineral nutrients and it is now established that the vital physiological processes involved in the development of the plant are absorption, photosynthesis, respiration, transpiration, formation of protoplasm and storage. Intimately linked up with the physiology of the plant are also continuous processes of building up (*anabolism*) of complex compounds of carbon and nitrogen and breaking down (*catabolism*) of these into simpler products in which water and oxygen are intimately concerned. These processes together comprise *plant metabolism*. In the course of these metabolic processes innumerable substances such as sugars, starch, cellulose, proteins, lignin, tannins, amino acids and amides are formed and as a result of these activities plants develop special organs of growth and reproduction, each of which has its special characters and makes particular demands on the nutrient supplies of the plant.

For the normal functioning of the above physiological processes water is required in very large quantities and is really the basis for the innumerable chemical reactions which support plant life. From the tip of the roots which reach into the soil to the extremities of the most remote leaf a plant is one continuous water pipe and this water system is the medium through which the nutrients pass to the various plant compartments to be built up into such foods as sugars and proteins which in turn are transformed into cellulose and other compounds that make up the solid structures of the plant. The air supplies plants with gaseous oxygen as such, and with carbon from gaseous carbon dioxide. The soil serves as the place in which plants anchor their roots and in addition supplies the mineral nutrients in varying amounts.

Though, nearly 60 elements have been detected in plants at the present time, yet not all of them have been found to be essential for their normal growth. Some of these are present in extremely small traces measurable in parts per billion while some of the radioactive elements are present in still smaller concentrations. Plants have the faculty of assimilating large amounts of certain elements out of proportion to their abundance in the soil with the result that some species may show unusual concentrations of a particular element. As much as 0.01 per cent of silver for example has been found in some mushrooms while the scouring rush (*equisetum palustre*) from the Danube Basin is reported to be a 'gold digger'. Similarly, selenium in concentrations toxic to feeding animals have been reported in some plants in America. It is generally agreed at the present time that the 15 elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium calcium, magnesium, sulphur, iron, manganese, copper, molybdenum, zinc and boron are universally indispensable to the normal growth of plants. It is only in comparatively recent times and largely as the result of work carried out in the last 35 years that the essential nature of the last five elements mentioned (which along with iron are required by plants in trace quantities) has been fully established. Though this list is not regarded as a final one, it can be stated with confidence that any others to be added to the category of essential elements will be required only in minuscule traces. In any case, very highly refined experimental techniques coupled with improved methods of purification and analysis would have to be applied before the absolute essentiality of any new elements could be demonstrated. It would be appropriate however, to mention that the group of elements: sodium, chlorine, silicon, aluminium and nickel though not proved essential have been found to have beneficial effects on *certain* crops by indirectly helping in growth processes.

There are many problems concerned with the supply of essential mineral nutrients to crops and this phase of the subject has been discussed in some detail by the writer in an earlier publication in this journal[1]. In the present context however, it should be adequate to mention that the main problems of mineral status concern deficiencies and excesses of the mineral nutrients and their interrelationships, often referred to as nutrient balance or interactions. Deficiencies may exist either as single or multiple deficiencies and when elements are present at very low levels they may act as limiting factors to growth. To produce healthy growth it is essential that the nutrient elements should be present in the various organs of the plants not only in sufficient quantity but also in fairly well defined proportions. The nutrition of the plant like everything else in nature is very complex (as adumbrated above) and is affected by many factors besides the actual nutrients themselves and a complete discussion of the subject would really involve so many other sciences. In the sphere of practical agriculture however, it should be remembered that the supply of mineral nutrients to crops concern both yields and quality which are not necessarily attained by the same treatment. These relationships can only be learned by experience and the best results are usually obtained by an understanding of the special effects of the individual elements and their interactions on growth of the plant under study.

THE SOIL.—The soil is a natural body, the product of geological and weathering agencies and is by no means a simple medium providing anchorage for the plant and serving as the source of its mineral requirements. It is indeed a very complex entity consisting of inorganic mineral constituents along with humified and unhumified organic matter of the utmost chemical and physical diversity. The mineral phase of the soil results from the disintegration and decomposition of rock whilst the organic or energy phase is produced from the residues of plants that have grown there in the past. These phases exist in many states of sub-division, including the relatively inert particles of sand which largely form the physical framework of the soil, and the more reactive clay and organic constituents which show colloidal behaviour and important ion exchange properties. A further point to remember is that a study of the chemical and physical properties of a soil is never complete without considering the teeming population of soil micro-organisms which play fundamental roles in the transformation of both organic and inorganic constituents of the soil. These organisms also compete with the plant roots for available nutrients. Actually as a medium for plant growth, the conditions existing in the soil may be described as a complex dynamic biochemical equilibrium which are profoundly affected by changes in climate. It will thus be understood that the soil is not a dead or static system but is really a nourishing medium consisting of the five main components: mineral matter, organic matter, soil water, soil atmosphere and a population of micro-organisms.

As regards the intake of mineral nutrients by plants from the external medium, it should be mentioned that this too is a complex phenomenon dependent upon a variety of factors of which soil moisture, soil reaction (pH), concentration and physiological balance of mineral nutrients, age and development of plants, extent of roots and intensity of meteorological factors are the main. The organic matter of the soil however, is important only for secondary reasons and is not directly essential from the point of view of the nutrition of the plant.

The absorptive system of a plant can be very extensive in its ramifications throughout the soil. When it is considered for example that the total surface area of the root system of a well developed rye plant is about 7,000 square feet with a total length of 350 miles, the difficulties confronting the agricultural chemist will be appreciated, who on the basis of chemical studies on the uptake of nutrients from the soil, has to formulate cropping programmes and manurial treatments. Added to this of course is the fact that the regulation of availability of nutrients in the soil is also profoundly affected by soil temperature, aeration, drainage and other management and cultural practices. In short the problems affecting the soil phase may be said to be even somewhat more intricate than those affecting the plant itself.

THE CLIMATIC ENVIRONMENT.—The plant environment is made up of both edaphic (soil conditions) and climatic factors and we have already considered the former. Many investigators have suggested that factors of climatic environment might be at least as important as genetic and soil factors in affecting the nutrition and composition of plants. The evidence for this view is based on comprehensive studies undertaken by them in recent times with the specific objective of determining the interrelationship between soil, climatic environment and plant growth. It is now generally agreed, that within limits, the performance of a plant is affected by a complex interrelationship between many chemical, physical and biological factors associated with the general environment, which includes both soil and the atmosphere. It will thus be seen that the study of plants growing under a natural or agricultural environment is dependent on difficult and capricious natural phenomena.

Since it is essential to consider nutritional problems of plants in relation to *all* the factors affecting their performance, the plant scientist naturally has to contend with all the difficult problems associated with the uncontrolled aerial surroundings in which plants grow. For example, plants of the same genetic origin may have nutrient contents that differ with the season and with the location where they are grown, and in fact the important phenomenon of biological variability has now been recognised as a function of both heredity and environment.

Though a detailed discussion would be superfluous, yet brief mention should be made, in turn, of the principal factors of the atmospheric environment of plants, viz. air, light, temperature, humidity and rainfall which are known to have a significant bearing on plant growth. These factors are usually uncontrolled or uncontrollable under natural conditions. Since carbon dioxide is one of the raw materials needed for plant growth, the importance of air which provides unlimited supplies of this and other gases, should be self evident. The carbon dioxide is generally taken in by the plant through the stomata of its leaves. The importance of light for the basic photochemical reactions (such as photosynthesis and chlorophyll synthesis) which take place within the plant is well known. Apart from its intensity, the actual duration of the daily period of illumination (photoperiod) also affects plant growth. There are for example, plants which require 'long day' conditions and others which need only 'short day' conditions. When these requirements however are not forthcoming to the respective classes their growth cycles become abnormal and the plants may fail entirely to produce flowers, fruits and seeds. Light however, is not known to play any direct role which is indispensable for the absorption, movement or metabolism of the mineral nutrients. Another essential requirement of plant growth is an optimum temperature, which may vary according as to whether the plant is young or old. It has been shown[2] that though it is difficult to separate the effects of low temperature on the absorption processes, from its effects on translocation and utilization of the nutrients within the plant, yet temperature actually affects the absorption of solutes from the soil. Indirectly therefore the rate of growth of the plant would be affected, as this is so dependent on the rate of nutrient uptake from the soil. The humidity of the atmosphere as distinct from the water supply in the soil is a vital factor in the determination of the water conditions within the plant. Further, air humidity also largely influences water loss from the leaves by transpiration. The importance of rainfall for plant growth is only too well known to warrant any emphasis. It plays a kep role in the maintenance of soil moisture which determines the intake of water by the roots of the plant.

The problems associated with atmospheric and climatic environment are indeed very complicated because they do not act independently and their effects are modified by one another. All the same, control of environment has invariably been found to be essential in order to obtain significant results from experimentation with plants. Control has been found necessary not only while each experiment is being carried out but also during the raising of experimental plants. No matter how uniform plants are genotypically (genetically) the possibility of achieving phenotypic (characters due to environmental stimulus) uniformity has been found practical only when they are grown under strictly controlled conditions. Modern techniques have now been developed for the construction of greenhouses to fulfil the basic requirements of a reproducible environment in the growth of mature plants. In spite of such refinements in technique, the control of the aerial surroundings still presents great difficulties and consequently the study of the effect of climate alone on plants is much less developed than that of the other limiting factors involved in plant nutrition studies.

Diagnostic Methods

Though the right perspective has been attained towards the numerous problems of scientific interest and technical importance in the field of plant nutrition, yet many of the difficult phenomena are still inexplicable on present knowledge. There is no doubt sufficient understanding of what plants do and how the system operates but there does not exist the possibility of setting down in the precise and elegant terms of the physical scientist the course of events in a growing plant under study. The reason for this should no doubt be abundantly clear from the above survey where the multiplicity of factors which impinge on the central problem have been discussed in some detail.

The time is now past for the agricultural scientist to be satisfied with rule of thumb methods and empirical tests for the critical evaluation of practical and economic problems involved in crop production. Since empirical methods of approach in the past have often been found to be very inefficient, expensive and frequently ineffective a variety of diagnostic methods, embracing different lines of approach have been evolved over the years, and are in vogue for the evaluation of problems affecting faulty nutrition of plants. Plants usually slow down in their normal rate of development and show other signs of trouble whenever any one of the many factors that contribute to their well being get out of balance. Naturally therefore the attack on such problems has to assume wide dissimilarity of method, depending on the specific problem or problems that require diagnosis. Whilst the appropriate tool to employ at one time may be a spade or a soil auger, yet at another time some highly specialised and refined implement of chemistry or physics may become necessary. Similarly, whilst crude estimations and measurements may suffice for one purpose, meticulous accuracy and precision may be a *sine qua non* for others. Diagnostic techniques have now been extended to cover both aspects and the methods adopted at the present time to provide guidance on crop nutrition problems may be categorised into three groups as follows:—

(1) Field experiments with fertilizers and manures in which yield responses resulting from their application to soils are measured.

(2) Soil analysis (relating yields to soil data) using chemical and biological methods, to assess the nutrient or fertility status of the soil. Pedological studies coupled with physical examination of the soil are also generally employed as useful auxiliaries to this technique.

(3) Plant diagnostic methods to determine the nutrient status of the crop plant.

Depending on the nature of individual problems most workers in the field of plant nutrition use methods in all three groups. This practice has been found particularly necessary because diagnostic methods should as far as possible take account of, and provide information regarding all the factors, the interplay of which are likely to influence yields and quality of the crop under investigation. At this stage it might be appropriate to discuss briefly each of the above diagnostic methods in turn.

METHOD OF FIELD TRIALS.—Ever since Liebig enunciated his mineral theory of crop nutrition, the method of field experimentation with chemicals has been applied with increasing importance to various crops for the purpose of evaluating optimum fertilizer dosages. These experiments have been conducted mainly in two ways. The *first* is the classical method of the Rothamsted Experiments, known all over the world, first laid down in 1843 by Bennet Lawes and Henry Gilbert on Broadbalk field in Hertfordshire, England, on wheat. In this method of experimentation the crop is grown on a particular piece of land which is divided into plots each of which is given a different fertilizer treatment. Whilst the control plots receive the complete range of fertilizers, the others receive a similar range with each of the essential nutrients omitted in turn.

To make the effects cumulative and to produce extreme conditions the treatment assigned to each particular plot is continued throughout each experiment. In the *second* method, an area of crop failure is divided into plots each of which is then treated with different fertilizers. Observations are then made on the treatments which produce beneficial responses. Though both methods are reckoned to be effective in giving useful information, they have had only a somewhat empirical status in the past.

At the present time, due principally to recent advances in statistical method, the technique of field experimentation has been revolutionized so that this branch of research is now a somewhat exact science. Improvements have been effected simultaneously both in the field technique itself and the final evaluation of the experimental results. Accordingly modern field experiments are generally designed with suitable precautions to offset to *some extent* the effects of the various environmental agencies at work, in order to arrive at an accurate appreciation of the relative merits of the various characters or treatments which have to be compared. This has been found essential because soil fertility is such a variable factor changing not only with depth but also from acre to acre and foot to foot in any one field or plot. Consequently the problem of producing identical soil conditions for the various treatments would appear an almost insuperable difficulty. Similarly with season, it is not impossible that the conclusions of one season's work are practically reversed in the next. The problem that the field experimentalist has to contend with being necessarily complex (due to the interaction of factors resulting from heterogeneity of soil and season), practical details such as plot size, plant population, cultivation, harvesting and units of measurement are all carefully considered in the design and execution of the present day field experiments. Since the chances of obtaining a true evaluation of results are greater when the experimental site is uniform, great care is usually exercised in the choice of land. Further, it is ensured that the land selected would conform to the general soil and environmental conditions under which the crop would be grown commercially. High standards of accuracy in field practice are maintained so as to give each plot as nearly as possible identical environmental conditions. The accuracy of any results obtained are further enhanced by using the system of replicated treatments. It should be mentioned in this connection that although with modern plot arrangements the effects of soil heterogeneity can certainly be reduced in the analysis of the data, yet they cannot by any means be eliminated.

The field experiment is one of the most direct methods for the determination of the optimal dressings of manures and fertilizers that should be applied in relation to yields and quality of crops. A further practical merit of the technique is that wherever favourable results are obtained the cause and remedy are simultaneously indicated. Modern statistical designs for field experiments are also very useful in studies on nutrient interactions, which are of importance particularly where deficiencies are induced as a result of nutrient imbalance. Since field experiments represent the final test nearest to agricultural practice, it is always to this method that one turns in order to judge the validity of the results of other techniques. As a rule however, the field experiment is an expensive form of research because it involves heavy work, and further the high standard of accuracy required coupled with the voluminous experimental results that have to be maintained, can only be guaranteed where skilled supervision and labour are available.

METHOD OF SOIL ANALYSIS.—The principle of using soil analysis in relation to problems of plant nutrition was recognised no sooner it was established that plants obtained their mineral nutrients from the soil. The immediate object of soil analysis is of course to determine quantitatively the potential supplies in the soil, of the various elements necessary for the nutrition of plants, with a view to making good any deficiencies. A further object could be to obtain an accurate characterization of the soil for purposes of soil survey, classification or general pedological studies (soil science). Chemists however, soon realised that the problem was not so simple as it appeared, because they found that in some cases plants could show deficiency symptoms even when grown on soils which on chemical examination were found to contain adequate quantities of the requisite nutrients. This problem naturally raised the question of availability of the various elements in different soils, to crop plants. Both chemical and biological methods are now in use for the determination of 'availability' and perhaps these could be considered in turn.

Chemical Methods.—When it is realised that the soil is such a complex medium, some of the difficulties associated with the chemical determination of availability of nutrients can be appreciated. The chemist attempts to correlate known crop responses with the composition of soil extracts prepared by using relatively simple reagent solutions. In this context, C.S. Piper 3 states that 'It is improbable that any simple form of chemical analysis can simulate the conditions existing in the soil throughout the period of active growth of a plant and so give a reliable measure of availability under all conditions'. In spite of the empirical nature and other shortcomings of these chemical methods yet it can be said that the diagnostic value of soil analysis is considerable. It certainly provides one of the useful tools for ascertaining the most profitable returns from fertilizers, in addition to its diagnostic value in ascertaining causes of crop failure.

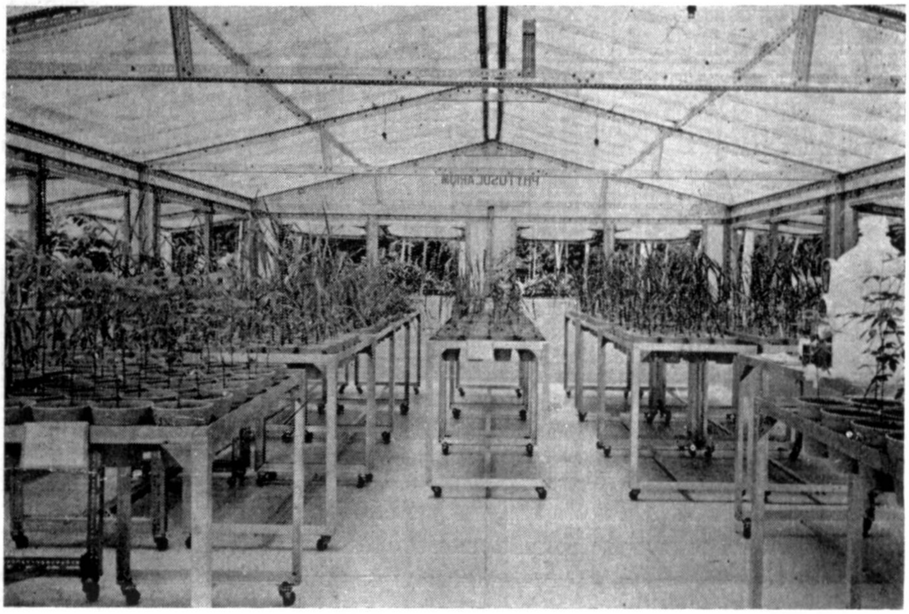
Since all soils show considerable variation, the first step in soil analysis is to obtain a sample of soil representative of the land, field or plot which is under study. Once this is done the physical determinations or chemical analysis (whether for purposes of soil classification or the measurement of some soil factor) are next carried out. The examination for chemical composition would usually include estimations of organic carbon and nitrogen along with 'total' and 'available' concentrations of the mineral constituents on which information is sought. In interpreting analytical results however, both field and laboratory errors would have to be taken into account.

Chemical analysis of the soil is certainly very useful for giving indications of deficiencies of mineral elements and for providing information on soil reaction (pH) and organic matter content. The analytical data however usually require expert interpretation, because there are many factors which must be taken into account when considering the general question of availability. The absolute concentration of the nutrient in the soil solution is not by any means the sole criterion involved.

Biological Methods.—Since there is always an element of uncertainty regarding the conclusions to be drawn from the chemical analysis of the soil, certain other methods have also been developed for the assessment of the fertilizer requirements of a soil. Whereas chemical reagents are used for the determination of available plant nutrients in the method just described, plants are employed as extracting agents in biological methods of soil analysis, to achieve the same objectives. The plant which explores a very large volume of soil with its extensive root system can absorb nutrients not only from the soil solution but also directly from the colloidal complex, by direction exchange. Since this process of absorption covers the entire life cycle of the plant, the material absorbed really represents an integration of the conditions existing throughout that period of time. The analysis of the plant as a measure of availability actually rests on this principle.

The biological methods that are generally employed at the present time could be divided into two groups: *first*, those in which higher plants are used as the nutrient extracting agent; and *second* those in which micro-organisms such as fungi and bacteria are used for determining the availability of certain of the mineral nutrients in soil. The two principal experimental methods now in use, where higher plants are used, are the 'soil pot culture' techniques originally proposed by Mitscherlich of Königsberg and by Neubauer of Tharandt. In both methods the conclusions are drawn from plants grown in samples of soil under regulated conditions in pots, and not from plants growing in the field. Though Mitscherlich used oat plants and Neubauer used rye plants, these methods have now been modified and improved in the choice of the test plants, and have also been extended to nutrient elements other than those originally estimated by these techniques. Plate I shows 'soil pot culture' experiments in progress at the Coconut Research Institute.

PLATE I



'Soil Pot Culture' experiments using indicator plants

The Mitscherlich method is essentially a fertilizer test in pots in which the soil requirements for the various nutrient elements are determined simultaneously on the basis of the dry matter produced in the various treatments. The Neubauer method however, proceeds upon a different principle and attempts to estimate the available nutrients in the soil by determining the amounts of these constituents taken up by young seedling plants during a specified growth period. Extensive comparisons have been made in Germany between these biological methods and the results of field experiments and the correlations have been found fairly satisfactory.

An entirely different approach to the problem of determining the plant nutrient status of soils is to employ bacteria and fungi as test organisms. This method includes the *Azotobacter* soil-plaque technique originally suggested by Winogradsky, and the *Aspergillus niger* method developed by *Benecke* and *Söding*. In effect this biological method is essentially a laboratory variant of the 'soil pot culture' technique. The difference is that the yield of mycelial growth is measured instead of the yield of a standard crop plant. The method itself is somewhat tedious and would require standardization against results of field experiments.

Brief mention should be made of certain *quick soil tests* which have been developed in recent times on the basis of colour reactions. These methods are finding increasing application particularly in the field. They are no doubt very valuable for obtaining quick semi-quantitative indications regarding the various mineral nutrients in the soil, on the experimental site itself.

The method of soil analysis (whether chemical or biological) has the particular advantage, that it can provide valuable forward indications regarding any possible deficiencies in the soil, so that preplanting correctional treatments could be used, if necessary. The method however is expensive, and requires apart from trained personnel, extensive laboratory facilities and scientific equipment.

PLANT DIAGNOSTIC METHODS.—Though undoubtedly there is a close and interlocking linkage between the plant and the soil, yet for diagnostic studies the nutrient status of the plant can be considered independently of, and as distinct from the nutrient status of the soil. Two fundamentally different approaches are therefore possible, one in relation to the plant—a research field for the Crop Physiologist, and Plant Chemist and the other in relation to the soil—a research field for the Soil Chemist (Physicist, Microbiologist) and the Agronomist. We are concerned here with the principal diagnostic methods based on the use of plants, which are now in vogue. These methods could be categorised conveniently into the following groups.—

(1) The Visual Method of Diagnosis (Symptomology), including the use of special indicator plants.

(2) Plant injection and spraying methods.

(3) Sand and water culture methods.

(4) Chemical methods of diagnostic plant analysis, including foliar diagnosis and leaf analysis.

The Visual Method.—The basis of the method is that plants suffering from deficiencies and excesses of mineral nutrients usually develop well defined and characteristic signs of these disorders. Though these visual symptoms of malnutrition appear particularly in the leaves, yet in some instances they also become evident in various other organs of the plant. The method can only be used when signs of disorders are present in the plants, and care and experience are required in the recognition of symptoms. There are some to whom the language of plants is a relatively open book—they have learned the meaning of the discoloured, gnarled or curling leaf and symptoms such as the outer markings or the inner imperfections of the sickly fruit. The speech of plants as revealed in visual symptoms tells a story of crop injury due to mineral nutrient deficiencies, and the ability to recognize these particular effects forms the basis, of the visual method of 'symptomology' for the diagnosis of faulty mineral nutrition in plants. Comprehensive work has been done on this technique by Wallace[4] and reference could be made to this publication for further information on the subject.

The visual method has much in its favour, and its usefulness can be extended by the use of special indicator plants and indicator plots, whereby symptomology could be combined with fertilizer treatments. Though the technique is essentially qualitative, it is extremely rapid and can be applied to detect both deficiency and toxicity effects of all the essential elements. The method is of particular value as a preliminary or supplement to more tedious and exacting diagnostic procedures. It is particularly advantageous in rapid survey work as it requires no expensive or elaborate equipment.

Injection and Spraying Methods.—It is a remarkable fact that plants can absorb mineral nutrients (either in solution or in the form of solid salts) when they are injected into the vascular tissues of the plant or are painted on (or sprayed) on the foliage. This forms the basis for the recently developed spray/injection technique of diagnosis. Like the visual method this too is essentially a qualitative method, but unlike that method it is applicable only to deficiency problems. Precautions regarding dosage and timing of operations are essential in order to avoid damage and to ensure effective action. Whether the treatment should be given in the form of spray or injection is purely a matter of choice and convenience depending on the crop under study.

Spraying methods afford ease of manipulation and Wallace (loc.cit.) suggests concentrations between 0.1 and 1.0 per cent as suitable for compounds of micro-nutrients, and between 1 and 4 per cent for those of the major nutrients. He recommends that in order to facilitate response to treatment, sprays should be applied at an early stage of growth to young leaves only.

Roach,[5] at East Malling has made a thorough study of the subject of liquid injections into leaves, shoot tips, petioles and stems. Unlike spraying, the injection method requires manipulative dexterity and considerable experience in the interpretation of results.

The spray/injection technique can be applied to trees and all varieties of crop plants and usually gives spectacular results within a period of one to two weeks. The method is particularly useful in making preliminary diagnoses, and in confirmatory roles to the visual method. It has also been found valuable in making tests with micro-nutrient elements. Where fixation processes in the soil are pronounced for certain elements, it has been found that the most efficient method of providing nutrients to the plant is by foliage sprays or tissue injections. This is a further point in support of the usefulness of this diagnostic method.

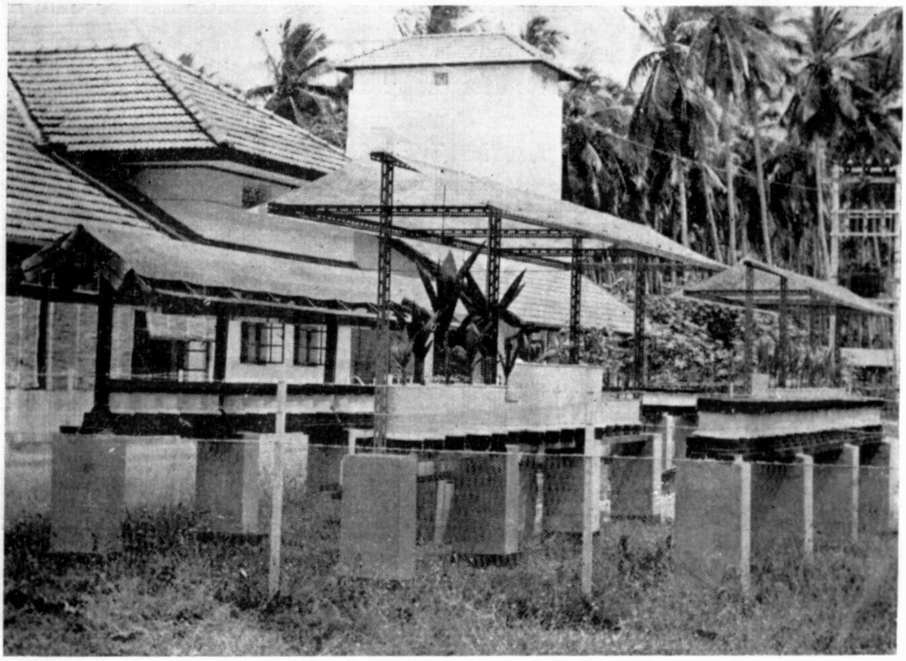
Sand and Water Culture Methods.—It has been deemed appropriate to include this important technique with the plant methods, because its application, whilst being associated directly with the plant, is always dissociated from the complex medium which we term soil.

Though the methods of sand and water culture actually originated in pure scientific inquiry more than a century ago, yet plant scientists have used, and are continuing to use them, to great advantage in the pursuit of fundamental knowledge in the field of plant nutrition. The methods have been found invaluable not only in assessing the actual mineral nutritional requirements of plants but also in the evaluation of the role of these essential nutrients in the physiology of the plant. Further, the methods provide a practical means of culture that avoids the sources of complication, error and infection that a soil may and often does provide in the different experiments and diagnostic procedures already discussed.

The discovery of the significance of the micronutrients gave a distinct fillip to the importance of these methods, and it can be said that physiological studies on the micro-nutrition of plants would be a virtual impossibility in the absence of sand and water culture methods. For a proper study, it is essential for a plant diagnostician to understand the fundamental reactions of his crop plant to simple combinations of factors. Sand and water culture methods alone provide wide scope for this form of experimentation. For example, whilst it is impossible to ensure absolute deficiencies of any elements in any field experiments, it is feasible by using sand and water culture techniques to induce visual symptoms of such deficiencies artificially and also determine the chemical composition of a crop plant characteristic of each particular deficiency so induced. It will thus be seen that this method besides providing the answers to such fundamental questions as what, when, how, why and how much of certain mineral elements are necessary for the growth of a particular crop, it is also a handy accessory to some of the other diagnostic methods (such as the visual and chemical methods) that are applied in this field.

Plates II and III show sand culture experiments on coconut seedlings in progress at the Coconut Research Institute. In Plate III, the contrast in performance between seedlings grown under conditions of absolute deficiency and optimum nutrition should be noted. Sand culture experiments in pots such as these make it possible to keep under check those variable factors (such as water and soil conditions) which are not controllable in the field.

PLATE II



'Sand Culture' experiments on Coconut Seedlings

Chemical Methods of Plant Analysis.—Chemical methods of plant analysis for the diagnosis of faulty mineral nutrition of crops have gained increasing popularity during the past 20 to 30 years and are now widely used on a variety of crops in all parts of the world. Direct correlations for example, have been drawn between the chemical composition of various plant organs and the fertilizer applications accorded to the soil.

From what has been stated and discussed above, the use of plant analysis in complementary and confirmatory roles to some of the aforementioned techniques should be self evident. In fact, for the accurate evaluation of the nutritional status of the *plant* one cannot find a more satisfactory direct method or index. Further, the importance of chemical plant analysis in diagnostic work will be abundantly clear, when it is recognized that the concentration of nutrients in the soil does not have a direct effect on the growth of the plant whereas it is only the nutrients that enter the plant and are finally assimilated that are unquestionably significant for growth. Even as the various soil methods employed in diagnostic work involve an approach on principles of soil chemistry, so surveys on the use of plant chemical methods have shown that the technique is based on certain basic and fundamental theoretical concepts of plant physiology.

The earlier views (especially of those influenced by the dogmas of traditional agronomy) to the effect that the composition of plants cannot be used as an index of fertilizer requirements have now been proved to be untenable. On the contrary, at the present time the chemical examination of plant tissue for the assessment of (*a*) nutrient needs of plants and (*b*) as a guide in fertilizer practice has reached an active stage in the research programmes of agricultural institutions in many parts of the world. The available experimental evidence is overwhelming that the transformations which take place in certain plant organs constitute determinative processes that regulate growth and development. These plant organs are therefore recognized now as index tissues in the integration of all factors that influence the availability of soil nutrients and their uptake by the plant.

At least two reasons could be adduced for the great expansion during the past 25 years in work on plant analysis for diagnostic purposes. One is unquestionably the increasingly recognized importance of micro-nutrient deficiencies, the diagnosis and quantitative evaluation of which would be frankly impossible in the absence of high-precision chemical methods. The other reason is doubtlessly associated with the incredible improvements that have been introduced into analytical methodology—particularly those which have facilitated the speedy and accurate analyses on large numbers of samples employing macro, semi-micro and micro-chemical techniques.

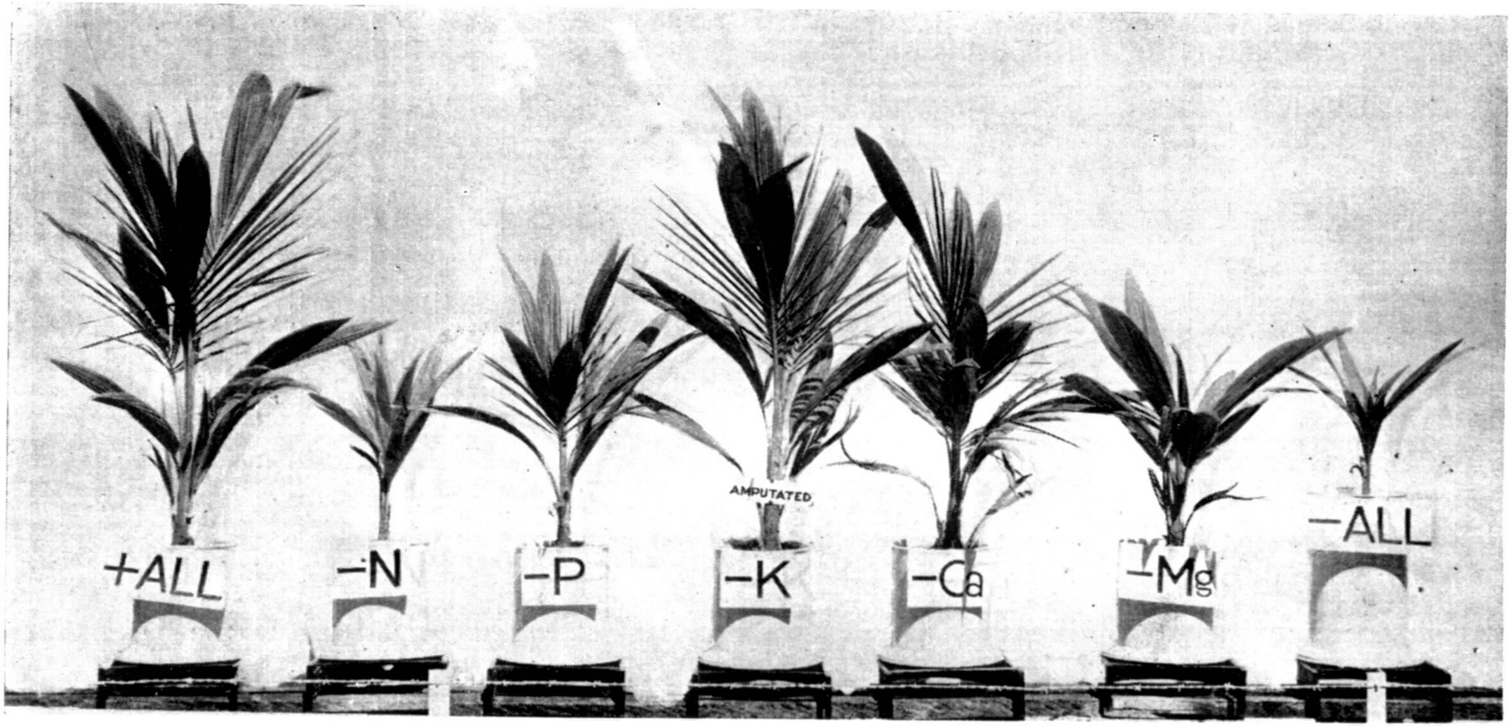
Some workers believe that errors of experiment arising from the methods of chemical analysis of plants cannot be reduced in the same way as those of field experiments. On the other hand, *Lundegardh and Thomas* are of opinion that the estimates of probable yield obtained by chemical analysis are at least as good as those based on field experiments.

The recent development of the method of rapid chemical tissue tests on fresh plant material, marks a further advance in the plant analysis technique whereby it has been extended to the field, for plant nutritional survey work on location.

Foliar Diagnosis and Leaf Analysis.—Among the numerous methods employed to control the nutrition of plants under practical field conditions, the technique of foliar diagnosis, which is based on accepted principles of plant physiology, has been found to give consistent experimental results over considerable periods of time. For purposes of control in the field, the analysis of the entire plant has doubtless been found a cumbersome procedure. Further, different plant organs have different functions and accordingly the results of gross analysis of a mass of heterogeneous organs do not comprise a sufficiently sensitive comparative index, either of the responses of the plant to changes in environment, or the nutrient inter-relationships between the plant and the soil.

It has been found that coincident with the seasonal growth cycles of plants there are well defined chemical cycles of the nutrient elements (and elaborated products) in the leaves, stems, roots and other plant organs. These cycles have been found to be of great significance both in considering deficiency effects and in diagnosing their causes. Since the classical investigations of *Isidore Pierre* in 1869, who pointed to the delicate sensitivity of the leaves to changes in composition associated with environmental factors, the leaf (which is the laboratory of synthesis and the seat of active growth processes) has been found the most satisfactory part of the plant for

PLATE III



Coconut Seedlings grown in 'Sand Culture' diagnostic analysis. The development of the chemical method of leaf analysis as a means of studying the course of the absorption of mineral nutrients under the influence of different growth factors may be attributed principally to the labours of *Lagatu* and *Maume* in France and *Lundegardh* in Sweden. A detailed discussion of this subject or the method of interpreting results (which is somewhat involved), is outside the scope of this review. It would be appropriate however to mention, that the concept of foliar diagnosis rests on a principle of comparative nutrient status, usable only with a key of interpretation. The method is actually based on the comparison of chemical data obtained from comparable leaves (i.e. leaves of the same physiological or metabolic age) of plants, one set of which is taken from high-yielding plants and the other from plants under test.

On the basis of extensive field trials in various parts of the world, the method has been reported to yield valuable quantitative assessments of the effects of the principal fertilizer elements on the yield of particular crops.

Phytosociology.—Passing reference should be made to this new diagnostic technique which is still under scrutiny. This method is based on a study of natural plant associations wherever they are linked up with deficiencies or toxic concentrations of particular elements in the soil. For example, the presence of certain plants like *Rumex acetosella* and *Sclerantus annuus* has been suggested by *Kuhnholtzlordat* as an index of lime deficiency. Again, the presence of specific flora including plants like *Astragalus, Stanleya, Oonopsis* and *Xylorrhiza* has been found in U.S.A., to characterise soils containing concentrations of selenium toxic to grazing animals.

The possibilities of applying phytosociology as a technique for diagnostic purposes is being investigated at the present time in France, England, Germany and the United States.

Limitations of the Techniques

In crop nutrition research the various diagnostic methods described above have their respective applications to different phases of the mineral nutrition of plants, depending on the specific problems involved. Under certain circumstances however, they can all be applied in relation to a central problem, and in this respect therefore the categories cannot be regarded as being mutually exclusive. The methods have all been estimated to have their special points of value, but they are also known in practice to have discernible weaknesses and limitations. The correct criterion however, for assessing the value of any diagnostic method purporting to be applicable to the determination of fertilizer requirements lies, in the agreement between the responses that have been forecast and the actual yields obtained with the prescribed treatments. It might be appropriate at this stage to amplify on the limitations inherent in some of the diagnostic methods which we have considered.

Whilst it is known that any fertilizer *only* becomes of real value when it is effective in the field, it does not follow that the field experiment is the best method for investigating the fertilizer requirements of a crop. In fact, it is subject to a number of disadvantages as a diagnostic method. In many countries, field trials over a number of years using mineral fertilizers have been reported to give erratic results. Although analysis revealed very low contents of some of the macro-nutrient elements, responses to their application in the field have been found to be small or absent. On the other hand, where positive responses were obtained again the findings were found to be conflicting and erratic in relation to analytical assessments of soil fertility status.

It is well known that field trials are subject to a severe leaching hazard which may well vitiate a fertilizer application. Thus a negative result in the field under conditions of heavy tropical rain may not be taken to mean that the element is not deficient. It may merely mean that the element was washed away before it could become effective. A positive response similarly may require cautious interpretation. Further, the elements under test in field trials may not be the only ones whose deficiency is currently limiting growth. In fact, it is now known that in the presence of micro-nutrient deficiencies erratic results with macro-nutrient fertilizers may be due entirely to chance contamination with minor elements. For example, accidental and variable contamination of a macro-nutrient fertilizer used in field trials can clearly have a very important bearing on results when molybdenum, copper and zinc deficiencies are possible in the soil. Unless it is known that the soil is not deficient in any of the minor elements (a fact which can only be demonstrated by more refined techniques), responses to major element applications, may accidentally include responses to minor element impurities in the fertilizer. Wherever such a possibility cannot be excluded, it would doubtless be difficult to interpret satisfactorily any response with major fertilizers. In any case the need for the accurate assessment by special techniques of the real operative or functional elements in a fertilizer is of the utmost importance.

Again, the possible presence of several simultaneous deficiencies may well produce a situation with which the usual field experimentation technique is not competent to deal for diagnostic purposes. In fact, the implications of multiple deficiencies operating simultaneously are now recognized to be more than academic in importance. If one admits the possibility of multiple deficiencies even of four, five or more elements whose identities are unknown at the outset, one must reckon on carrying out very involved trials to elucidate double, triple and high order interactions that would be difficult to disentangle. When one adds to this complexity the risks mentioned earlier, fertilizer research on field crops could present formidable problems. Since the aim must be to correct all possible limiting elements, such a conclusion only implies the use of more refined experimental techniques capable of detecting a limiting deficiency uncomplicated by the presence of other deficiencies. The summation of the experience of workers at the present time has led to the conclusion that field trials capable of yielding unambiguous results can only be conducted in conjunction with collateral chemical analysis of the plant and soil, coupled with much work in pots. At this stage, it should be recalled that we started on the premise that a positive response to an application in the field is the *only* result likely to be of constructive and economic value to the investigator. Consequently field experiments are indispensable, and the converse statement of the above conclusion would mean that the rational planning of field fertilizer trials would become possible only when the ability of the soil under test to supply the mineral elements essential for plant growth is known. For instance, it is of no special service to advocate a large application of a given fertilizer to achieve a small application of a chance impurity. It is more important to know what the trace impurity is which is limiting growth in a critical degree. Hence the need for supplementary techniques in the diagnostic stages of fertilizer investigations which would give decisive indications and a knowledge of the limiting deficiencies.

The so-called 'Soil Pot Culture' techniques of Mitscherlich and Neubauer purport to measure the nutrient status of the soil, the former by relating soil nutrient content to the growth (yield) of the crop and the latter by determining 'soluble nutrients' in the soil by their uptake by seedling plants. In contrast, the plant chemical methods, such as the method of foliar diagnosis deal with certain relationships between the concentration of nutrients in the leaves (or other plant organs) and growth factors associated with the general environment. In spite of the fact that these methods are suitable and accurate for use where precision is required yet they too have been found to have certain shortcomings. For example, for purposes of nutritional diagnosis, these pot techniques cannot claim to differ in principle from the much simpler expedient of soil analysis methods, in which acids are used as extractants of 'available' nutrients. They can all be regarded only as pointers to probable mineral deficiencies, since the intake of minerals by plants is not determined solely by chemical supplies in the soil. Further, there is the possibility that the structure and composition of the soil in the pot can be different to conditions in the field, and the problem of restricted root growth in pots could result in enhanced root competition. This condition does obtain because, even at only one plant per pot the stand is 'close' by field standards, and the scope of rooting is generally restricted only to the top foot of soil. In other words, in pots one cannot exactly reproduce the conditions under which the plant grown in the field absorbs nutrients from the soil. A further point which needs stressing is that the soil pot technique is invariably an indirect one because indicator plants are used in place of the crop plants themselves. This constitutes a tangible weakness in the method because it is known that species differ somewhat in the relative balance of nutrients required for their optimum growth. It would therefore not be plausible to infer that the formula best suited for the 'indicator' will necessarily be applicable to all species of crop plants.

In the present state of knowledge it is impossible from an analysis of the soil to predict deficiencies with absolute certainty. Consequently the results of chemical methods of soil analysis cannot be used too rigidly or categorically. This method has the further practical disadvantage that it can be used only by scientifically trained workers who have considerable experience in interpreting results.

Regarding the visual technique, it should be pointed out that all crop plants do not necessarily show characteristic foliar or other symptoms. Further, deficiency symptoms are sometimes indistinguishable and are sometimes masked entirely by those produced by pests and diseases.

The spray/injection technique too has its weaknesses. It has been found ineffective in some cases and has been reported to give unreliable and misleading results with others. The technique generally gives its best results with crops in their early stages of growth.

Like all the other diagnostic techniques the chemical method of plant analysis too has certain limitations. Since the method has significance only in a comparative sense it is (as are the data for entire plants) usable only after the norms have been established. It is essential to evolve these on the basis of exhaustive surveys of the crop under study for different seasons, and for different environmental conditions. Unless this is done plants grown at widely separated centres or in different seasons cannot really be compared. According to *Thomas* and *Mack*[6]; 'No physiological significance is to be attributed to the foliar diagnosis of any one fertilizer treatment (plot) considered alone'. The principal weakness of the method would seem to be that it serves rather to explain past happenings than to provide information regarding the future performance of a crop.

Conclusion

The problems of crop nutrition are legion and in attempting to solve them for a particular crop, research ranging under many branches of study may become necessary in order that a clear, complete and valid picture could be built up. When this is done, precise information for the soil, plant and climatic environment may become available. If the findings from the various facets of investigation converge or dovetail then any conclusions to be drawn could be regarded as incontrovertible. If however, they are conflicting and divergent, then any claims to an understanding of the central problem would necessarily lack foundation.

Viewing the field as a whole, the various diagnostic methods that have been discussed may be said to represent an integration of different lines of approach, and they have been found most valuable for co-ordinated studies on difficult and stubborn problems in crop nutrition. Though, the most successful diagnosis of problems have been obtained by using various combinations of the methods, yet the application of individual techniques is not precluded, and could in fact be employed for a ready diagnosis of relatively simple problems. The choice of the method would of course depend on the conditions in the field, and the facilities and personnel available. The use of single methods however, cannot be expected to give a complete and unequivocal answer in all circumstances.

Perennial and semi-perennial plants (particularly tree crops) present to the field experimentalist additional problems not usually encountered with the ordinary annual arable crops. The extreme type of these perennials usually lack uniformity of genetic composition and may also show great variability as regards potential yield capacity. The differential response of the individual plants to yearly changes in weather conditions also introduces a further uncontrollable variation factor. Since with such crops the quality of the produce might be equally important as the quantity, management practices may involve detailed operations even on individual trees. Thus diagnostic approaches (including experimental designs) and studies on the mineral nutrition of such perennial crops raise many difficult practical problems which are yet a challenge to the imagination and ingenuity of the plant diagnostician.

The luxuriance of a plant is not necessarily a criterion of its nutritive value or an index of optimum mineral status. Consequently, the importance of a thorough knowledge of all the growth manifestations of the crop under study, and the need for many approaches to proceed toward a goal of increased understanding of soil-plant interrelations, is abundantly clear. In our present state of knowledge, (giving critical consideration to practical and economic factors), it can be said that a skilful use of the diagnostic methods described, would ensure an appraisal of the nutritional status of crops and also provide invaluable guidance to investigators endeavouring to solve problems encountered in modern horticultural and agricultural practice.

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