BIOLOGICAL METHODS FOR DETERMINING FERTILIZER REQUIREMENTS OF CROPS

by

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PEOPLE-PACKED PLANET

"If the United States seems to be filling up--and it's certain that Americans have less elbow room than they once did--the phenomenon is not exactly unique. So is the world. New statistics gathered by the United Nations say that with emphasis.

During the last three decades, according to U. N. 'population experts,' the number of human beings on this planet has risen from an estimated 1,834,000,000 to an estimated 2, 378,000,000. The total population of the globe rose by an average of approximately 1 percent a year during that period. If this rate of increase is maintained there will be twice as many people a century from now as there are today.

In the year 2051, then, there may be around 4,750,000,000 individuals on this terrestrial ball--to use what sounds like a most appropriate phrase from an old hymn. That's a lot of humankind. It's fairly obvious that if the earth cannot support its present population in reasonable comfort, tremendous advance will be necessary if it is to feed, house and clothe twice as many people.

Is such an advance possible? Merely to live in the United States is to realize that the world can be made to produce more abundantly than some pessimists can imagine. Americans have made their share of this continent support 150,000,000 people at an unprecedented standard of comfort. Perhaps the miracle can be accomplished.

But future generations will inhabit a more crowded sphere. Those unfettered souls that must have the great open spaces will have only the deserts or the oceans."--Editorial from the State Journal, Lansing, Michigan

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I. HISTORICAL BACKGROUND OF THE MODERN CONCEPTS OF FERTILIZER USE

A. Early theories of the nutritional requirements of plants

1. Introduction

Since man first planted a seed and produced a crop, the problem of his conscious control of the conditions affecting plant growth has occupied his attention. The mysterious development of a vigorous plant from an apparently lifeless seed has excited the curiosity of mankind for hundreds of years. In recent time, the problem of assisting nature to provide for the plant's needs has become vitally important because of the increase of population and the great complexity of its requirements. It is obvious to agricultural workers that the world acreage of usable agricultural soils is decreasing at an astonishing rate. Inquiry into the methods of determining the proper use of fertilizer for crop production is one of the most important problems of modern agriculture. The current scientific approach to this problem was not developed in recent time, but has evolved slowly over a period of at least two thousand years. While a narration of the errors of early scholars should not be given undue prominence, a knowledge of some of their efforts to understand plant growth is necessary if we are to fully comprehend the problems confronting us at the present time. Erroneous theories often have been the stepping stones to the discovery of scientific truth.

The purpose of the present study is to inquire into the various biological methods for the determination of the fertilizer needs of crops. This study involves careful inquiry into how early work was in error, an evaluation of the current procedures, and the development of necessary improvements of these procedures. It is erroneous to assume that the present methods are necessarily correct because they are modern and that they satisfactorily serve the needs of agriculture. A re-investigation of the major aspects of the problem based on original literature is essential if we are to avoid the propagation of faulty theories and inadequate technical procedures.

2. The Theory of the Four Elements

Many of the fundamental aspects of the nutritional requirements of plants are now so well known that they are frequently taken for granted, without any realization that their historical development involved the efforts of many people over a very long period of years. There is no precisely defined beginning of man's effort to understand the nature of plant growth, but certainly Aristotle's philosophy that the four "elements", fire, air, earth, and water, were the units comprising all matter might be considered the point of departure which led ultimately to our modern concepts.

Aristotle believed that the union, or mixis, of the four "elements" in the soil generated the nutritive organic matter of plants in the form of exceedingly minute particles, or homoiomeria. These particles were supposedly assimilated through the roots of plants and deposited unchanged in tissues and organs. Aristotle thus laid the foundation for the work of many of his successors for his theory was the earliest expression of what is now known as the humus theory. The humus theory, or belief that plants were nourished by preformed organic matter in the soil, dominated the study of plant nutrition for over 2000 years. Even at the present time, this concept, in modified form, is the basis of the so-called "organic gardening" theory of plant nutrition.

3. The Theory of Water as the Nutrient of Plants

About 300 years ago, a Flemish physician and chemist named John Baptista van Helmont (1652) used quantitative chemical experiments for the first time in the study of the nutrition of plants. He ridiculed the ancient philosophers for their failure to utilize experimental data. An early experiment of van Helmont, which was fundamentally very sound, convinced him that water was the primary substance from which plants were formed. This conclusion was the result of a now famous experiment which has been described many times as an example of the improper interpretation of essentially accurate observations.

Van Helmont stated, "I was able to show by the following experiment that all vegetables are produced immediately and materially from the single element water. I took an earthen vessel in which I placed 200 pounds of soil previously dried in an oven. I then watered it with rain water and planted therein a willow branch weighing 5 pounds. After an interval of five years the tree which had sprung up weighed 169 pounds and some 3 ounces. The earthen vessel which was always watered when necessary with rain water was large and embedded in the ground; lest any flying dust should get mixed with the soil, an iron cover plated with tin and provided with a large opening closed the mouth of the vessel. I did not determine the weight of leaves which fell during the four months. At the end of the experiment I dried the soil again in the vessel and obtained the same weight of 200 pounds lacking about 2 ounces. The 164 pounds of wood, bark, and roots were therefore derived from water alone."

In as much as 98 percent of the fresh weight of many plants, lettuce for example, consists of water, it is not surprising that the comparatively crude quantitative data of van Helmont led him to an incorrect conclusion.

4. The Theory of Earth as the Nutrient of Plants

Jethro Tull (1733), commenting some 150 years later on the willow branch experiment conducted by van Helmont, said that those who agreed with the conclusion drawn by van Helmont "were deceived in not observing that water has always, in its intervals, a charge of earth from which no art can free it." In Tull's opinion, it was not the liquids in the soil, but the very minute particles of earth suspended in it that constituted the "proper pablum" of plants. Tull believed that the pressure exerted by the growing roots forced these particles into the "lacteal mouths of the roots" and into the translocation system of the plant. He summed up the opinions of the agricultural workers of his period when he stated, "It is agreed that all the following materials contribute in some way to the increase in plants, but it is disputed which of them is that very increase or food: nitre, water, air, fire, earth." The great contribution to the knowledge of plant nutrition made by Tull was his emphasis on the soil itself as the source of plant nutrients. That he was wrong in concluding that the earth particles were the nutrients, rather than the minerals which they contained, does not detract from the value of his contribution, for from his time onward, the study of the nutrient requirements of plants was centered on the soil.

5. The Theory of Humus as the Nutrient of Plants

The humus theory had its origins in the homoiomeria of Aristotle, as noted earlier in our discussion, and it was gradually expanded and developed for a period of 2000 years. The theory that humus was the nutrient of plants reached its greatest importance during the time of Thaer (1844), Berzelius (1842), and Humphrey Davy (1813). According to this concept, plants feed on substances similar to their own organic components. The organic matter of the soil, or humus, was regarded as the chief nutrient of plants and the major source of soil fertility. It is interesting to note that the proponents of this theory failed to concern themselves with the origin of the humus, and like many farmers today, assumed that there was an inexhaustible native supply of this substance in the soil. The roots of plants were believed to extract the humus from the soil and to transform it into plant structures by combining it with water. Some of the followers of the humus theory believed that minerals were not essential for plant growth, and others believed that they acted as stimulants to growth rather than as essential nutrients.

The application of more accurate chemical methods made it obvious that the large amounts of carbon in plants had to be explained. Nothing was known of the role of carbon dioxide in the atmosphere, and as a result the proponents of the humus theory firmly believed that the carbon component of plant structures originated from the humus in the soil.

Many workers opposed the idea that the humus in the soil provided the carbon in the plant, and they began to accumulate information pointing towards the true importance of the leaves as the organs involved in carbon assimilation and to the roots as the organs involved in nutrient absorption.

Ingen-Housz (1779) and Senebier (1782) came to the conclusion that "purification" of the air occurred during periods of light, while "vitiation" took place in darkness. Senebier argued that the increased weight of the willow tree in van Helmont's experiment came from the "fixed" air. He said, "If then the fixed air dissolves in the water of the atmosphere, combines itself in the parenchyma with the light and all other elements of the plant; if the phlogistine of that fixed air is precipitated in the organs of the plant, if this precipitate remains, as we think, since this fixed air goes out of the plant in the form of dephlogistine, it is clear that the fixed air, combined in the plant with the light, leaves there a material which was not there previously, and my experiments on etiolation demonstrate this."

Lavoiser (see Browne 1944) concluded in his discussion of the origin of carbonaceous matter in crops that it might seem logical to suppose, superficially, that plants obtain their carbon from the vegetable earth, the humus, soil and manure. But because some plants live in apparently pure water and air, Lavoiser considered it more reasonable to assume that this carbonaceous material is derived from the decomposition of carbon dioxide obtained from the air.

It remained, however, for de Saussure (1804) to establish beyond a doubt that green plants assimilate the carbon from the carbon dioxide in the atmosphere. He was the first to state that the soil furnished a small but essential part of the essential nutrients of the plants, and that the fertilizing value of humus was due chiefly to its mineral content.

Wiegmann and Polstorff (1842) conducted a series of precise experiments on the growth of plants in extracted humus, and they found that the residue in 100 grams of the extract exposed to the air for a month was 136 milligrams, while the residue in 100 grams of extract in which plants had been grown for a month was 132 milligrams. Because of this slight difference of only 4 milligrams, these workers concluded that humus plays an insignificant role in plant nutrition.

The theory of the humus nutrition of plants received serious blows from many investigators, but it remained for Liebig (1840, 1852) to complete its overthrow and to clarify the problem of plant nutrition in the minds of his contemporaries. Liebig stated in a series of lectures before the British Association for the Advancement of Science that "the primary source whence man and animals derive the means of their growth and support is the vegetable kingdom. Plants, on the other hand, find new nutritive material only in inorganic substances." Liebig so effectively disposed of the humus theory that from his day forward primary consideration has been given to the study of the ash of plants in relation to the mineral content of the soils on which they were grown. Liebig's successful attack on the humus theory of plant nutrition ended the profitless philosophical concepts originating from Aristotle's homoiomeria and made possible the scientific approach to the problem of soil fertility.

The foregoing discussion indicates that the efforts of the most brilliant men of a period of two thousand years were necessary to determine the materials serving as nutrients for plants. During Liebig's time, agricultural science attained a fairly sound scientific basis. Agricultural chemists, having attained a rudimentary knowledge of the chemical components utilized by plants, then turned to the study of the quantities of these nutrients required for the optimum production of crops.

6. The Beginning of the Biochemical Approach to the Problem of Plant Nutrition

At the same time that the various theories of plant nutrition were being proposed and discarded, the first true approach to the determination of the nutrient needs of the plant already was under way. Fully 150 years ago, Chaptal (1801) described the properties, extraction, and utilization of various mineral constituents of vegetables. At about the same time, Hermbstadt (1804) conducted what were probably the earliest large scale field experiments. Hermbstadt attempted to answer a number of pertinent questions. In his own words, "(1) Do the nutrient elements of the fertilizer reappear in the organs of plants? (2) Do the elements participate in the formation of such organs and of the plant constituents deposited therein? (3) Can the quantity of each plant constituent be increased by increasing the amount of the element adapted for its formation? (4) Can we determine whether the same field, without addition of new fertilizer, suffers a decrease in yield with each year and whether, by a rotation with other cereals or with seed crops etc., a better yield of grain can be obtained without adding new fertilizer?" Even the thought behind these questions was far ahead of the current thinking of his time.

In the first tabulation of its kind, De Saussure (1804), using what are regarded now as very inaccurate chemical procedures, gave percentages of water soluble salts, insoluble phosphates and carbonates, silica, alumina, and metallic oxides in the ash of leaves, branches, wood, bark, flowers and fruit of seven different trees and in the ash of the straw and grain of wheat, oats and barley. He came to the conclusion that the nature of the soil exerted a pronounced influence upon the percentages of the mineral constituents of plants.

Carl Sprengel (1837), one of the most meticulous chemists and experimenters of his time, established the foundations for the thought of many later workers in the field of fertilizer use when he expressed his belief that the chemical analysis of crops might indicate their nutritional deficiencies. He also believed that the quantities of the various nutritional ions in the plant are dependent on the chemical composition of the soil in which the plant was grown.

As early as 1842, Wiegman and Polstorff conducted pot culture experiments using sand and nutrient solution designed to attack the validity of some of the contemporary theories of their time, although these workers were not specifically concerned with the humus theory.

Liebig (1840, 1852, 1863) gave a great impetus to the study of plant nutrition and the use of fertilizers when he formulated his Law of Minimum and applied the law to the response of crops to fertilizers. From his time on, experimenters in the field of plant nutrition have continued the never ending search for the proper nutritional requirements of agricultural plants.

During the latter part of the nineteenth century, various workers utilized the chemical analyses of plants in their studies of the responses of crops to fertilizers. For example, Hellriegel (1869) studied the optimum concentration of nutrients in barley in this manner. Petersen (1875) and Wolffe (1877) studied oats, Hanamann (1878) studied various cereals. Many students during this period, in addition to those mentioned, also used chemical analyses of crops in their efforts to determine the proper concentration of nutritional ions in plants.

Some agricultural chemists of the period attempted to correlate the percentages of nutrients in plants with their quantitative responses to fertilizers. Liebscher (1893) compiled data from field studies; Hellriegel (1893) used nutrient solutions in quartz or sand; and Joulie (1894) carried out a systematic survey of farmers' fields.

The above brief description of the work of a few investigators is sufficient to show that practices in use today had their origin in the agricultural chemical revolution of the latter half of the nineteenth century. In many cases, the techniques used during the period were good, but the inadequate status of the current knowledge of plant physiology frequently permitted faulty conclusions to be drawn.

B. Development of biological methods for the determination of fertilizer requirements

1. Introduction

Since the work of Liebig laid the foundations upon which developed the later biological approach to the problem of fertilizer use, it is natural to regard this early work important, not only historically, but also as the stepping stone which led to the present day techniques. It will be recalled that De Saussure (1804) attempted to use the chemical compositions of plants as indications of their nutritional requirements, and he observed that these compositions varied with the soil, with the part of the plant, and with the age of the plant.

Liebig (1852, 1863) probably was the first of the many investigators who tried to give a mathematical expression to the magnitude of crop response to fertilizer. Although these expressions appear as a mass of formulae and geometric curves, one must remember that science speaks plainly only through mathematics. With this thought in mind, the development of Liebig's laws appears as an essential part of the historical background of the currently used biological methods for determining the fertilizer requirements of crops. Consequently, these early mathematical expressions of the responses of crops to fertilizers will be discussed briefly as a foundation for our understanding of the more recent advances of the twentieth century.

2. The Law of the Minimum

It is interesting to note that the Law of the Minimum was the earliest attempt to relate by precise mathematics the composition of the soil and the plant to the fertilizer requirements for maximum crop yields. While it is commonly assumed that this law was first expressed by Liebig (1852, 1863), a survey of the literature of his period reveals that as early as 1804 De Saussure had a vague concept of the effect of minimum factors, and Sprengel (1837) in his "Die Bodenkunde oder die Lehre von Boden" revealed that he probably had a clearer concept of the facts than did Liebig when he stated, "The soil is often neither too stiff nor too porous, neither too wet nor too dry, neither too cold nor too warm, neither too high nor too low; it may be situated under very advantageous climatic conditions, it may have an abundant supply of humus, and be located on a favorably inclined slope, and yet may often be unproductive because it is deficient in <u>one single element</u> that is necessary as a food for plants. Again it may also fail to bear good crops because it contains a very easily soluble plant food in too great an excess or because it contains substances that act as poisons to the growth of plants" (Browne 1944).

The concept of the effect of limiting factors, however, was not understood clearly by agricultural chemists until Liebig expressed it with clarity in his writings. In his first publication (1840) he expressed the opinion that the percentage of the nutritional ions in plants was constant and he assumed that this chemical composition represented their inherent nutrient needs. Consequently, many workers came to the conclusion that they could maintain the fertility of soils merely by returning to the soils the total amount of nutrients removed by the crop. This has been commonly referred to as the bookkeeping method of soil fertility, a procedure which was strongly supported by contemporaries of Liebig and which we still find at the present time featured prominently in the advertizing propaganda of the fertilizer industry. The popular term now is "mining the soil". Continuing his earlier study, Liebig (1852) arrived at the opinion that there exists a Law of Exhaustion for each cultivated plant. He stated, "This state of exhaustion inevitably happens even when there has been withdrawn from the soil, by a course of crops, only one of all the different mineral substances necessary for the nourishment of plants. For the one which is wanting or exists in deficient quantity, renders all others inefficient, or deprives them of their activity." Liebig expressed this concept mathematically by the equation, $P = F - R$, where P was the yield of the crop, F the amount of soil nutrients, and R the "resistance" exerted by other factors affecting growth. This formula gave a mathematical expression for the bookkeeping theory of plant nutrition.

The most concise and clear expression of this Law of the Minimum is found in Liebig's "Natural Laws of Husbandry" (1863). In his own words, "Every field contains a maximum of one or several, and a minimum of one or several other nutrient substances. It is by the minimum that crops are governed, be it lime, potash, nitrogen, phosphoric acid, magnesia, or any other mineral substituent: it regulates and determines the amount or continuance of the crops."

Lagatu and Maume (1927,d) quoted the French edition of this book, "Les Lois Naturelles de l'agriculture" (1863), by stating that nutrient ions are absorbed by plants in definite proportions so that if the maximum amount of one ion is absorbed, the accumulation of other nutrients is retarded.

The implications of the Law of the Minimum may be summarized as follows:

1. Plant growth increases with increases of the limiting factor until the factor ceases to be limiting.

2. If two factors are limiting or nearly limiting growth, an increase of one will have but little effect, while increases of both will exert a very considerable effect.

3. If the absorption of one nutritional ion reaches the maximum limit, the absorption of other ions is retarded.

From the time that the Law of the Minimum was first stated, it became apparent that it did not follow strictly the interpretation that Liebig himself had applied to it. The first adequate attempt to apply the law to experimental data was made by Hellriegel and his coworkers. In a series of papers, Hellriegel and Wilfarth (1888), and Hellriegel, Wilfarth, Romer, and Wimmer (1898), described the growth of barley in sand cultures which were supplied with all necessary nutritional ions, with the exception that the amount of one nutrient salt was varied. A portion of these data are assembled in table 1 and are shown graphically in figure 1. The results of varying the magnitude of the nitrogen supply indicate that the first increment of nitrogen produces a certain increase in yield; but the yield increases due to the second and third increments are proportionately greater. The yield is greater than is expected, if as Liebig assumed, the effect of nutrient supply is proportional to the amount present. The fourth and fifth increments of nitrogen produce a lesser increase in yield and a sigmoid curve results when the data are presented graphically. This sequence of effects are not restricted to nitrogen for Hellriegel obtained a similar curve when he varied the supply of potassium.

In the early years of the twentieth century, Mitscherlich (1909) subjected the relationship of nutrient supply to crop yield to a rigorous mathematical analysis and proposed a different statement of the Law of the Minimum. Mitscherlich's version states that the increase in yield per unit of the limiting nutrient supplied is directly proportional to the decrement of the yield from its maximum magnitude. This statement of the Law of the Minimum has been widely accepted as a more exact indication of the relationship between plant growth and nutrient supply than was Liebig's earlier statement.

Mitscherlich's restatement of the Law, in turn, has been severely criticized not only by his contemporaries Briggs (1925), Balmukand (1928) and Pfeiffer, Simmermacher and Rippel (1919), but also by later workers such as Macy (1936). These investigators upheld the principle

Table 1. The effect of successive increments of nitrogen fertilizer on growth of barley in sand cultures (Hellriegel and Wilfarth, 1888).

Milligrams of nitrogen supplied	0	56	112	168	280	420
Grams of dry plant material	0.742	4.856	10.803	17.528	21.289	28.727
Grams of increased yield per nitrogen increment of 56 milligrams	—	4.114	5.947	6.725	1.880	2.975
Percentage dry material produced	11.9	37.9	38.0	42.6	38.6	43.4
Weight per grain, in milligrams	19.5	30.0	33.0	32.0	21.0	30.0

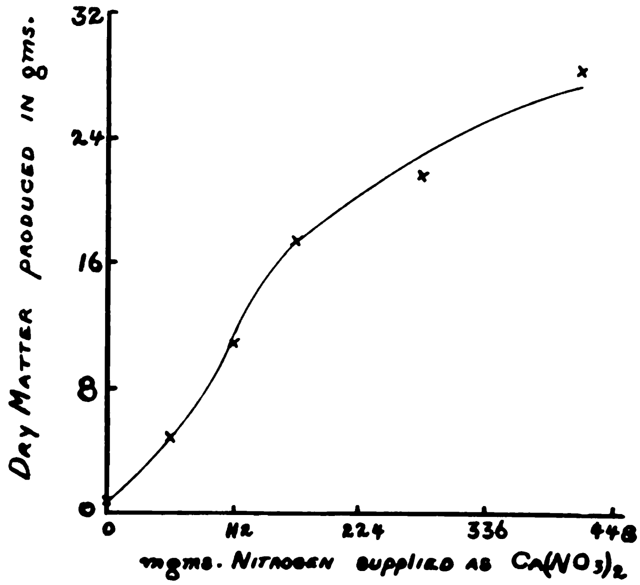

Figure 1. The effect of nitrogenous fertilizer on the growth of barley. (Hellriegel and Wilfarth, 1888)

of Mitschlerich's interpretation but differed as to its proper mathematical statement.

A further critical consideration of the Law of the Minimum is found in the work of Lagatu and Maume (1927,d) which involved the study of the ratios of the percentages of nitrogen, phosphorus, and potassium in plants grown at different levels of fertility. These workers noted that if one element was limiting, the ratios of the concentrations in the plants of the other elements to that of the deficient one increased. In other words, they believed that when one nutrient was deficient, the proportion of the others in the plant increased, while that of the deficient nutrient decreased. Such results obviously were in contradiction to those expected from the Law of the Minimum. Wallace (1928) working with apples and Bartholomew, Watts, and Janssen (1933) working with potatoes obtained data consistent with those of Lagatu and Maume, but Murneek and Gildehaus (1931) apparently obtained a reverse effect in their work on the nutrition of apples.

As already pointed out, the applications of the basic principle of the Law of the Minimum resulted in the procedure commonly referred to as the bookkeeping method of plant nutrition and which is now known as "mining the soil." This bookkeeping approach was supported strongly by Liebig and Wolff (1871) who catalogued the chemical analyses of many plants in order to return to the soil the nutrients which were removed by crops. This approach to the maintenance of fertility is short sighted, and it is unfortunate that at the present time many agriculturists still care for our agricultural heritage on this basis.

3. The Laws of the Minimum and Maximum Nutrient Content of Plants

Goodall and Gregory (1947) credit Weinhold (1862, 1864) as the first to use the chemical analysis of plants as an index of the amount of available nutrients in soil. At about the same time, Hellriegel (1867, 1869) studied barley in sand cultures and found that the percentage of potassium in the grain increased as the supply of potassium was increased. He concluded that maximum yields were obtained when the potassium content of the straw was 0.5 percent of the dry matter and 0.38 percent in the grain. Wolff (1868) reported a minimum percentage of CaO in oats, and on the basis of further work, he published (1877) data indicating the minimum percentages of the six principal nutrients in oats as well as percentages associated with satisfactory plant growth.

Heinrich (1882) made a significant advance in using this procedure for determining fertilizer requirements when he analyzed a specific portion of a plant, namely the roots of oats, when grown at various levels of nitrogen. The roots were chosen as they were "more depleted at maturity the poorer the soil. The whole of the available nutrients have migrated to the aerial parts of the plant, to be used for assimilation and formation of new organs." Heinrich proposed a "Law of the Minimum" which stated that if a nutrient was lacking or present in a limiting amount, the concentration of that element in the root was at a minimum value. On the basis of this statement, he gave minimum concentrations of the six major nutrients below which the nutrient was deficient.

Von Dikow (1891) also reported data based on root tissue, but he included as "roots" of barley all parts of the plant below the first node. The minimum percentages for P_2O_5 and nitrogen were reported as 0.13 and 0.63 percent respectively. These minima were based on the fact that material from three of his four plots exhibited approximately these values. Von Dikow also suggested that the range of the mineral content of the plant was limited by a maximum as well as by a minimum magnitude, and he proposed that Heinrich's Law of the Minimum be supplemented by a Law of the Maximum. This new law stated that until a certain maximum concentration of nutrients was reached, fertilizers would not produce the maximum growth. Helmkampf (1892), using oats and wheat subjected to differential fertilizer treatments, noted that the percentage of nitrogen in the roots of plants grown on all plots approximated Heinrich's minimum value and that the crop showed marked responses to the treatments. The potassium content increased with increased applications of potassium, and the phosphorus content remained constant between 0.40 and 0.48 percent calculated as P_2O_5, regardless of the treatments. He concluded that in this latter case, the maximal value suggested by von Dikow had been reached. Stahl-

Schroder (1904), on the other hand, reported results obtained with oats and wheat showing evidence of a luxury consumption, and consequently he rejected von Dikow's proposal of the existence of minimum and maximum concentrations of nutrients in the plant.

In a series of papers, Atterberg (1886, 1887, 1887a, 1887b, 1888, 1888a, 1889, 1901) published the results of numerous experiments with oats using sand cultures and field experiments. He reported in detail the results of chemical analyses of the various portions of the plants. He gave minimal and average values for the principal nutrients but did not accept the idea of a maximum concentration as postulated by von Dikow (1891). Atterberg's results (1888) indicated that very high supplies of the phosphate ion might continue to increase the phosphorus content of tissue even though there was a depression of the yield.

The true significance of a minimum percentage of a nutrient in a plant tissue from a nutritional requirement standpoint is still being debated. The Law of the Maximum has received considerable attention as it applies to the problem of the luxury consumption of nutrients. While some workers before the turn of the century did not accept the concept of luxury consumption of nutrients, the Breslau experiments of Pfeiffer, Simmermacher, and Rothmann (1915) showed that luxury consumption did occur.

Whereas the Law of the Minimum as proposed by Liebig (1852, 1863) and later by Wolff (1871, 1877) had a practical application in the bookkeeping concept of plant nutrition, the Law of the Minimum as proposed by Heinrich (1882) and the Law of the Maximum as proposed by von Dikow (1891) found their expression in concepts of the critical concentrations, threshold concentrations, and optimum nutrient ratios used very extensively at the present time. The greatest portion of this early work was based on cereals and many workers proposed minimum levels of nutrients in the plants. Hellriegel (1867) gave minimum values of 0.5 and 0.38 percent of potash in the dry matter of the straw and grain respectively. Wolff (1876, 1877) collected all the data available at the time for plant analyses and published them in his book "Ashen Analysis". From these data, he was able to postulate values for the "necessary minimal content" in plants and also for "good average development". The work of the later workers, Heinrich (1882), Haessner (1887), von Dikow (1891), and Helmkampf (1892), followed similar lines. Atterberg (1901) probably summed up the ideas of the period previous to 1900 in the report of his oat experiments already mentioned. He concluded that if the supply of a nutrient is increased, the nutrient is assimilated by plants in increasing quantities and its percentage in the plant is increased. He describes the use of his minimal values as follows: "The element which is present in the plant in minimum quantity may be determined as follows: The percent content as shown by the analysis is compared with the average and minimum content of oats. In general, the fertilizer ingredient whose percentage is farthest below or least above the average figure representing the minimum content is present in minimum quantity." Atterberg believed that this procedure made it possible to deduce the fertilizer deficiencies of the soil.

During the period just discussed, the majority of the possible approaches to the problem of estimating the fertilizer requirements of crops already had been considered and at least some experimental work had been carried out. The great value of the work of this period, however, was that it served as a fertile source of ideas on a subject which even today is obscure. Whereas no great scientific advance was made, the ideas that the work produced, although they in many ways were rather elemental, were the sources from which the investigators of the twentieth century were able to build the more scientific approach we know at the present time.

4. Summary of the Work during the Last Half of the Nineteenth Century which Was Instrumental in the Development of Modern Procedures

a. Field Trials

The majority of agricultural workers have at some time made use of field trials to determine the fertilizer requirements of crops. The best known, and one of the earliest field trials, was that designed by Lawes and Gilbert (1851) to test Liebig's (1840) theory of mineral fertilization. These investigators concluded that the nutrient composition of the crop removed is "no direct guide whatever" to fertilizer requirements. These trials have been continued to the present day at Rothamstead in the same manner as originally planned by Lawes and Gilbert. Joulie (1882) conducted work in France using the data obtained from soil analysis, tissue analysis, and field trials to design a program to build up the soil; consequently he might be credited as the first to apply data obtained in the laboratory to the practical management of farm lands. Many other workers conducted their nutritional studies on the basis of field plots, including Helmkampf (1892), Liebscher (1893), and Atterberg (1901). Some investigators, however, observed that the field trials did not yield as conclusive data as did pot experiments. For example Liebscher, Kretschmer, von Seelhorst, and Willms (1898) and also Atterberg (1901) recognized the inherent weakness of field trials. Atterberg, however, pointed out that plant analysis and pot culture techniques, while giving a better indication of the nutritional status of the plant, did not lead directly to the determination of the amount of plant nutrients available in the soil, and consequently admitted the necessity of the supplementary use of field trials.

b. Pot and Solution Cultures

The development of the use of pot cultures followed the overthrow of the Humus Theory by Liebig (1840) and the establishment of the mineral theory of fertilization. The difficulties experienced by the workers of the time in obtaining good results under natural conditions also encouraged the use of pot cultures under more or less controlled conditions. Any worker in the field of biological science realizes only too well the difficulties of obtaining consistent research data under uncontrolled natural conditions.

Sachs (1860) was one of the first to further refine experiments in plant nutrition by using water solutions of pure chemicals to grow plants. He published one of the first standard formulae for growing plants in culture solutions, and Knops (1865) soon after proposed a solution that has since become one of the most widely used in the study of plant nutrition. Other solutions proposed during the period were those by Tollens (1882), Schimper (1890), and Pfeffer (1900).

Hellriegel (1867) working with barley in sand cultures provided with nutrient solutions proposed minimum concentrations of potassium. He later (1869) obtained similar data using water cultures. Other workers of the period who used pot or solution cultures in their study of the fertilizer requirements of crops were Wolff (1876), Petersen (1876), Helmkampf (1892), Hellriegel and Wilfarth (1888), Hellriegel, Wilfarth, Romer, and Wimmer (1898), Hellriegel (1893) (1893a), Liebscher, Kretschmer, von Seelhorst, and Willms (1898), and Atterberg (1901).

It was apparent, at least to some of the workers, that there were certain limitations in the use of pot and solution culture techniques. Wolff (1877) stated that "the fact that the experiments were conducted in water culture somewhat invalidates the results and tends to prevent a valid field interpretation". Somewhat later Liebscher and his co-workers (1898) concluded that, while the experiments appeared to be inconclusive when the data were compared to those obtained from pots, care would have to be taken in the interpretation of the results obtained under artificial conditions. These views are recognized by most of our own contemporary workers in the field of plant nutrition, but unfortunately their implications are not always acted upon.

Certainly it must be agreed that the artificial conditions imposed by pot culture techniques are not conducive to the direct application of data obtained by their use to practical procedures in the field. Under the circumstances, it was logical to look directly to the plant, growing under various conditions, as the source of data concerning its nutritional status.

c. Plant Analysis *versus* Soil Analysis

The problem of whether to analyze the soil or the plant to determine fertilizer requirements still perplexes students at the present time. As yet, no definite answer can be given. As soon as the source of plant nutrients was settled by Liebig, the question of whether to analyze soil directly to determine their amounts, or to regard the plant as nature's ideal nutrient extracting agent troubled students of the fertilizer requirements of crops. The earliest concept of the use of plant analysis was based on the bookkeeping method of calculating fertilizer requirements proposed by Liebig (1840) and others.

As noted previously, Weinhold (1862, 1864) probably was the first investigator to consider the chemical analysis of the plant as an index of the availability of nutrients in the soil. He believed that the composition of the plants growing naturally in a given location indicated the amount and availability of the nutrients in the soil. He made the error, however, of considering that when more than one species was abundant in a given area, all such species should have identical chemical compositions. He tested this supposition, and when he found that it was not valid, he dropped the investigation, concluding that the physical properties of soils were of greater importance than were its chemical properties.

Weinhold's work stimulated other workers to correlate the chemical components of plants with the amount of added fertilizer. The most important contribution to this approach was that made by Wolff (1871, 1880) who collected all the chemical analyses published from 1800 to 1880 and compiled them in his classic book "Aschen Analysis".

Investigators did not give much thought to the use of plant analysis as a diagnostic procedure to determine the nutritional status of the plant until around 1890. Helmkampf (1892) stated the problem when he said, "plant analysis in connection with fertilizer experiments furnishes indications of whether or not a soil is deficient in one or more of the essential nutrients.......investigations concerning the proper part of the plant to analyze, the proper stage of its growth, etc., need to be conducted." Liebscher (1893) also concluded that "since the richness of the soil in phosphoric acid exerts an influence on the composition of the grain, the analysis of the grain may be taken as an indication of the richness of the soil."

Janussewski (1895) following the same line of thought as did Joulie (1894) stated that "plant analysis in fertilizer experiments can only be used when careful estimation and evaluation of the soil as to its physical properties is taken into consideration". Remy (1896) believed that the amount of nutrients absorbed in a given time was in no way dependent on the amount of plant tissue produced during that time, and he also believed that weather influenced the rate of nutrient absorption within certain limits as well as did the amounts of nutrients available.

Towards the end of the nineteenth century, the major workers had come to the conclusion that the chemical analysis of plants was a more reliable procedure for the determination of the nutrient requirements of crops than was the analysis of the soil. This view probably was given undue emphasis because of the generally unsatisfactory analytical techniques at their command for the proper analysis of soil.

d. Early Methods of Chemical Analysis

As the chemical methods of analysis used during the nineteenth century are obsolete and are of historical interest only, attention will be given here only to their general features. In this early work, the majority of the investigators based their results on the percentages of total ash in the plant. Lowry and Tabor (1931) credit Palladin (1864) as the first to use the sap of plants for the study of their nutrition.

The earlier work involved the analysis of the whole plant, but later many investigators recognized that different portions of the plant differed in their value as indicators of nutrient uptake. Heinrich (1882) concluded that "the roots are the most appropriate organ for comparitive investigations involving the determination of nutrient conditions in the soil." Hellriegel (1869) considered that the composition of the straw of cereals was the best indicator. Von Petersen (1876) found that the entire top was a better indicator than was the straw or grain taken separately. Other workers, such as Wolff (1877) and Helmkampf (1891, 1892), agreed with von Petersen. On the other hand, Januszewski (1895), and later Atterberg (1901), regarded the composition of the grain as the best indicator of the availability of nitrogen and phosphorus, and the straw as the best indicator of the potassium supply. It is apparent that by the end of the nineteenth century, sufficient progress had been made to indicate the necessity of using specific portions of the plant, if the chemical analysis of plants was to serve as a procedure for determining fertilizer requirements.

Our discussion indicates that nothing really new has been added in recent times. Frequently it is assumed that tissue analysis is a recently developed concept; but the advances since the end of the nineteenth century have been those of improved techniques, particularly analytical techniques, rather than the development of new approaches. If one were to search for the reasons why the early work failed to show good correlations between the chemical analysis of plant tissue and the nutritional status of the plant, it would be apparent that the chemical procedures used were inadequate and that too little was known about the factors affecting the absorption of nutrients. It is essential, therefore, to discuss in some detail the factors involved in the absorption and concentration of nutrient ions in the plant if a true understanding of the proper use of "tissue testing" is to be attained.

II. FACTORS AFFECTING THE ABSORPTION AND CONCENTRATION OF NUTRIENTS IN PLANTS

A. Introduction

Many factors influence the absorption of nutrients by plants and their percentages in plant tissues. Any attempt to utilize biological methods, that is to say, either the growth of plants or their chemical composition, to determine the fertilizer requirements of crops depends on the assumption that only the amount or availability of a nutrient in the soil influences its effect on growth or its concentration in the tissues of the plant. Strictly speaking, this hypothetical situation is never true. However, this is not equivalent to a condemnation of biological methods. The use of biological methods theoretically is the best of all procedures for the determination of the fertilizer requirements of crops, provided of course, that all factors affecting the results other than the availability of nutrients in the soil are constant or insignificant in their magnitude.

It is essential that the many factors affecting the responses of plants to nutrients be understood and constantly borne in mind if the use of biological methods is to serve as an adequate technique for the determination of the fertilizer requirements of crops. Consequently these factors will be discussed briefly.

B. Mechanism of Nutrient Absorption

A complete understanding of the mechanism by which plants absorb their nutrients from the soil would make it possible to evaluate accurately the influence of all factors on the response of crops to fertilizers. Unfortunately, a complete comprehension of this intricate biological phenomenon is not possible on the basis of present knowledge. However, such evidence as is available should be utilized in an attempt to determine how various factors affect the uptake of nutrients by plants.

The preponderance of evidence leaves no doubt that the process of nutrient accumulation by plants is intimately associated with the metabolic activities of plant cells, and it cannot be regarded merely as a passive entry of ions into the roots. The important question is the mechanism by which nutrient ions enter the plant because the process is affected by factors other than the amount present. The metabolic aspects of ion accumulation have been investigated almost exclusively by the nutrient solution technique. The application of this experimental approach to problems of fertility depends on the assumption that a basic similarity exists between the absorption of ions from a nutrient solution and from the soil. Such an assumption is based on the theory that the nutrients move unhampered by the surface forces residing on colloidal particles; but the solid phase of soil is regarded as the source of most nutritional ions, particularly cations, in the soil solution.

Recent research by Comber (1922), Truog (1928), and Jenny (1950) has shown that the extracted soil solution does not provide the answer to the problem of the mineral nutrition of plants. The attempts of Comber and Truog to attribute a more important role to the solid phase of the soil were unsuccessful because no theory was advanced which provided a convincing mechanism involving the solid phase.

Jenny (1950) has shown that the uptake of radioactive sodium, at higher concentrations, is decidedly greater when adsorbed on clay suspensions than when present as a chloride or bicarbonate salt in solution. In the case of an ammonium saturated clay versus a solution of ammonium chloride, the uptake of ammonia by the roots was nearly equal under both conditions. On the other hand, a solution of potassium chloride provided a better source of potassium than did a potassium saturated clay, as would be expected on the basis of the solution theory of nutrient availability. It is sufficient for the purpose of the discussion to emphasize that the absorption of cations adsorbed on clay particles bears no simple relationship to their absorption from aqueous solutions.

The theory of contact exchange proposed by Jenny and Overstreet (1938) involves a mechanism for the absorption of nutrients held colloidally on the clay particles. This mechanism can be deduced from theoretical considerations concerning the nature of colloidal surfaces. Specifically, it rests on the redistribution of ions when they find themselves in intermingling colloidally held ionic layers. Accordingly, the contact absorption theory embraces all colloidal systems and not only those in the soil and in the cells of roots. The absorption of cations by roots from colloidal surfaces in the soil by contact exchange and by direct absorption from solutions is diagramatically shown in figure 2.

The application of the theory of contact exchange to the mineral nutrition of plants postulates that swarms of ions intermingle in the electrical fields of the colloids in the soil and those of the absorbing cells of the roots. Inasmuch as an ion is attracted by two colloidal surfaces it goes to the surface possessing the greatest attractive force. If this be true, the transfer of ions from the soil colloids to the root does not involve a true solution. The uptake of ions adsorbed on colloidal surfaces by this mechanism is largely independent of the water content of the soil and of the transpiration stream of the plant.

Both solutions and contact mechanisms operate in the soil. As far as major nutrient cations are concerned, the soil solution would contribute a relatively smaller amount of the total absorbed by the plant. The lower the salt content of the soil solution, the greater will be the relative importance of contact exchange with the soil colloids. For those micro nutrient ions, such as iron, which are largely insoluble at higher pH values, contact exchange may well be the dominent mode of their acquisition by plants.

The direct application of the theory of the metabolic absorption of ions to the problem of nutrient absorption from soil is seen to be somewhat more difficult than to the problem of absorption from solutions.

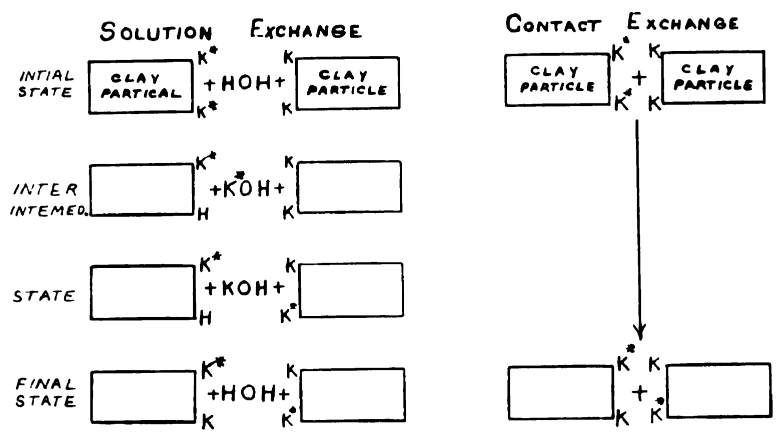

Figure 2. Diagrammatic representation of absorption by contact-exchange and from solutions. (Jenny and Overstreet, 1938)

But since the metabolic activities of the absorbing cell undoubtedly are involved in the entry of ions into the plant, irrespective of external conditions, it is apparent that all factors affecting metabolism will also influence absorption.

C. Internal Factors Affecting the Concentration of Nutrients in Plants

1. Species and Variety

Drake and Scarseth (1940) have emphasized that plants of different genera may differ profoundly in their nutrient uptake, and Wimer (1937) pointed out that this may be true also for species of the same genus or even for varieties of the same species. Many workers have reported their observations on the different amounts of the nutritional ions contained in plants of different species grown under identical conditions.

The differences in the composition of plants has been studied ever since chemical analyses of plants were first carried out. Arendt (1859) stated that "our literature is over rich in ash analyses of many kinds but they may be briefly summarized as follows: Plants must have a certain amount of inorganic nutrients. This amount is different for different plants. The number of inorganic elements is about the same in all plants, although their relative amounts differ due to the various chemical and physical natures of the soil and due to the kind of plant and due to the age of the plant and due to the various natures of the different organs." Wolff (1871, 1877) in his book "Aschen Analysen" compiled the results of innumerable analyses of plant materials reported by various authors from 1800 to 1880. Although

in some cases, the analytical methods were crude, Wolff's tabulated data indicated that the ash content varied in different kinds of plants, in different stages of their growth, and in different localities. Since this early compilation, differences in the chemical composition of different species have been noted by many workers, even when plants were grown under similar conditions. Richardson (1920) determined the composition of the ash of certain dune plants and in the sand in which they grew. The most striking differences observed in the proportion of elements absorbed was in the amounts of silicon, calcium, and aluminium, although differences were also noted in potassium and magnesium. Working with alfalfa and tobacco grown on soils containing both lithium and strontium, Headden (1921) found that the alfalfa had little lithium but a considerable amount of strontium in the ash, while in the case of tobacco the reverse was true. Skinner and Reid (1921) reported differences in the mineral content of wheat and clover grown in the same nutrient solutions. Greaves and Nelson (1925) obtained similar results. Newton (1928) grew sunflowers, beans, wheat, barley, peas and corn in the same container. He harvested the wheat and barley at the heading stage, the corn at the time of tasseling, and the peas and beans at the blooming stage, and then determined the percentage of the various nutrients present in the plants. His results are summarized in table 2. Not only did Newton detedt differences in the various species, but also found that the absorption of nutrients from the culture solution was greater than from a loam soil. He did find, however, that there was less variability in the amounts absorbed

Table 2. Chemical composition of various crops grown in nutrient solution in the same container. (Newton, 1928)

Crop	Composition as Percent of Dry Matter				
	Ca	K	Mg	N	P
Sunflower	2.2	5.0	0.64	3.6	0.56
Beans	2.1	4.0	0.59	3.6	0.55
Wheat	0.8	6.7	0.41	4.5	0.49
Barley	1.9	6.9	0.54	4.7	0.52
Peas	1.6	5.3	0.50	4.5	0.19
Corn	0.5	3.9	0.40	2.9	0.39

from the solution than from the soil.

Trelease and Martin (1936) reported that Western wheat grass, Agropyron smithii Regdb., grown on various soils accumulates from 1 to 60 parts per million of selenium, while Astragalus bisulcatus A. GRAY grown on the same soil accumulated from 200 to 4300 parts per million. Daniel (1934) found that legumes ordinarily contained 3.9 times as much nitrogen as did grasses. Plants that were low in calcium and phosphorus remained low in those elements, even when grown in fertile soils. Those plants containing relatively high amounts of these elements always contained relatively large amounts even though grown on a poor soil.

It has been established that the variety of a plant also exerts an effect on its composition. Wolff (1877), from his study of the data in "Aschen Analysen", suggested that there were differences in composition depending upon variety. Since 1930, much information on the varietal difference in chemical composition of plants has been published. Such differences, probably governed by genetical factors, have been reported in oats, (Lundegardh 1931, 1932); apple, (Wallace 1932; Hill, Johnson, Heeney and Buckmaster 1951; Piper, 1936); wheat, (Maume and Dulac 1934, 1934a, 1935a, 1936, 1937, 1937a, 1938a); and sugar cane, (Borden 1936; Beauchamp and Alvarino 1940). Even the rootstock has been shown to have an effect upon the composition of the plant budded or grafted on it (Roach 1931; Hill unpublished data).

The work of Yates and Watson (1939) showed that the differences between some varieties of a species may not be significantly great, in contrast to the rather large differences reported by most workers.

These varietal differences in the chemical composition of plants make it essential to consider their magnitudes in any attempt to develop biological techniques for evaluating fertilizer requirements. The effects of variety on the percentages of nutrients in plant tissues implies the necessity of determining standard concentrations for each variety. The published literature seems to indicate that these varietal differences are merely expressions of the ability of plants to absorb nutrients, and do not reflect necessarily their specific physiological needs.

As with any biological study a definite opinion cannot be expressed concerning the necessity of setting up standards of composition for each variety of plant. A number of workers have considered that their data indicate that, in some cases at least, varietal differences and even species differences may be so small that general conclusions are possible. For instance Craig (1941) concluded the same optimal values for nutrient concentrations in sugar cane could be used for any of the studied varieties of this crop. The results obtained by Lundegårdh (1941) for oats were considered by him to apply to other cereals as well. Wallace (1941) and Wallace and Osmond (1941) considered that, in general, the minimum levels for the concentrations of potassium and magnesium found for apple trees would be generally applicable to other fruit trees. Crowther (1936), working with cotton, showed that while some varieties had a particularly high nitrogen content, they responded to nitrogenous fertilizer to the same extent as did varieties normally containing much lower percentages of nitrogen.

Even if it is admitted that there are a few instances in which generalities can be made, one must conclude that critical values must be related to a comparatively closely related group of plants, and must be applied to different varieties of the same species with care.

3. Tissues

There has been a considerable number of data reported that indicate that the various tissues within a plant differ in their chemical composition.

As early as 1804, De Saussure noted that differences existed in chemical composition of the ash of leaves, branches, wood, bark, flowers, and fruit of seven different species of trees and also in the ash of straw and grain of wheat, corn, barley and oats. This tabulation probably was the first of its kind and it led to the sampling techniques used at the present time. During the nineteenth century the significance of De Saussure's data was not fully recognized and only isolated workers paid attention to them.

In 1859, Arendt studied oats, and he analyzed the ears, the two upper leaves, the three lower leaves, the highest stem internode, the two middle internodes, and the three lower internodes of the plant at five different stages in the development of the plants. Arendt's data not only confirmed the variations reported by De Saussure, but also indicated the striking differences between the samples taken at different periods during the growing season. Wolff (1877) also conducted experiments with oats growing in water cultures. He supplied successive increments of single nutrients under conditions of an adequacy of the others. After analyzing the grain, straw, chaff, and the roots, he came to the conclusion that minimum concentrations of nutrients could be specified for the aerial parts of the plants. A condensation of his data appear in table 3.

The results of Wolff, Arendt, and De Saussure convinced many workers that the analysis of the whole plant provided the best index to its nutritional status. Consequently, before 1900 Helmkampf (1892) and others followed this procedure because, they argued, a composite sample of the plant averaged the variations in its tissue. Other workers, for example Heinrich (1882) and Atterberg (1901), verified the work of De Saussure and believed that consideration should be given to the differences in the nutrient concentration occurring in specific portions of the plant. Atterberg concluded that the differences in the percentages of nitrogen and phosphorus were greatest in oat grain, while analysis of the straw, or of whole plants, indicated the greatest differences in potassium content. Remy (1906, 1906a), working with various plants, reported that the concentrations differed in different tissues and believed that a

Table 3. Necessary minimal concentrations of the six major nutrients in oats, as well as concentrations required for good average development. Based on analyses of the whole mature plants grown in water cultures. Concentrations expressed as percentages of the dry material. (Condensed from Wolff 1876, 1877)

	Composition					
	N	P	K	Ca	Mg	S
Necessary minimal concentrations	0.6	0.15	0.42	0.11	0.06	0.04
Necessary concentrations for good average development	1.0	0.22	0.66	0.18	0.12	0.08

selection of tissue would indicate the nutritional status of the plant. Pfeiffer, Simmermacher, and Rippel (1919), working with oats, and Cook (1930), working with wheat and rye, again showed that the different tissues varied in their chemical composition at various stages during the growth of the plant.

In a search for more accurate correlations between the nutrient contents of crops and their responses to fertilizers, workers have shown, incidentally, many examples of differences in the percentage compositions in closely related tissues. Wallace (1928, 1929), working with gooseberries, found significant differences in the laminae and petioles of leaves. Emmert (1931), working with tomatoes and lettuce, found that the conducting tissues, petioles of tomatoes, and midribs of lettuce, all contained higher concentrations of nutrients than did the blades of these crops. Lagatu and Maume (1930d) even showed differences in the concentration of nutrients in the leaves on a single branch of the grape. The concentration was greater in younger than in older leaves. Ulrich (1948) reported that the potassium content differed in the petioles and leaf blades of grapes and that the effect of fertilizer applications was expressed more clearly in the petioles. Whereas there were only small differences in the nutrient concentrations in the blades of grape leaves as the result of potash fertilization as indicated in figure 3, the differences evident in the petioles were quite large as indicated in figure 4. Ulrich's data also show that the leaf blades seldom contained nitrates, whereas the petioles did.

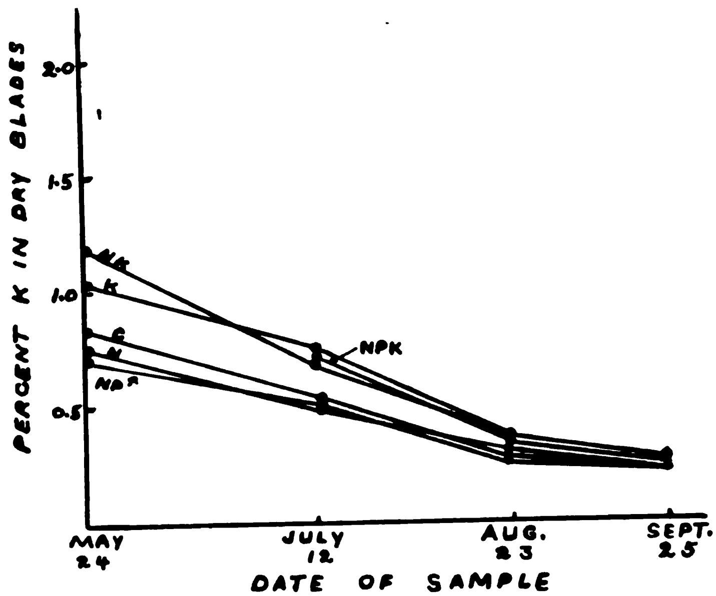

Figure 3. Potassium concentration in the blades of recently matured leaves of Petite Sirah grapes in Alexander Valley, California in 1940.

(Ulrich, 1942)

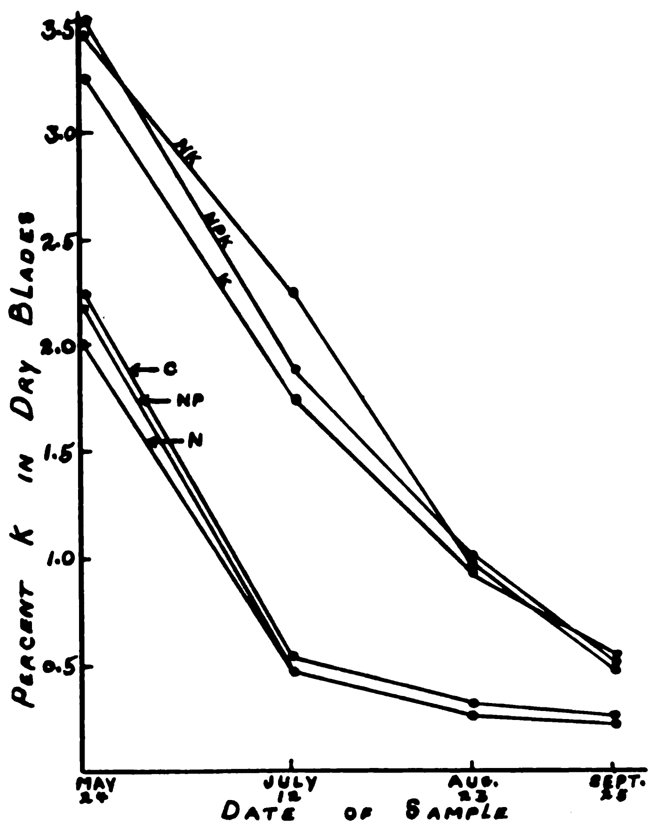

Figure 4. Potassium concentration in the petioles of recently matured leaves of Petite Sirah grapes in Alexander Valley, California in 1940, showing differences in its concentration induced by potash fertilizer. (Ulrich, 1942)

Thornton (1932) and Hoffer (1926) considered that their field testing methods could be properly interpretated only if specific tissues were sampled. Lagatu and Maume (1933) found differences between the leaves on fruiting and non-fruiting branches on the same grape vine. Sturgis and Reed (1937) reported that the nitrogen and phosphorus content of the leaves and stems of rice was only one-third that of the panicle. Raleigh and Chucko (1944), studying tomatoes, concluded that variations in the nutrient supply exerted a smaller effect on the composition of fruits than on either the vines or the roots of the plant.

A few recently reported data reveal differences in the concentrations of nutrients even within an organ itself. Emmert (1931) presented evidence that the composition of the edge of a leaf of lettuce more nearly indicated the nutritional status of the plant than did the central region of the lamina. Nightingale (1942, 1942a) found differences between the meristematic base of the leaves of pineapple and the remainder of the leaf. Subsequently he sampled only the meristematic region as it appeared to reflect differences in the effects of fertilizers to the greatest extent.

The differences in percentages of nutrients in various tissues presents a serious problem to the use of tissue analysis for the determination of fertilizer requirements, and the most suitable tissue for analysis must always be selected. The significance of this situation will be discussed in a later section, and it is sufficient for our purpose to emphasize that the majority of investigations have shown that the analysis of whole plants is unsatisfactory.

3. Diurnal Rhythms

a. Nitrogen

It was apparent to the early workers in the field of plant nutrition that consideration should be given to the effect of the time of day on the nutrient content of plant tissues, but in this early work, there were expressed differences of opinion concerning the nature of these variations. Kosutany (1897) (see table 4 and figure 5), Otto and Kooper (1910), Kooper (1910), and Susuki (1897) found increased amounts of nitrogen in plants during the night, while Schulze and Schutz (1909), and Pigorini (1914) found a decrease. Nightingale (1927) found that the period of daily illumination of tomato plants caused variations in their carbohydrate and nitrogen contents. Scarseth (1943) found that plants suffering from inadequate nitrogen had virtually no nitrate during the afternoon although he obtained positive tests during the early morning.

Diurnal variations were shown also by Woodward, Shepherd and Tysdal (1944) in the percentages of nitrogen in the dry matter of alfalfa. Their data are summarized in table 5 and figure 6.

b. Phosphorus

McCool (1926) working with the extracted saps of various plants, and Phillis and Mason (1942) working with cotton leaves found that the concentrations of phosphate in the sap and the percentage in the leaves were higher during the morning than after mid-day, which indicated a

Table 4. Variations in the percentage of nitrogen in the leaves of the grape during the day and night through the growing season. (Kosutany 1897)

Date	Percentage nitrogen in dry material	
	Day	Night
June 4	4.161	4.616
June 19	4.254	4.276
July 3	3.829	4.031
August 14	3.406	3.461
August 28	3.994	4.048
September 25	3.153	3.119
October 9	2.680	2.258
Average	3.581	3.687

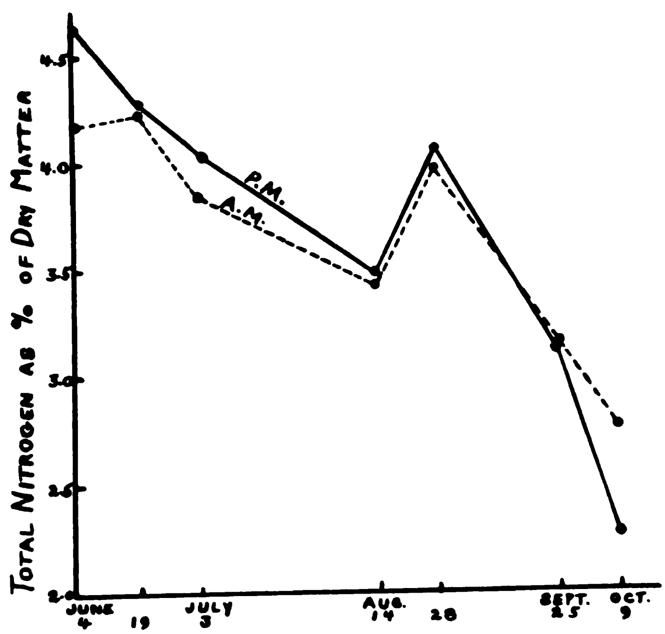

Figure 5. Variations in the percentage of nitrogen in the leaves of the grape during the day and night through the growing season. (Kosutany, 1897)

Table 5. Percentages of nitrogen in the dry matter and of the ash in the dry matter of alfalfa harvested at 2 hour intervals over a 24 hour period. May 30 and 31, 1944. (Woodward, Shepherd, and Tysdal 1944)

Hour of day	Percentage	
	Nitrogen	Ash
7 AM	2.55	7.20
9	2.68	6.48
11	2.60	6.12
1 PM	2.60	6.14
3	2.47	6.09
5	2.54	5.84
7	2.50	5.99
9	2.52	6.93
11	2.54	7.34
1 AM	2.60	7.92
3	2.66	8.67
5	2.62	8.08
7	2.49	7.66

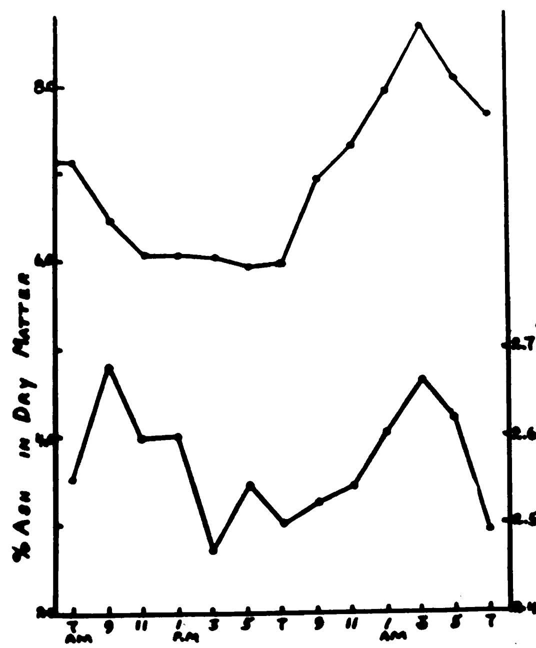

Figure 6. Variations in the percentages of nitrogen and of ash in the dry matter of alfalfa harvested at two hour intervals over a 24 hour period. May 30 and May 31, 1944. (Woodward, Shepherd, and Tysdal, 1944)

decrease during the day followed by an increase during the night. Previously, Schulze and Schutz (1909) had reported that the percentages of phosphate in the leaves of the Box Elder were higher in the afternoon throughout the growing season.

c. Potassium

A few data showing the diurnal variation in the potassium content of plants have been reported in the literature. Schulze and Schutz (1909), working with the Box Elder, noted that the potassium content in the leaves expressed as the percentage of the dry matter was higher in the afternoon than in the morning. Penston (1935, 1937, 1938) found that the amount varied from 2.5 to 4.1 percent in potato leaves and from 3.7 to 4.9 percent in maize over a 24 hour period.

d. Ash

Less attention has been paid to the diurnal rhythms of the total ash content of plants than to its individual components. Woodward (1944) found that the total ash in alfalfa varied by as much as 40 percent of the mean depending upon the time of day when the material was harvested. His data, presented in table 5 and figure 6, show that the lowest percentage of total ash occurred at five P.M. and the highest at three A.M.

While there are conflicting reports concerning the magnitude of diurnal variation of the total ash content of plants, such variations do exist. Consequently, the diurnal factor must be considered in the use of any procedure for diagnosing the nutrient status of plants by the chemical analysis of their organs or tissues.

4. Season and Stage of Development

a. Introduction

Data showing seasonal variations in the chemical composition of plants frequently are misleading for there are two unrelated factors involved.

The seasonal factor actually is a composite of such individual factors as length of day, temperature, humidity, precipitation, etc. If a seasonal effect on the chemical composition of plants becomes evident, it is difficult indeed to generalize as to the cause. For example, the high temperatures in August, or the midsummer drowth, or some other factor, or combinations of factors may exert the dominant influence.

But while the biotic factors comprising the seasonal environment of plants is passing through its normal, or abnormal phases in some cases, the plants themselves are changing as they proceed with their development. It becomes impossible, therefore, to separate the relative importance of seasonal factors, *per se*, from the effects of the advancing maturity of the crop.

Strictly speaking, seasonal effects should be regarded as separate from the developmental factor associated with the plants themselves, but this is not possible when crops are studied under field conditions.

Perhaps true seasonal effects on the composition of crops could be better studied by comparing crops grown in successive seasons, rather than by comparing them through a given season. But arbitrarily, the data that have been reported on the basis of the date of sampling are grouped as seasonal in our discussion.

b. Nitrogen

Over a period of years much information has been obtained indicating that the concentration of the components of plants vary with the date of sampling throughout the season. Remy (1896) using wheat, oats, and barley, Kosutany (1897) using the grape, and Schulze and Schutz (1909) using the leaves of the Box Elder, all reported decreases in the percentage nitrogen in the dry matter of the plants as the season progressed and the age of the plant increased.

Liebscher (1887), using oats, barley, and wheat, reported that in the early part of the season the accumulation of nitrogen was relatively higher than of total organic matter, whereas in the period of seed formation the accumulation of total organic material was greater. His data are presented in abbreviated form in table 6 and figure 9. Adorjan (1902) also showed that wheat, previous to the jointing stage, contained the greater percentages of nitrogen. In fact, he reported that the uptake of nitrogen was three times greater during this stage than during the later stages of growth. This worker stated, "In youth,

Table 6. Relation between the number of days after germination and the relative yield in dry matter, nitrogen, P_2O_5, and K_2O for various crops as reported by various authors. The maximum amount of each constituent is taken as 100.

Oats (Liebscher, 1887)					Barley (Pfeiffer et al, 1921)					Corn (Salter and Ames, 1928)				
Days after germination	Dry matter	N	P_2O_5	K_2O	Days after planting	Dry matter	N	P_2O_5	K_2O	Days after planting	Dry matter	N	P_2O_5	K_2O
18	20.3	45.8	46.3	74.0	17	0.3	1.3	0.6	0.6	10	0.3	0.1	0.6	0.9
47	37.9	67.9	63.3	93.0	35	2.8	12.0	5.5	8.1	34	1.8	2.0	2.7	4.1
67	80.3	84.1	86.8	100.	49	14.7	37.1	23.2	49.8	48	4.1	10.1	4.5	10.0
93	100	100	100	74.7	68	49.3	67.8	46.2	77.7	62	9.9	19.8	12.6	25.2
--	--	--	--	--	88	89.2	82.5	79.6	100	76	22.5	29.7	30.6	42.3
--	--	--	--	--	107	100	100	100	92.9	90	34.2	44.1	50.4	51.3
--	--	--	--	--	--	--	--	--	--	104	64.8	67.5	72.0	56.7
--	--	--	--	--	--	--	--	--	--	111	100	100	100	100

wheat takes up most of its nutrients, stores them, and then uses them for kernel formation at a later time."

More recent work, however, has presented the opposite view. Salter and Ames (1928) concluded that in a general way, the rates at which nutrients are absorbed bear no constant relationship to the rate at which plants increase in dry matter and that the nutrient absorption proceeds at an ever increasing rate throughout the season. The view of Salter and Ames is supported by their data which have been partially reproduced in figure 7 and table 6, and also by the data of Pfeiffer, Simmermacher and Rippel (1919), and of Pfeiffer and Rippel (1921) which are shown in table 6 and figure 8. These data present a striking contrast to those of Liebscher presented in table 6 and figure 9. This earlier work indicated that the greatest portions of dry matter, nitrogen, P_2O_5, and K_2O were accumulated during the initial stages of plant development. The more recent work of Salter and Ames (1928), of Pfeiffer and his co-workers, and of others shows evidences indicating that not only is this not true, but that the reverse situation actually is a more accurate picture. The true significance of these data is that they show not only different levels of the various components in the plants through the season, but also show that these components are absorbed at varying rates through their life cycles.

The majority of the work discussed was based on cereals. At the present time, more consideration is being given to the horticultural annual and perennial crops. It is apparent, that the seasonal fluctuations of the nutrient components in cereals also occur in other

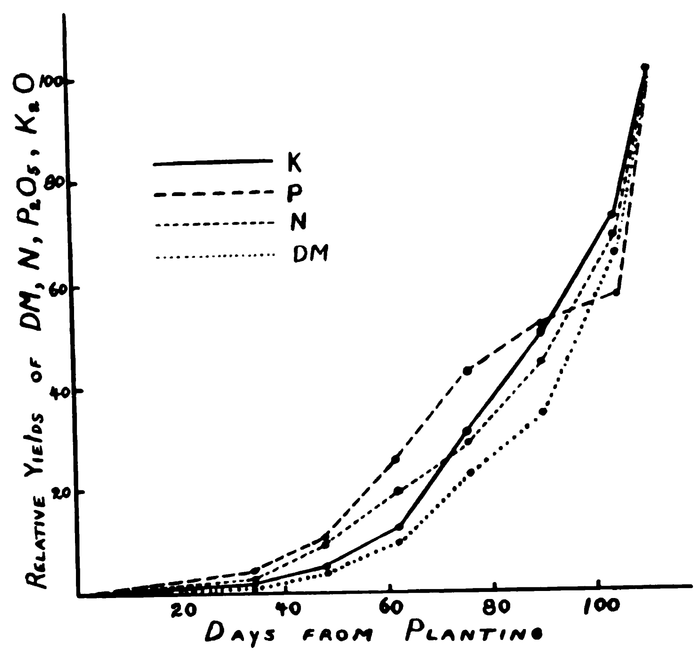

Figure 7. Total dry weight, nitrogen, phosphorus, and potassium in corn grown at Wooster 1927, and sampled at various times throughout the season, expressed as percentages of their maximum values. (Salter and Ames, 1928)

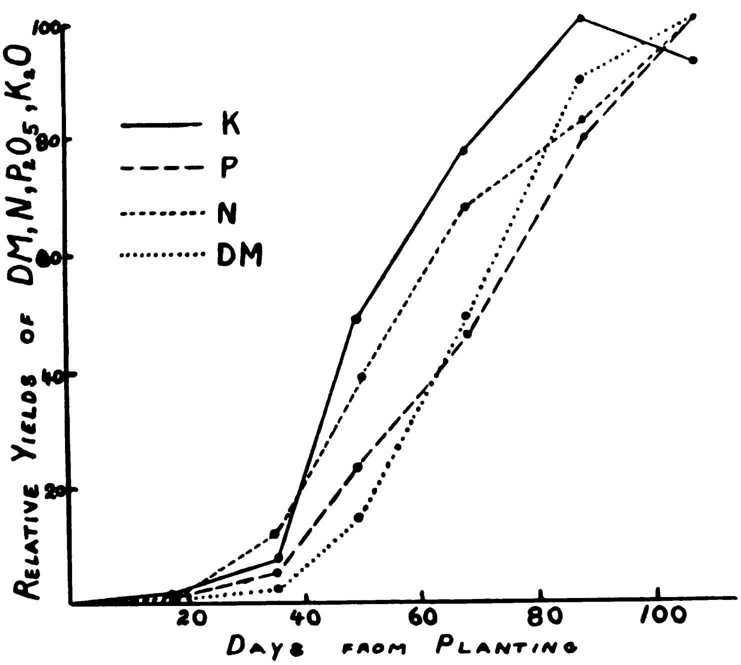

Figure 8. Total dry weight, nitrogen, phosphorus and potassium in barley grown at Breslau, 1919, expressed as percentages of their maximum values. (Pfeiffer and Rippel, 1921)

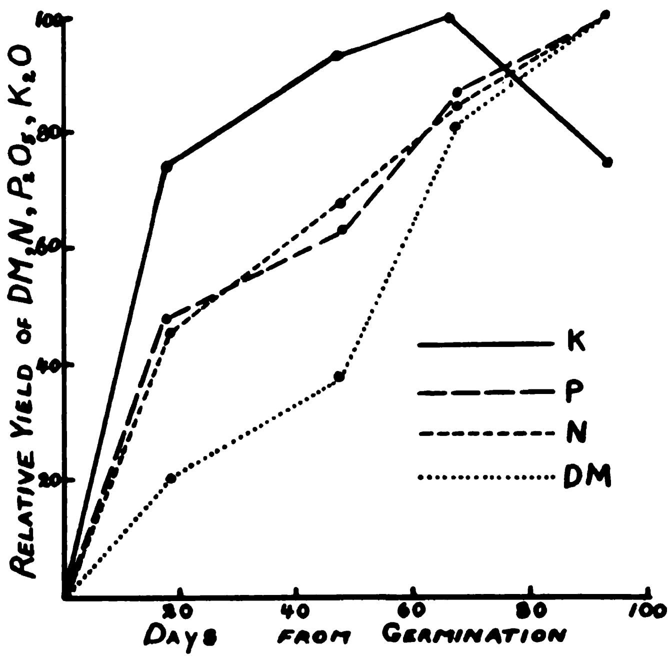

Figure 9. Total dry weight, nitrogen, phosphorus and potassium in oats, expressed as percentages of their maximum values. (Liebscher, 1887)

crops. Murneek (1930) concluded that the growth and development of the apple tree in early spring is largely supported by the nitrogen stored in the tree during the previous year. Consequently, all woody structures of the tree showed a progressive decrease in nitrogen content from early spring until active growth had ceased. The non-bearing spurs had a maximum nitrogen content at the time of bud swelling and a minimum content when vegetative elongation had ceased. There was a seasonal percentage decrease in the nitrogen in the leaves. The value decreased from 4 to less than 1 percent. Murneek found that from 35 to 45 percent of the total nitrogen in the leaves was transported back into the tree prior to abscission. The soluble nitrogen in the leaves usually increased during this period while the insoluble fraction decreased.

Mulay (1931, 1932), studying a six year old Bartlett pear tree, found that the percentage of total nitrogen in the shoots began to increase at the end of October, reached a peak in December, remained constant until February, and sharply decreased in March when new growth began. In May it had reached a minimum.

The "foliar diagnosis" technique developed by Lagatu and Maume (1934, 1937, 1937a), Thomas (1945), and Thomas and Mack (1940, 1944) has been used over a period of years to show that the concentration of nitrogen and the relative amounts of nitrogen in the group of components, NPK, vary through the season. An example of this seasonal variation is shown in table 7 and figure 10. These data were obtained from

Table 7. Concentrations of calcium, nitrogen, phosphorus, and potassium, expressed as percentages of the dry matter, in the third leaf from the base of the main stem of the potato at various dates through the growing season. (Lagatu and Maume 1930)

	May 2	May 22	June 2	June 11	June 19
Check plot					
Ca	5.65	5.28	8.40	9.40	9.40
N	3.45	2.56	2.22	1.98	1.92
K_2O	5.60	4.61	4.72	4.62	5.01
P_2O_5	0.88	0.77	1.04	0.82	0.87
Nitrogen treated plot					
Ca	6.60	8.47	9.74	9.31	9.12
N	4.35	3.11	2.80	2.52	2.55
K_2O	4.80	4.90	3.81	3.66	3.81
P_2O_5	0.62	0.51	0.79	0.39	0.38

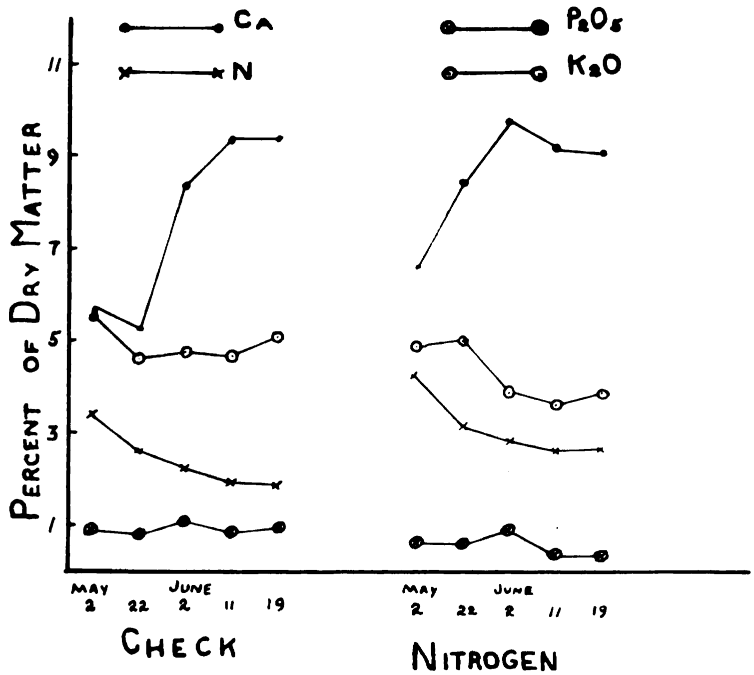

Figure 10. Concentrations of nitrogen, calcium, potassium and phosphorus expressed as percentages of the dry matter of the third leaf from the base of the stem of the potato through the growing season. Samples taken from a plot receiving nitrogen and from one receiving no fertilizer. (Lagatu and Maume, 1930)

two plots, one unfertilized and one treated with nitrogen. The fertilized plot yielded 264 pounds more potato tubers than did the unfertilized. Table 7 and figure 11 show that the nitrogen decreased in its percentage of the dry matter as well as in its relative amounts with respect to the other elements.

c. Phosphorus

Analytical data based on many samples of many plants reported by various workers indicate that the concentration of phosphorus in plants exhibits less variation than do the other major nutrients.

In a study of the composition of the leaves of the Box Elder, Schulze and Schutz (1909) found a gradual decrease in the percentage of phosphorus from 1.697 percent of dry matter in May to 0.374 percent in September. Since this early work, the gradual decrease in the phosphorus concentrations in tissues during the growing season has been confirmed by many investigators. Pfeiffer et al (1919), for example, reported data showing that the phosphorus concentrations, expressed as percentages of P_2O_5 in the dry matter of barley leaves, decreased from 01462 percent in May to 0.178 percent on July 23. The phosphorus content of the straw showed the same relative decrease during the progress of the season. The percentage in May was 0.624 and on July 23 it was 0.2626. In a later report covering the three-year period from 1918 to 1920, Pfeiffer et al (1921) confirmed the previous

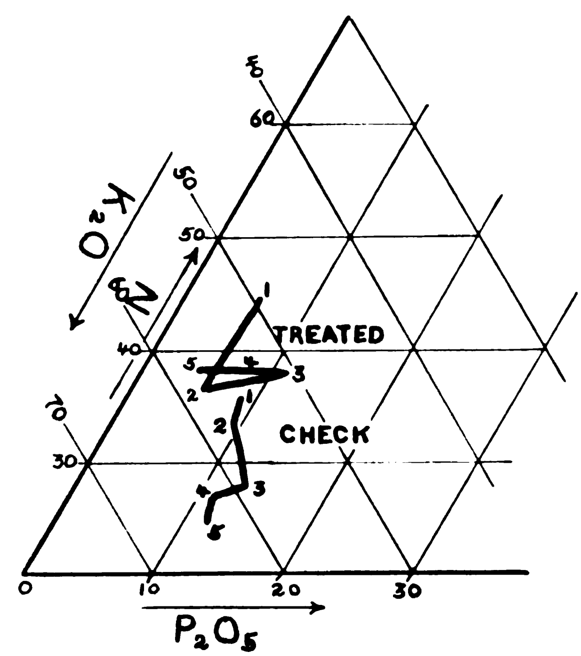

Figure 11. Seasonal effect of the amount of nitrogen, P_2O_5, and K_2O in the NPK-unit in the third leaf from the base of the stem of the potato through the growing season , Montpellier 1928. The numbers 1, 2, 3, 4, and 5 represent the sampling dates given in table 7. (Lagatu and Maume, 1930)

conclusion that the percentage of phosphorus in the dry matter decreased through the season.

However, there are data conflicting with those of Pfeiffer, for Adorjan (1902) showed that, during the jointing stage, there was a high rate of phosphorus absorption which was related to the requirements of the inflorescence. After jointing, no significant amount of phosphorus was absorbed.

More work has been done on the seasonal fluctuations in the concentration of phosphorus in plant tissue by the use of the foliar diagnosis methods than by other procedures. Data of this type have been reported by Lagatu and Maume (1934), Thomas (1945), and Thomas and Mack (1940). The work of Lagatu and Thomas does, in a general way, confirm the decrease in the concentration of phosphorus with the advancing age of the plant tissues; they also make it evident that this decrease may not always occur because of the influence of climate. Some of their data showing the complexity of seasonal phosphorus fluctuations are given in table 7 and figures 10 and 11. In 1928, the phosphorus concentration in potatoes reached a maximum value in the tissue on about June 2 in both the treated and the untreated crops, and decreased after this date. Figure 11 illustrates this same effect is seen in the relative amounts of phosphorus in the unit, NPK, in the plants. During this work, the authors noted that the temperature rose sharply around May 24. They believed that this sharp rise in temperature caused the deviation from the normal gradual decrease in phosphorus as growth progressed.

d. Potassium

Of the three nutrients, nitrogen, phosphorus, and potassium, potassium exhibits the most consistent relationship between its percentage in the plant and the advancing season. Investigators consistently have reported decreases in potassium concentration with advancing season, among them Schulze and Schutz (1909) working with the leaves of the Box Elder, Liebscher (1887) working with oats, Pfeiffer et al (1919, 1921) working with barley, and Salter and Ames (1928) working with corn. Some of the data of these workers are assembled in table 6 and figure 7.

Lagatu and Maume (1930, 1934) considered that, while there was a gradual decrease in the potassium concentration in potato tissue, this decrease was not as striking as that of nitrogen. This conclusion was based on the data in table 6 and figure 10. These workers also showed by the data presented in figure 11 that the amount of potassium relative to total nutrients of the group NPK decreased as the season progressed. Ulrich (1942) concluded from his data based on the blades and petioles of Petite Sirah grapes in 1940 that the percentage potassium in the dry matter decreased through the season even though his experimental plots had been variously fertilized. Many workers have described this effect of season, among whom are Burd (1919), Duley and Miller (1921), and Knowles and Watkins (1931).

While only the three major fertilizer components, nitrogen, phosphorus and potassium, have been discussed, the data presented emphasize that one of the most important factors involved in the determination of the fertilizer requirements of plants by biological responses in tissue composition is the seasonal variation of the concentrations of nutrients in the plants. The resolution of this problem is complicated further by seasonal variations in the supply of nutrients in the soil. Realization that such variations exist makes it apparent that the date of sampling influences the data obtained. Careful standardization of the sampling date, therefore, is necessary if comparable and interpretable data are to be gathered. A correct sampling date must take into consideration all of the factors affecting the concentration of nutrients in plants.

D. External Factors Affecting the Concentration of Nutrients in Plants

1. Climate

a. Illumination

A little work has been reported on the effect of illumination on the composition of plants. Although the data are not conclusive, they should be taken into consideration in any attempt to relate the chemical composition of plants to their fertilizer requirements.

Mitchell (1936) reported that the percentages of phosphorus and potassium in White Pine seedlings were influenced by the amount of illumination the plants received. Studies carried out by the Rhode Island Experiment Station showed that the nitrate content of the beet also was considered to be affected by light (R.I. Agric. Exper. Sta. 1935). Wark (1938, 1939) believed that cereals were so influenced by illumination that samples for analysis should be taken only in bright weather, if they were to be used for nutritional diagnosis. Pfeiffer, Blanck, and Friske (1913) and Pfeiffer, Blanck, and Flungel (1912) showed that shading of oats growing in pot culture induced an increased percentage of nitrogen in the straw. These data are presented in condensed form in table 8.

b. Water

Tollens (1902) reported the results of his work on potatoes, which had been published previously by Drszewski and Tollens (1900), which indicated that the percentages of total ash increased in potato tubers as the available water in the soil increased; and he concluded that the percentages of nitrogen, potassium, and phosphorus in the ash, as well as the amount of dry matter, are all influenced by plant food in the soil and also by its water content. Pfeiffer, Blanck and Flungel (1912) grew oats in pot cultures receiving different amounts of nitrogen, and he observed that excess water appreciably decreased the percentage of nitrogen in the dry matter of the straw. The data are condensed in table 8. Pfeiffer, Blanck, and Friske (1913) carried out an extensive investigation covering the growth of oats in seven different soils, each at four levels of moisture. The data summarized in table 9 show that the percentage nitrogen is decreased while that of phosphorus is increased by additional water. Potassium appears to

Table 8. Percentages of nitrogen in the straw of barley, grown in pots, as influenced by increasing amounts of water. (Pfeiffer, Blanck, and Flungel 1912)

Nitrogen added (grams)	Percentage nitrogen in dry straw			
	Intermittent water	Moderate water	Excess water	Excess water Plants shaded
0	0.58	0.50	0.11	0.57
0.355	0.63	0.57	0.108	0.57
0.710	0.82	0.55	0.308	0.62
1.065	0.76	0.79	0.506	0.76
1.420	1.01	0.87	0.659	0.91
1.775	1.71	1.09	0.885	1.01
2.110	2.00	0.98	0.835	0.87

Table 9. Effects of different moisture levels on the relative concentrations of nitrogen, phosphorus and potassium in oats. The concentrations obtained at the lower amount of water are taken as 100. (Pfeiffer et al 1913)

Year	Water level	N	P_2O_5	K_2O
1911	Low	100	100	100
	High	77	126	109
1912	Low	100	100	100
	High	81	134	112

be only slightly increased. These data confirm those of a similar nature obtained by Seelhorse and Tollens (1901), and later also those obtained by Pfeiffer et al (1919), Richards (1932), Yates and Watson (1939), Lilleland and Brown (1941), and by Greaves and Carter (1923). It is not possible, however, to know if this effect is due to the water alone or to the greater amounts of nutrients made available to the plant by the water.

c. Temperature

The literature does not reveal any information on the direct effect of temperature on the composition of plants. However, the fact that temperature might influence the percentages of the elements in the plant ash has been considered by a number of workers. For example, Tollens (1902) believed that the percentage of potassium, phosphorus, etc. in plant ash as well as the amount of dry matter are influenced by variations in temperature, perhaps as they affect the soil. Fraps (1931) also noted that "the interpretation of chemical analysis as related to the capacity of the soil to supply plant food is affected by various factors including the temperature." In working with potatoes in 1928, Lagatu and Maume (1930a) obtained the following experimental data from which an effect of temperature may be deduced. The phosphorus content tended to reach a maximum value in all curves at a period between June 2 and June 19. A burst of growth of the potato plants also was apparent during the same period. The

average temperature until May 22 was 14° C but between May 22 and June 2 the average temperature increased rapidly to 21° C and remained approximately constant at 21° C after June 2. These workers considered that the deviations from the normal concentrations of phosphorus in the plants which occurred could be attributed to the influence of temperature alone.

d. Weather

Weather represents the combined effect of moisture, temperature, illumination, and all the other physical factors comprising climate. Certainly, this complex of factors induce important changes in the composition of plants and unfortunately its influence cannot be controlled in field experiments.

Lagatu and Maume (1934) aptly stated the influence of the physical factors constituting weather on the accumulation of nutrients in leaves as follows: "There exists what may be designated a 'chemism' due to the influence imparted by the fertilizer itself and also a 'chemism' due to the effects of meterological conditions."

Workers in the field of plant nutrition are well aware that the same fertilizer treatment may produce very different crop yields in meterologically different years. The "foliar diagnosis" technique of Lagatu and Maume has shown different percentages of nutrients in plants grown in different seasons varying in weather. They detected these differences in potatoes (1934a), and in the grape vine (1928).

Their data based on grapes are condensed in table 10 and figure 12, and they clearly show the effect of the climatic factors on the concentrations of nutrients in the leaves, even though the same plants were sampled during successive seasons. In a season of adequate rainfall and optimum temperatures (1924) the yield and the levels of nutrients in morphologically similar leaves were greatly increased (1925). These workers placed great stress on the necessity of evaluating climatic factors in any procedure involving the determination of nutrient requirements of plants by foliar diagnosis techniques. They stated (1934) that, "in an area which can give, for the same season, meteorological conditions that are very different from year to year, the problem of fertilizing a cultivated plant can not be definitely solved. Employment of fertilizers is speculative only, depending for its value on many factors."

Thomas and Mack (1941) also demonstrated the influence of the climate on the nutritional status of tomatoes grown in the greenhouse in the spring and fall, and reported striking differences between the percentage of nitrogen, phosphorus and potassium in the dried foliage. This work was conducted under the differences in such climatic conditions as light intensity and length of day occurring in the spring and fall in contrast to the previous work of Lagatu and Maume (1928) which was conducted under different conditions of moisture and temperature. The climatic effects are seen in table 11 and figures 13, 14, and 15. The yield was always higher in the spring than in the

Table 10. The effect of two meterological different seasons (1924 and 1925) on the nitrogen, phosphorus and potassium in the third leaf from the base of the main stem of potato plants grown on a check plot and a plot receiving a complete fertilizer. The data are expressed as percentages of dry material. (Lagatu and Maume, 1930)

	Season 1924 Annual rainfall 725.1 mls.				Season 1925 Annual rainfall 529.1 mls.			
	Date of sampling	N	K_2O	P_2O_5	Date of sampling	N	K_2O	P_2O_5
				Unfertilized plot				
Composition	May 10	4.40	2.05	0.89	May 11	1.98	1.66	0.50
	June 14	2.64	1.88	0.59	June 14	1.65	1.39	0.32
	July 12	2.55	1.65	0.51	July 12	1.50	1.50	0.26
	Aug. 9	2.00	1.42	0.44	Aug. 9	1.20	1.45	0.14
	Sept. 13	2.20	1.14	0.44	Sept. 13	1.35	0.83	0.13
				Fertilized plot (Annual application since 1919--------80 Kg. N, KNO_3 consisting of 80 Kg. nitrogen 90 Kg. K, K_2SO_4 75 Kg. P, Superphosphate				
Composition	May 10	4.5	4.37	0.74	May 16	2.59	1.98	0.80
	June 14	3.14	4.12	0.58	June 14	2.15	1.71	0.70
	July 12	3.04	4.05	0.54	July 12	1.95	1.66	0.50
	Aug. 9	2.78	3.15	0.51	Aug. 9	1.30	1.73	0.36
	Sept. 13	1.62	2.32	0.32	Sept. 13	1.75	1.80	0.25
Yield	Unfertilized	560 Kg.					440 Kg.	
	Fertilized	795 Kg.					540 Kg.	

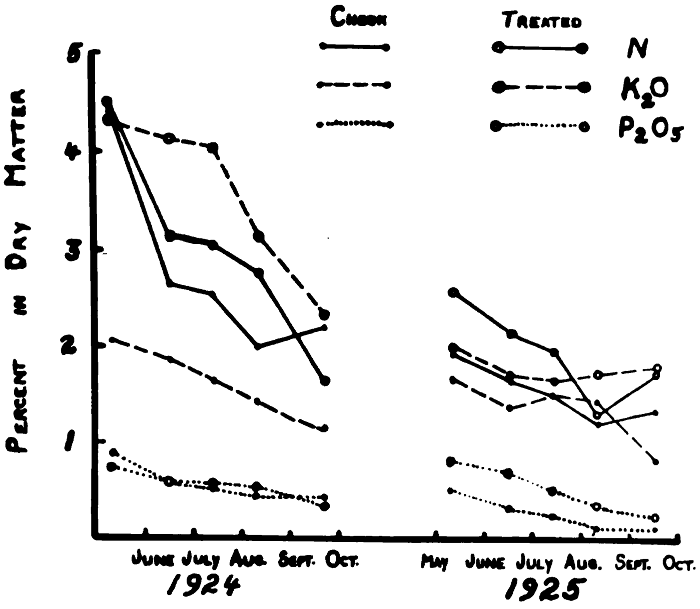

Figure 12. The effect of different climatic seasons on the yield and chemical composition of the leaves of the grape during the seasons 1924 and 1925. (Lagatu and Maume, 1928)

Table 11. Percentages of N, P_2O_5, and K_2O in the dried foliage, milligram equivalents, and the composition of the NPK-units of the fifth leaf in the spring and in the fall of tomato plants receiving the treatments shown. (Thomas and Mack, 1941)

Date of sampling	Mineral content of foliage				Milligram equivalents				Composition of NPK-units		
	N	P_2O_5	K_2O	N P K	N	P_2O_5	K_2O	S	N	P_2O_5	K_2O
	%	%	%	%	me	me	me	me			
Check Plot--no fertilizer plots											
April 5	3.96	1.87	1.49	7.33	282.7	79.1	31.8	393.7	71.8	20.1	8.1
April 29	2.90	2.50	0.69	6.09	207.1	105.7	14.1	327.5	63.2	32.3	4.5
May 27	2.14	3.07	0.65	5.85	152.8	129.7	13.8	296.3	51.6	43.8	4.6
Sept. 3	4.84	1.52	1.29	7.65	345.6	64.3	27.5	437.4	79.0	14.7	6.3
Sept. 23	3.66	1.85	0.90	6.41	261.3	78.3	19.3	358.7	72.8	21.8	5.4
Oct. 21	2.90	2.24	0.63	5.77	207.1	94.7	13.4	315.2	65.7	30.1	4.3
Phosphorus fertilized plots											
April 5	3.9	1.9	0.85	6.6	278.5	79.8	18.1	376.4	74.0	21.2	4.8
April 29	2.4	2.4	0.67	5.4	169.9	100.9	14.2	285.1	59.6	35.4	4.9
May 27	1.8	2.8	0.65	5.3	131.4	119.5	13.8	264.7	49.6	45.2	5.2
Sept. 3	4.9	1.6	1.7	8.2	349.8	67.9	36.3	454.1	77.0	14.9	8.0
Sept. 23	4.0	1.7	1.4	7.1	287.0	72.7	29.6	389.4	73.7	18.7	7.6
Oct. 21	3.1	1.9	1.0	6.0	224.2	80.6	21.3	326.1	68.7	24.7	6.5
(2N)PK fertilized plot											
April 5	4.6	1.2	1.9	7.7	328.4	51.3	39.9	419.6	78.3	12.2	9.5
April 29	3.3	1.6	0.98	5.9	237.0	67.7	20.9	325.6	72.8	20.8	6.4
May 27	2.7	2.2	0.92	5.8	194.2	91.1	19.7	304.9	63.7	29.8	6.5
Sept. 3	5.2	1.3	1.6	8.1	372.7	56.7	33.0	462.4	80.6	12.3	7.1
Sept. 23	3.9	1.8	1.3	6.0	282.7	74.7	27.5	384.9	73.4	19.4	7.1
Oct. 31	3.4	1.7	0.84	6.0	242.8	73.8	17.9	334.5	72.6	22.1	5.3

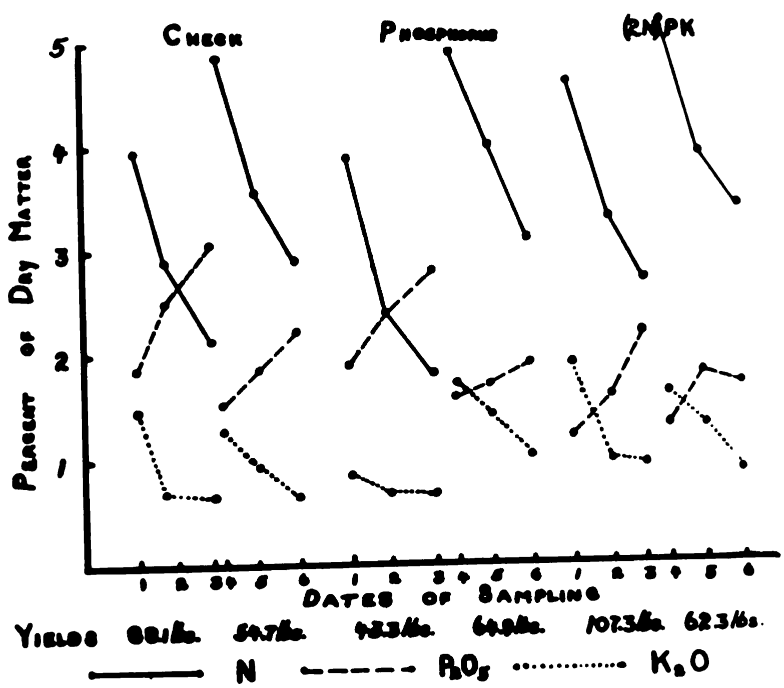

Figure 13. Changes during growth in the percentages of nitrogen, P_2O_5, and K_2O in the fifth leaf of Spring and Fall crops of tomatoes receiving the treatments indicated. Yields expressed in pounds per plot are indicated. (Thomas and Mack, 1941)

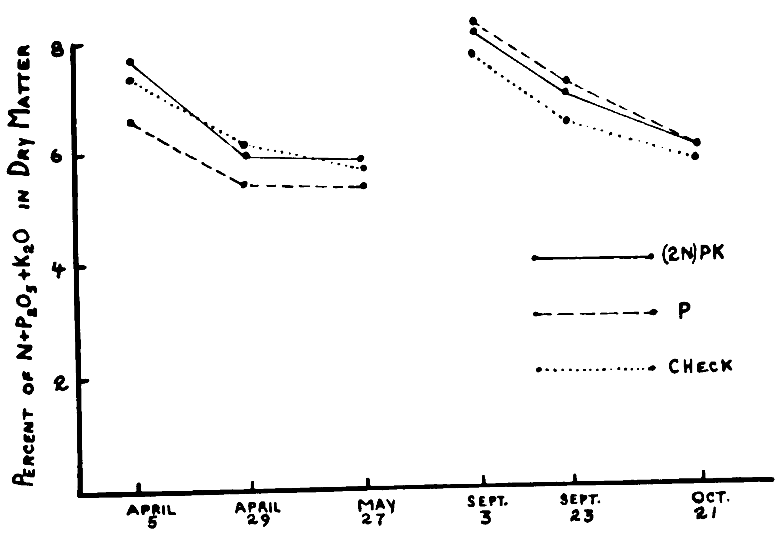

Figure 14. Percentages of $N + P_2O_5 + K_2O$ in the fifth leaf of tomatoes in the Spring and Fall, fertilized as indicated. (Thomas and Mack, 1941)

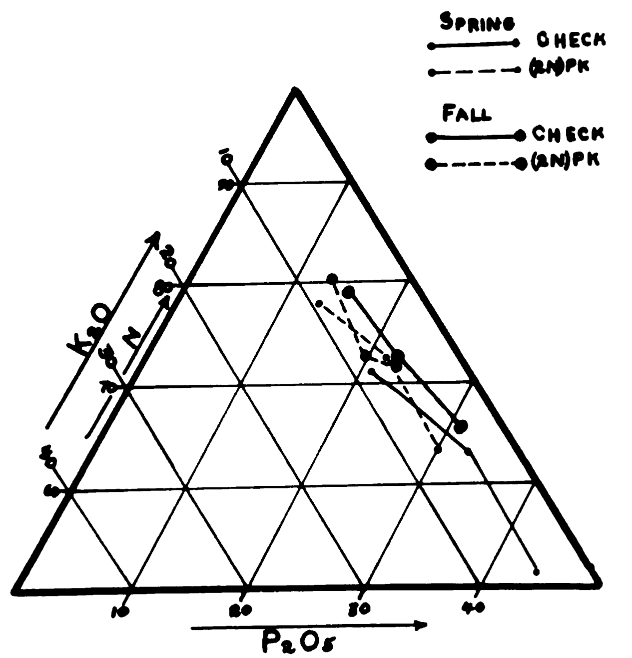

Figure 15. Changes during the growth of tomatoes in the N-P_2O_5-K_2O equilibrium in the fifth leaf. The plants were grown in Spring and Fall with fertilizer treatmnets as indicated. (Thomas and Mack, 1941)

fall except in the phosphorus series which suffered from the *Fusarium* wilt (figure 13). The concentrations of nutrients in the fifth leaf from the base was always higher in the fall than in the spring although its yield of dry matter is lower (figure 14). The relative percentage of nitrogen is higher and that of potassium is lower in the NPK unit during the fall (figure 15). The percentage of nitrogen in the dry matter of the leaf tends to be higher in all treatments in the fall, while that of potassium tends to be variable (figure 13).

2. Pests and Diseases

The effects of pests and diseases on the chemical composition of plants probably are those most frequently overlooked by students of the nutrient concentrations in plants. Eaton (1944) has shown that nutrient deficiency may sometimes result in an increased susceptibility to disease and this situation makes it difficult to separate the effects of nutrition from those of disease. It is, of course, true that diseases and pests may upset the nutritional status of the plant. The more apparent diseases and pests are easily recognized and may be avoided in the collection of samples, but real problems may arise when pathological conditions occur which are not evident to the person collecting the samples, and which may lead him to erroneously interpret nutritional data based on chemical analysis.

3. Management

The management, or "farm practice", frequently is overlooked as an important factor influencing all biological tests of soil fertility. The quantitative effect of this indefinite but important aspect of fertility studies is difficult to determine. Only a few data will be presented to indicate the subtle influence of the mechanical routines involved in field management.

Thomas, Mack and Cotton (1942) studied the effects of irrigation practices on tomatoes, and Thomas, Mack and Rahn (1944) reported differences in crop response due to the placement of fertilizer. A number of workers have reported differences in crop response and in the composition of plants due to the type of crop rotation and cover crop; and it is of course obvious that legumes will fix nitrogen which can be used by succeeding crops. Some crops have a considerable power of extracting nutrients from compounds in the soil that are relatively unavailable to other crops. Russel and Russel (1950) have shown that lupins are good extractors of phosphate. Rogers, Gall and Barnett (1939) suggested that the best way of preparing land for maize was to allow the native weeds to cover the land and then to plow them in. In this way the weeds were shown to accumulate and then to supply available zinc to the succeeding crop. They suggest also that alfalfa, which is a strong extractor of zinc, would be a valuable cover crop in orchards.

The effect of irrigation has been noted previously and examples have been given from the work of Pfeiffer et al (1912, 1913). This work, however, was carried out in pot cultures. Data based on field irrigation studies are presented in table 12, and they may serve as examples of the type of effects that might be attributed to this phase of field management. These data, taken from the publications of Greaves and Carter (1923) and Greaves and Nelson (1925), were based on the growth of wheat under irrigation at Logan, Utah. The results show that increasing amounts of irrigation water decreased the percentages of calcium, magnesium, phosphorus, potassium and total ash in the dry matter of both the straw and grain.

Lagatu and Maume have conducted experiments showing the effect of different sources of the three major nutrients on their concentration in the dry material of various crops; for example, the effect of various sources of nitrogen on the composition of grape leaves (1930b), various sources of phosphorus on tobacco (1935), and various sources of phosphorus on the grape (1936, 1936a) all were investigated. Their work shows that equivalent amounts of phosphorus or of nitrogen exerted a different effect on the concentration of these elements in the plants, if they are supplied to the plants in different compounds.

Lagatu and Maume (1934b) observed an increase in the percentage of nitrogen from 0.3 to 0.5 percent of the dry matter and an increase in the phosphorus from 0.1 to 0.3 percent due to tillage practices alone. They also observed that tillage practice increased the relative amounts of nitrogen and phosphorus in the NPK unit in the plants, while it decreased the relative amount of potassium.

Table 12. Percentages of nitrogen, phosphorus, potassium, calcium, magnesium, and total ash in wheat grain under irrigation at Logan, Utah. (Greaves and Carter, 1923)

Inches of irrigation water applied	Percentages in dry material					
	Ca	Mg	N	P	K	Ash
0	0.103	0.170	2.39	0.295	0.396	1.56
5	0.107	0.171	2.16	0.301	0.414	1.56
10	0.122	0.172	2.18	0.306	0.439	1.57
15	0.165	0.172	1.99	0.323	0.491	1.71
20	0.195	0.198	1.98	0.371	0.490	2.01
35	0.211	0.207	2.01	0.458	0.534	2.28
67.5	0.262	0.224	2.06	0.424	0.535	2.19

The time at which fertilizer is applied also is a phase of field management studied by Lagatu and Maume (1930c). Using the grape, variety Rupestris, they established five plots which were cultivated on Nov. 21, Dec. 21, Jan. 21, Feb. 22 and March 28 respectively. One-half of each plot received a complete fertilizer while the remaining half served as a control plot. The data show a significant increase in the amount of potassium absorbed from the fertilized plots when the fertilizer was applied Nov. 21, but this effect decreased gradually and steadily as the time of application was delayed from November to March.

The subtle effects of management factors often are overlooked in experiments with fertilizers. Certainly it must be borne in mind that the mere addition of fertilizers to a soil does not ensure a predictable rate of absorption of the nutrients by the plants.

4. Soil

The theory of the use of biological techniques to determine the amounts of plant nutrients in the soil, and to determine thereby the fertilizer requirements of crops depends on the ability of plants to truly reflect the nutrient content of the soil. Even if all factors were constant except the amount of available nutrients, the development of the root system would still influence the results. It is obvious that, if the development of the root system itself is a reflection of the availability of nutrients, then no real problem is involved, but if factors other than nutrients are acting upon the root, then a very real problem of interpretation may appear. Such non-nutritional factors as the presence of a hard-pan, a high water table, or a zone of toxic salt accumulation have been investigated by Lillel and Brown (1941).

Soils with adequate quantities of a given nutrient may be unable to supply an adequate amount for plant growth because of interference of other ions. For example, Johnson (1924) reported that the absorption of iron was prevented by high concentrations of manganese in the soil, and Boynton and Burrell (1944) found that magnesium deficiency was induced in apple trees on soils repeatedly fertilized with potassium salts. Pineapple plants have been shown to be unable to absorb nitrates from soils which were low in potassium. In this case, Nightingale (1942) considered that an adequate supply of potassium was necessary for normal nitrate absorption. Ulrich (1942) found that the addition of nitrates to the soil stimulated the absorption of potassium by the roots of grapes. It is probable that this was due to the greater development of the root system induced by the nitrogen.

These data emphasize that a very complex set of conditions controls the type and quantity of the various nutrients absorbed from the soil by the roots of plants. The ability of the plant to absorb nutrients in such a manner as to permit the proper interpretation of the complexity of the response of crops to fertilizers will be discussed in the succeeding sections of the present discussion.

III. FIELD TRIALS

A. Introduction

The basis of all biological methods for the determination of fertilizer requirements of crops is the use of plants as extracting agents. These procedures are in contrast to chemical methods of analysis which utilize various chemical solutions to simulate the extraction power of the plant. Both of these methods are attempts to analyze the soil so that estimates of the fertilizer requirements of crops can be made.

The field plot experimental procedure is the oldest, as well as the best known, of the biological methods of determining fertilizer requirements. Fundamentally, the method involves the application of fertilizers to the soil in various combinations and in various amounts, and then using the responses of the crops as indications of the proper kinds and amounts which may be profitably added to the soil.

It has already been pointed out that the historical experiments of Boussingault (1841) were carried out either in the laboratory or in small pots. About 1830, however, Boussingault began a series of field experiments in France. He weighed and analyzed the fertilizers used and the crops he obtained. At the end of each crop rotation, he prepared a balance sheet to show to what extent the fertilizer had satisfied the requirements of the crop. The results of one such experiment are given in table 13, and they illustrate his application of the bookkeeping method to the maintenance of soil fertility. This

Table 13. Statistics of a rotation. (Boussingault, 1841)

Crop	Yield in kilograms per hectar					
	Dry matter	Carbon	Hydrogen	Oxygen	Nitrogen	Mineral matter
Beets	3172	1357.7	184.0	1376.7	53.9	199.8
Wheat	3006	1431.6	164.4	1214.8	31.3	163.8
Clover Hay	4029	1909.7	201.5	1523.0	84.6	310.2
Wheat	4208	2004.2	230.0	1700.7	43.8	229.3
Turnips	716	307.2	39.3	302.9	12.2	54.4
Oats	2347	1182.3	137.3	890.9	28.4	108.0
Total during rotation	17478	8192.7	956.5	7009.0	254.2	1065.5
Added in manure	10161	3637.6	426.8	2621.5	203.2	3271.9
Difference not accounted for taken air, rain, or soil.	+7317	+4555.1	+529.7	+4387.5	+51.0	-2206.4

bookkeeping method was used also by Liebig who strongly advocated it as a procedure to study mineral fertilizers some 20 years later.

Field plot techniques obtained their greatest impetus from the difference of opinion between Lawes (1847) in England and Liebig in Germany concerning the importance of ammoniacal fertilizers. Liebig had developed a patent fertilizer containing phosphtes and alkalis but not nitrogen, for he believed that the nitrogen used by the plant came from the atmosphere. Lawes did not believe that the atmosphere was the true source of the nitrogen, and to prove his point established field experiments at Rothamstead. He obtained greater crop fields when ammoniacal fertilizers were used in addition to Liebig's patented fertilizer. The Rothamstead experiments were essentially the same as those performed by Boussingault but they had the added advantage of continuity. The meticulous methods used by Lawes and Gilbert in recording the results obtained from their field trials have given a constant source of valuable information to statisticians studying fertilizer use and crop production.

The success obtained by these early workers with their field experiments led many investigators to question the value of pot and solution cultures for the estimation of the fertilizer needs of the crop. Wolff (1877) concluded, after a series of water culture experiments on oats, that "the fact that they were conducted in water culture somewhat invalidates the results and tends to prevent a valid field interpretation."

A report published in the "Journal de Agriculture de Paris" by Joulie (1889) presented the details of the field techniques he used in the improvement of the soils on his farm. Using diagnostic soil and plant analysis, he made recommendations concerning the fertilizers to be applied to the soil. He then grew the crops, analyzed them, and compared the data with "composition types" based on averages of numerous data from plants of good growth. Any nutritional deficiencies becoming apparent were remedied. Joulie was able to reclaim soils which previously had been unable to support good crops by following this procedure.

During the nineteenth century, the development of field trials progressed rapidly. In spite of the additional work and expense involved and the uncertainty of the generalizations drawn from them, field trials have been used on an ever increasing scale during the past one hundred years. When used properly, their value is unquestioned, and when data obtained by them are subjected to statistical analysis, their usefulness is very great.

B. Statistical Analysis of Data

1. Introduction

The application of field trial techniques to crop responses was very haphazard until the relatively recent advances in plot design and the statistical methods of analysis developed by Fisher (1935). These newer techniques were applied by such workers as Snedecor (1937), Goulden (1939), and Paterson (1939). Fisher's techniques have so revolutionized the use of field trials that they now represent an exact science, at least in comparison to their previous empirical status.

Field trials may not be regarded as a fundamentally sound biological method, but more accurately, they represent an overall attempt to improve cultural practices in which the ultimate measure of improvement is profit per acre. The general practices of field cultivation used today are of a relatively high level of efficiency, and any improvements in crop yields cannot be as great as those obtained by early workers. Small differences in yield are difficult to evaluate without proper statistical study which takes into consideration all possible measureable causes of variation in the data. Since it is apparent that there are factors over which man has no control, involved in the use of field trials, it is essential that conditions be controlled as uniformly as possible during the progress of the field trials.

The results of any one field trial have only a limited application, for they theoretically are true only for the particular soil and season involved. To establish a general law, a number of separate experiments on various soil types and during several seasons must be conducted, and this mass of data used to prove the authenticity of the conclusions.

The variable nature of both soil and season poses an unavoidable obstacle to the easy solution of fertilizer problems by field trials. Soils vary in fertility, not only from acre to acre, but even from foot to foot. This makes it impossible to use identical soil conditions for various fertilizer treatments. It is quite conceivable that an apparent difference may be due to the difference in the original fertility of the plots. Similarly the prevailing climatic conditions in any season may favor certain of the treatments. Even slight changes in climate might be sufficient to cause significant changes in the relative merits of the experimental treatments. The theory of statistics and experimental design permit the development of techniques which, although they cannot completely eliminate extraneous effects, can minimize them to such an extent an approximate appreciation of the relative merits of the experimental treatments can be realized. Since the application of statistical methods to field trial data is so essential a general discussion of the methods of minimixing error will be presented.

2. Experimental Area

The importance of the careful selection of the site for experimental field plots cannot be overemphasized. The area should conform to the general soil and environmental conditions under which it is intended to apply the results of the experiment. At the same time, it is essential to select the most uniform area available in order to lessen the effect of the variable soil fertility of plots which are to be compared. Recently, an erroneous concept has developed that this is not of vital importance because of current improved techniques. While it is true that the currently used plot arrangements considerably reduce the effect of soil heterogeneity, they cannot eradicate its effect entirely. The more uniform is the site, the greater will be the probability of obtaining a true evaluation of the fertilizer treatments. On the other hand, it must be borne in mind that a field trial is conducted under artificial conditions, even though the site is uniform. The results must be interpreted as to permit their application to areas not so uniform as the plots themselves, for uniform agricultural areas are the exception rather than the rule.

3. Replication

Statistical theory states that a single large plot cannot give data of any value for comparative purposes, and consequently the experimental treatment should be divided into a number of smaller but similar plots. With replication, the increase of plot size is effective, to a certain extent, in the reduction of error, but replication reduces the standard error of the mean of one treatment at a rate proportional to the square root of the number of its replications.

While the value of the replication of experimental fertilizer applications is realized by the majority of agricultural workers, there are cases where it is not only not necessary but may even be undesirable. For example, the techniques developed by Lagatu and Maume (1934) involve such masses of similar data accumulated over many years that these workers now consider further replication to be unnecessary.

4. Randomization

Statistical significance is based on the assumption that the estimates of the means and standard deviations obtained from the data approximate the true values that would be obtained from an infinitely large number of replicated plots. Such a situation requires that plots duplicating any one treatment be located at random. Fisher (1934) states that "an estimate of error will only be valid for its purpose if we make sure that in the plot arrangement, pairs of plots treated alike are not nearer together, or further apart than, or in any other relevant way, distinguished from pairs of plots treated differently."

On the other hand, there tends to be a close similarity between the fertility of adjacent plots, and in consequence there is a greater probability of demonstrating real differences between treatments if they are carried out on adjacent plots. Bray (1948) in his work with corn in Illinois, considered that his techniques require that the plots are not randomized.

One must conclude the necessity of each experiment being considered on its own merits, and the fertilizer treatments or the collection of ~~samples~~ samples be randomized or not as seems best under the circumstances.

5. Local Control

A great many types of field plan have been designed and used at various times in an attempt to obtain a more accurate control over the error and to permit more accurate interpretations. There are three basic plans for field plot experiments, the complete randomization which has no local control and consequently is of limited value, the randomized block which is the simplest type having a local control and which permits the removal of error in one direction, and the latin square which permits the removal of error over two directions. The latin square is the most efficient design for small numbers of treatments. In addition to these three basic types, more complex plot designs such as factorial, split-plot, and confounded designs all permit a more accurate elimination of error and permit a correspondingly more accurate interpretation of the data. The difficulty of the more intricate plot designs is that the mathematical complexity of their interpretation is so great that inexperienced workers become confused in their final interpretations and may be led into errors by the complexity of the calculations designed to help them.

Fisher (1934) has summarized the principles of plot design in the diagram reproduced in figure 16 which shows the interrelationship of methods for minimizing the effects of factors other than that being studied.

C. Applications

1. Introduction

Field trials have been conducted for more than one hundred years. Every worker in the field of plant nutrition has resorted on occasion to their use for the purposes of his investigations. The majority of the chemical methods of analyzing soils or plants to determine fertilizer

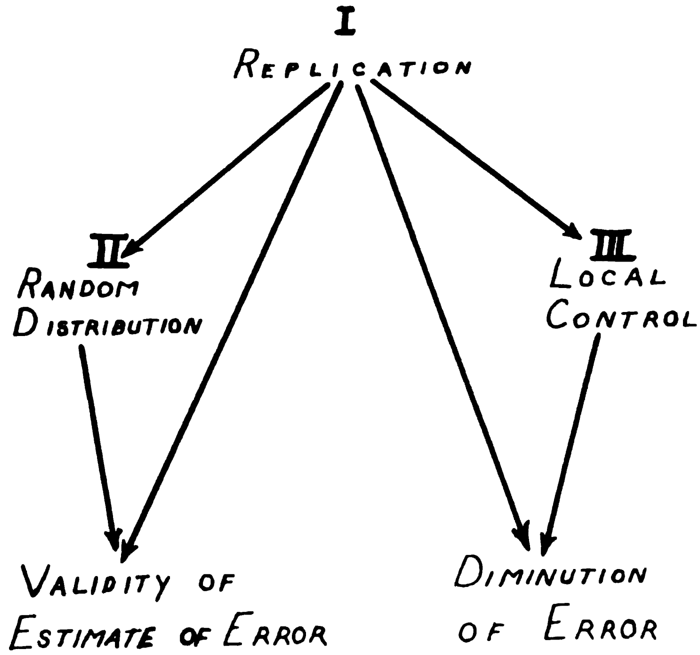

Figure 16. Diagram summarizing the principals from which statistical methods have evolved. (Fisher, 1934)

requirements, and other biological methods have been developed in order to avoid the necessity of field plot trials. The value of these additional procedures has been subjected to criticism. Alway, Shaw, and Methley (1926) state that "crop analysis does not appear promising as a practical method of detecting the phosphate hungry fields on prairie soils and is not to be compared in desirability with the use of small plot trials." On the other hand, Hardy, McDonald, and Rodriquez (1935) consider that "greatest reliance should be placed on the results of a statistical analysis of yield and chemical analytical data derived from a properly planned manurial experiment."

Field trials are being used currently either to correlate yields directly with fertilizers applied as Alway thought best, or to correlate the concentrations of nutrients in the tissue with yields from properly planned fertilizer experiments carried out on field plots, as proposed by Hardy.

2. Correlation of crop yields with fertilizer applications

Parker's work (1933) at the Virginia Truck Garden Station on tomatoes illustrates the procedure used and the inferences which may be drawn from the field plot trials of fertilizer applications. Parker conducted experiments from 1928 to 1932 in order to find the correct fertilizer applications for the maintenance of optimum yields of tomatoes under the local conditions. He applied eleven treatments on the variety Marglobe. The average yields for the five year period are shown in figure 17. It was concluded that under the local conditions

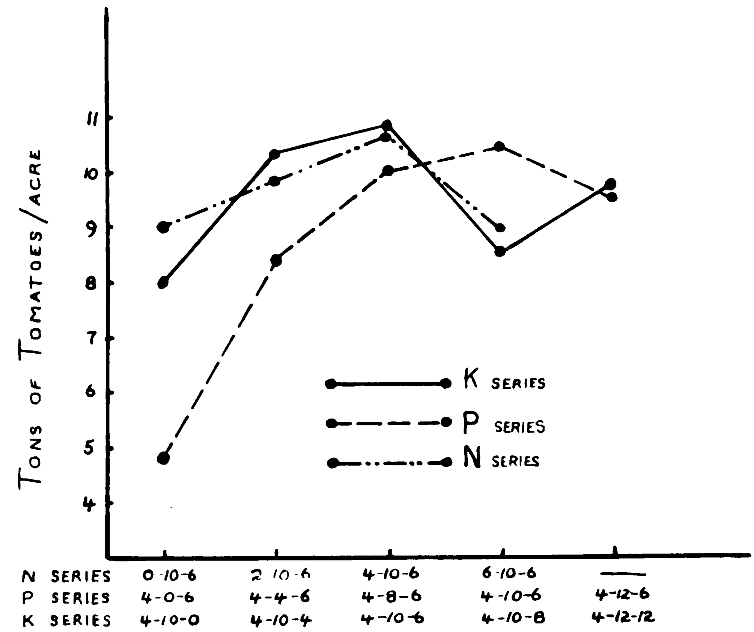

Figure 17. The effect of increasing amounts of nitrogen, phosphorus and potassium in a fertilizer mixture on yields of tomatoes in Westmoreland county, 1928-1932. (Parker, 1933)

of the experiments, the 4-10-6 fertilizer produced optimum yields when it was used at the rate of 1000 pounds per acre.

The weakness of the technique used by Parker is the same as in the majority of field trials, namely the use of treatments consisting of definite fertilizer ratios. It has already been noted that the absorption of each plant nutrient and its concentration in the plant is affected by a complex set of conditions. Surely, the arbitrarily selected combinations of nitrogen, phosphate, and potassium fertilizer units will be affected by these conditioning factors, and interpretation of the results will be difficult. If one considers the possibilities of combining three factors at various levels, it is apparent that this procedure cannot determine the required amount of fertilizer for optimum yields. There can be only a suggestion of the amounts and combinations giving the best results under the conditions of the test. Unfortunately, although this weakness is apparent to most agricultural workers, little has been done to apply better techniques which would overcome the difficulty. It cannot be said that this is due to the lack of proper available procedures when the Law of Diminishing Returns as put forth by Bray (1948) and by Willcox (1930, 1937) are recalled. Also the use of chemical analysis of plant tissue represents another more adequate approach.

3. Correlation of Tissue Analyses with Fertilizer Applications

Chemical analyses of plant tissues has attained wide usage during the past few years, especially since simplified chemical procedures permit approximations of the compositions of large numbers of samples in a relatively short time. This technique was used by Carolus (1933) to study the same plots reported on by Parker (1933). In 1932, Carolus analyzed the plant material obtained from the plots for nitrogen, phosphorus, potassium, magnesium, calcium and total ash. The samples were taken at the beginning of fruit setting and the results were expressed as milligrams per plant as suggested by Bartholomew, Watts, and Janssen (1933). These analytical and yield data are partially summarized in table 14. These data show that there was a great relative increase in both the fresh and dry weights when phosphate fertilizer was used. The effect was smaller in the case of nitrogen and there was some depression in the yield when potassium was used. Carolus believed that more phosphate could have been utilized by the crop since values in the BB and CC lines (table 14) were negative. Since the method of calculation yielded positive values in BB and CC, there is some evidence of luxury consumption of nitrogen and potassium. The final conclusion drawn from these data is that the optimum fertilizer should contain at least 12 percent P_2O_5, less than 6 percent nitrogen, and less than 12 percent K_2O. These results confirm in a general way those obtained by Parker but are of added value in that they indicate how the fertilizer ratio recommended by Parker could be improved.

Table 14. A comparison of the effects of the ingredients of the fertilizer mixtures indicated on the absorption of certain nutrients by the tomato plant expressed on the per plant basis and on the yield per plot in 1932. (Carolus, 1933)

	Fresh Wt. gms.		Dry Wt. gms.		N mgms.		P mgms.		K mgms.	
Nitrogen series										
Fertilizer Ratios	0-10-6	6-10-6	0-10-6	6-10-6	0-10-6	6-10-6	0-10-6	6-10-6	0-10-6	6-10-6
Yield	1265	1580	152.4	194.2	3422	4706	403	481	5211	5363
A	----	+315	-----	+42	----	+1284	---	+78	----	+152
AA	----	+ 24.9	-----	+27.6	----	+ 37.5	---	+19.4	----	+ 2.9
B	----	----	-----	+ 4.1	----	+ 431	---	-22	----	-1147
BB	----	----	-----	+ 2.16	----	+ 10.1	---	- 4.4	----	- 17.6
C	----	----	-----	----	----	+ 388	---	-33.3	----	-1288
CC	----	----	-----	----	----	+ 7.75	---	- 6.43	----	- 19.4
Phosphorus series										
Fertilizer ratios	4-0-6	4-12-6	4-0-6	4-12-6	4-0-6	4-12-6	4-0-6	4-12-6	4-0-6	4-12-6
Yields	272	1387	43.2	161.2	1273	4355	120	535	1550	5027
A	---	+1115	----	+118	----	+3082	---	+415	----	+3477
AA	---	+ 410	----	+273	----	+ 242	---	+346	----	+ 224
B	---	----	----	-59.2	----	-2140	---	- 76.9	----	-2880
BB	---	----	----	-26.9	----	- 33	---	- 12.6	----	- 36.4
C	---	----	----	----	----	- 395	---	+ 87.5	----	- 760
CC	---	----	----	----	----	- 8.3	---	+19.6	----	- 13.1
Potassium series										
Fertilizer ratios	4-10-0	4-10-12	4-10-0	4-10-12	4-10-0	4-10-12	4-10-0	4-10-12	4-10-0	4-10-12
Yields	1019	1081	146.5	134.4	4222	3383	442	411	3707	5138
A	----	+62	-----	-12.1	----	- 839	---	-31	----	+1431
AA	----	+ 6.1	-----	- 8.3	----	- 19.9	---	- 7	----	+ 38.5
B	----	----	-----	-21.1	----	-1090	---	-57.9	----	+1201
BB	----	----	-----	-13.6	----	- 24.5	---	-12.3	----	+ 30.1
C	----	----	-----	----	----	- 490	---	+ 5.8	----	+1739
CC	----	----	-----	----	-----	- 13	---	+1.4	----	+ 51.0

Table 14. (Concluded)

A Change due to the addition of N, P, or K.

B Deviation from the theoretical growth and absorption

C Deviation from the theoretical absorption on DM basis

AA % change based on the non-treated plot

BB Deviation of B expressed as a percent

CC Deviation of C expressed as a percent

<u>Calculation of B and BB</u> Example phosphorus content in phosphorus series.

Untreated plot yielded 272 grams fresh weight and 120 mgm. phosphorus.
1387 gms. material on the treated plot absorbing phosphorus at the same rate should take up 611.9 mgms. (272:1387::120:x). Actual content of phosphorus is 535 mgms. Therefore the absorption is 76.9 mgms. less than theoretical. BB is therefore (76.9/611.9)100 equals 12.6%.

<u>Calculation of C and CC</u>

Same as for B and BB except on a Dry Weight basis.

4. Discussion

It has been stated previously that the field trial technique is the oldest, as well as the best known, biological method used for the determination of the fertilizer requirements of crops. Field trials are not, however, without their limitations and disadvantages. Probably the most important of these is the heavy expenditure in labor and money required to conduct adequately such a trial. Assuming that the design of an experiment is technically sound, the accuracy of execution of such details as cultivation, harvesting, units of measurement, developmental studies, etc., is essential for its success. Such accuracy can be guaranteed only where skilled supervision and labor are available, and consequently field trials are too expensive to use on a scale suitable for the needs of individual farmers. Field trial experiments also are subject to substantial error if an improper plot design is used, and in some cases even when the correct design is used authentic data may not be obtained. Another disadvantage is that the results cannot be used during the year the field trial is conducted. Some workers have attempted to overcome this difficulty by making observations at an early stage of crop development on such characters as leaf color or rate of stem growth (Wallace 1943).

There is no doubt that field trials are the most direct way of diagnosing the fertilizer requirements of plants and it is equally

without doubt that they are the ultimate test to which the findings of any other method must be submitted. It is recognized by all workers that if predictions founded on the results of any other method fail to be confirmed by the results of field trials, such a method is unsatisfactory. While an extensive set of field trials is essential for the standardization of other techniques, these other techniques frequently will be found to be of greater practical value.

IV. LAW OF DIMINISHING RETURNS

A. Introduction

It was pointed out in the discussion on the use of field trials that many of the inherent difficulties of that approach to the determination of the fertilizer requirements of crops could be overcome by the application of the Law of Diminishing Returns. An understanding of this law in principle as well as in application is fundamental to the study of the fertilizer requirements of crops.

Von Liebig first emphasized the need of the various mineral nutrients for plant growth, and immediately thereafter attempts were made to formulate mathematically a law expressing the relationship between increments of plant nutrients and the yields resulting from them. The first of these attempts was the Law of the Minimum, already suggested by Sprengel (1837), but emphasized by Liebig (1863). This law recognized the importance of a limiting factor governing plant growth. Unfortunately, the Law of the Minimum as proposed by Liebig was poorly stated and numerous workers became involved in the effort to develop a sufficient body of data under controlled conditions to show that it was incorrect, and later to formulate a more correct statement.

Mayer as early as 1869 realized that there were limitations to Liebig's statement of the Law of the Minimum, for he said that "it is not possible to increase the production of plant substance at will, since a further application of labor and capital gives rise to a smaller and smaller increase in production." This probably was the earliest disagreement with the straight lime relationship between nutrient supply and growth expressed by Liebig's law. Somewhat later, Heinrich (1882) noted that increased amounts of fertilizer not only failed to produce correspondingly great increases in yield but actually lessened the crop yield. Wagner (1883) at Darmstadt confirmed Heinrich's observations experimentally and reported his own data to show that "the first 25 parts of nitrogen added to soil produced 1277 grains of spring wheat; the second 25 parts, 563 grains; the third, 318 grains; the fourth, 39 grains." At the end of the nineteenth century, Wollny (1897-1898) laid the foundation for an interpretation of the Law of Diminishing Returns which was later proposed by Mitscherlich. Wollny concluded from his investigations that "with addition of nutrients a rise occurs in the productive power of plants which is at first progressive and then becomes gradually smaller up to a certain limit, beyond which a further increase in the supply of nutrients causes a corresponding reduction in yield." This early work interested Mitscherlich who soon became interested in the relationship of fertilizers to the yield of crops, and he carried out a series of precise experiments from which he formulated his own statement of the Law of Diminishing Returns.

B. Development of the Law of Diminishing Returns

1. Basic Assumptions and the First Approximation

Mitscherlich (1909) considered it necessary in order to obtain the most accurate expression for the relationship between increases in yield and amounts of fertilizer used to determine to what degree the crop yield was dependent on the vegetative factor operating at the minimum intensity.

Mitscherlich's original proposal (1909) of the Law of Diminishing Returns was based on pot culture experiments with oats. Six kilograms of sand were fertilized with the following nutrient salts at time of planting:

Salt	Grams per pot
$Ca(NO_3)_2$	3.76
NH_4NO_3	2.09
$CaCl_2$	1.27
$CaCO_3$	1.28
$MgSO_4$	3.66

In addition to these salts, each pot received a top dressing of 376 grams of $Ca(NO_3)_2$ and 2.09 grams of NH_4NO_3 on June 4 and August 10, or approximately one and three months after planting. In this experiment, phosphorus was the limiting nutrient and was supplied as mono-, di-, and triphosphate, each at four levels. The averages of the data from four experiments, expressed as grams of dry matter in the straw and roots, are reproduced in table 15 and in figure 18.

The smooth curves found experimentally suggested to Mitscherlich that they could be expressed by a mathematical equation. He assumed

Table 15. Yields of oats fertilized with different amounts of phosphate in each of three forms, together with the probable errors of the yields. (Mitscherlich, 1909)

Fertilizer expressed as grams of P_2O_5	Yields of dry material, expressed in grams		
	With monobasic phosphate	With dibasic phosphate	With tribasic phosphate
0.10	44.3±1.4	45.4 ± 0.4	8.4 ± 0.9
0.25	60.5 ± 0.8	62.7 ± 1.0	14.0 ± 3.5
0.50	78.5 ± 3.6	78.0 ± 1.3	25.0 ± 1.7
1.00	88.5 ± 3.4	91.4 ± 3.9	44.1 ± 1.2

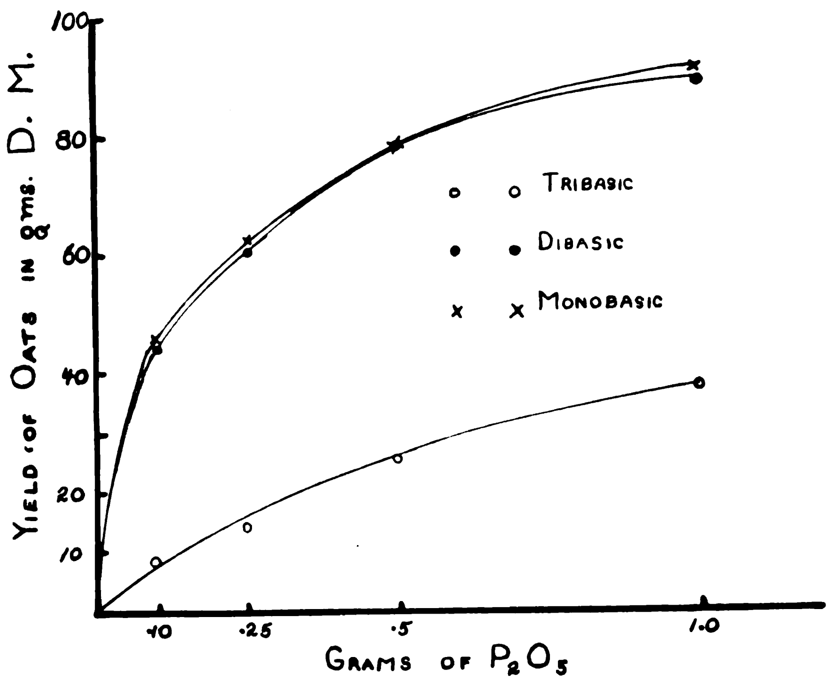

Figure 18. Yield of dry matter of oats fertilized with different amounts of phosphate supplied as the monobasic, dibasic, and tribasic salts. The points indicated were obtained experimentally, but the curves were drawn according to the equation $y = A(1-e^{-\varsigma x})$. (Mitscherlich, 1909)

that a plant or crop should produce a certain maximum yield if all conditions were ideal and that when an essential factor is deficient, there is a corresponding reduction in the yield. He further assumed that the increased yield produced by one unit of the deficient factor is proportional to the decrement of the yield from its maximum. That is to say:

$$\frac{dy}{dx} = C_1(A-y)$$

where y is the yield obtained when x is the amount of the limiting factor present, A is the maximum yield obtainable if the factor were present in excess, and C_1 is a constant. On integration, and assuming that $y = 0$ when $x = 0$, it follows that

$$\log_e(A-y) = \log_e A - C_1 x, \quad \text{or}$$

$$\log(A-y) = \log A - cx \quad \text{where } c = 0.4343C_1, \quad \text{or}$$

$$y = A(1-10^{-cx})$$

Mitscherlich claimed that the proportionality factor was constant for each plant nutrient, independent of the crop, the soil, or other conditions. This means that if such were truly the case, the yields obtainable from any given quantity of fertilizer could be predicted from a single field trial. It would further be possible to estimate by pot experiment the amount of available plant food in soil. Mitscherlich (1923) did use this formula for these purposes. In 1911, Mitscherlich published further results obtained when phosphorus was the limiting nutritional factor and confirmed the conclusions based on his earlier work reported in 1909.

Mitscherlich considered that his statement of the Law of Diminishing Returns applied also to factors other than nutritional which affected crop yield. In 1912, he reported data, condensed in table 16, showing the quantitative relationship between yield and water supply. These data are significantly close to the theoretical values calculated from his equation.

Spillman (1924) working independently arrived at the same basic conclusion as did Mitscherlich, and he expressed the Law of Diminishing Returns in the form

$$Y = A - R^x, \quad \text{or} \quad Y = M - AR^x$$

where Y is the actual yield obtained with x units of fertilizer, M is the theoretical maximum yield that can be attained with the fertilizer in question, and R is a constant. The fundamental difference in these statements was that Mitscherlich arrived at his formula by assuming that the slope of the curve of yield increase is proportional at all points to the amount of increase theoretically yet possible from the use of the fertilizer in question, while Spillman (1924) arrived at the same expression by assuming that the successive increments in yield due to successive increments in fertilizer applied constituted the terms of a decreasing geometric series. Spillman (1924) has shown mathematically that there is no difference between

Table 16. Yield of tops of oats and peas grown in pots containing various percentages of soil mixed with sand, maintained at the water holding capacity. Experimental results and those calculated from Mitscherlich's formula are arranged in adjacent colums. (Mitscherlich, 1912)

Percent soil in the sand	Water added mls.	Yield of oats		Yield of peas	
		Obser.	Cal.	Obser.	Cal.
20	470	43.7	43.4	22.4	22.8
30	690	61.1	60.2	32.4	31.7
45	1035	81.0	80.7	33.3	42.5
70	1610	104.0	105.1	55.3	55.3
100	2300	123.0	123.7	71.0	65.1

the expression 1^{-c} in Mitscherlich's equation and R in the equation he himself proposes.

2. Criticisms of Mitscherlich's Equation

a. Major Criticisms

Mitscherlich's work stimulated a veritable flood of controversy and it was subjected to many criticisms, both as to the way in which he applied it, and as to the way he mathematically expressed it. One severe criticism was that his maximum value, A, was determined by mathematical means and not experimentally. The question of whether the constant, C, for a particular nutrient is independent of other conditions of growth is still a matter of controversy. Finally, the logarthmic equation itself was criticized because it was confined to the expression of the effect of a single factor on crop yield, and also because it did not account for depressions in yield caused by large applications of fertilizers. Critics also pointed out that other, and simpler, equations could be used.

b. Criticisms by Pfeiffer and his Co-workers

Mitscherlich's theory, that the increase in yield with increasing applications of fertilizer gradually diminishes, was subjected to experimental test by Pfeiffer, Blanck and Flugel (1912). They investigated the effect of increasing amounts of nitrogen on the growth of oats in pots of soil containing excess water, containing intermittent periods of adequate and inadequate water, and containing excess water with the plants self-shaded. The results are shown in table 17, and it was maintained that the yield followed a parabolic curve expressed by the equation

$$y = a + bx + cx^2$$

The use of the equation of a parabolic curve accounted for depressions in yield resulting from the use of excessive amounts of fertilizers. The "comparison of fit" of Pfeiffer's data to the two curves

$$y = a + bx + cx^2$$

and

$$\log(A - y) = \log A - cx$$

is shown in figures 19 and 20. The parabolic function appears to show a closer fit to the experimental data.

Mitscherlich (1912a) in reply to his critics, pointed out that the validity of any law of the minimum ceases when no further increase in yield occurs, because the injurious effects occurring when excessive amounts of fertilizer are used bear no causal relation to limiting factors. He also pointed out that his formula, which contains constants of physiological significance, theoretically is better founded physiologically than that of Pfeiffer which contains constants of only mathematical significance. Finally, Mitscherlich pointed out that all studies of crop growth from the time of Liebig had shown an ever diminishing rate of increase in yield with successive increments of

Table 17. Average yields of four pots of oats (grain and straw) with various amounts of nitrogenous fertilizer and under various moisture conditions. Actual data and those calculated from the Mitscherlich and Pfeiffer equations are arranged in adjacent colums. (Pfeiffer, Blanck, and Flugel, 1912)

Fertilizer added (grams of nitrogen)	Calculated and observed yields of grain and straw, expressed in grams								
	Excess water			Moderate water		Intermittent water		Excess water plus shade	
	Observed	Calculated 1	Calculated 2	Observed	Calculated 3	Observed	Calculated 4	Observed	Calculated 5
0.00	8.5	8.4	8.9	14.8	20.0	8.0	9.1	9.1	11.3
0.335	44.0	44.2	44.1	48.7	44.8	36.3	34.4	44.0	41.9
0.710	74.2	65.9	72.9	72.8	65.2	52.2	53.2	69.7	65.8
1.065	96.4	80.9	95.1	73.3	81.6	65.8	65.3	80.6	83.1
1.420	107.5	89.8	111.1	87.0	93.5	70.8	70.8	89.6	93.9
1.775	122.2	95.5	120.5	95.8	101.2	68.1	69.7	101.1	98.2
2.130	124.7	99.0	123.5	110.3	104.6	62.9	61.9	117.4	95.8

Calculations:

1. $\log(105 - y) = 4.5706 - 0.0365x$ x = gms. N/0.028
2. $y = -3.221x^2 + 38.426x + 8.932$ x = gms. N/0.355 y is Max. when x is 2.118 gms. N
3. $y = -2.145x^2 + 26.976x + 19.958$ x = gms. N/0.355 y is Max. when x is 2.231 gms.
4. $y = -3.307x^2 + 28.707x + 9.052$ x = gms. N/0.355 y is Max. when x is 1.536 gms.
5. $y = -3.282x^2 + 33.775x + 1.333$ x = gms. N/0.355 y is Max. when x is 1.826 gms.

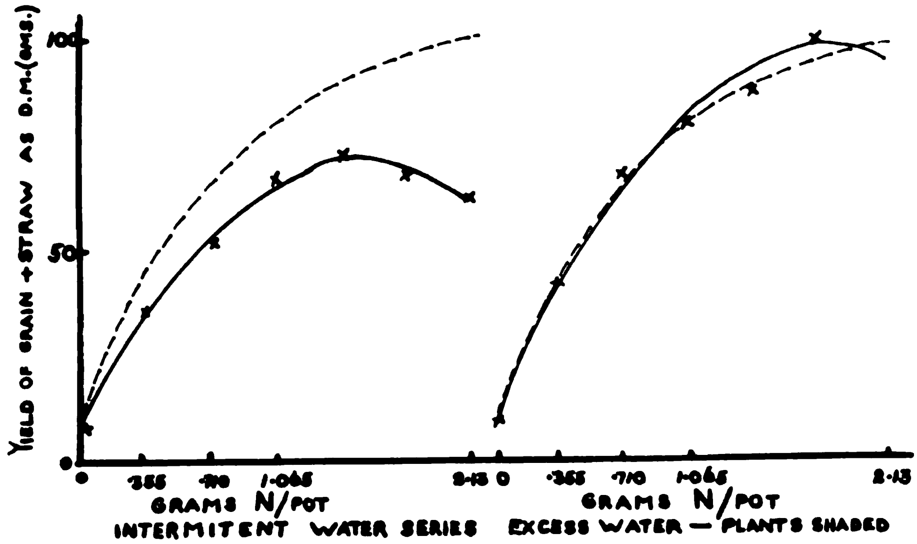

Figure 19. Yield of oats plotted against grams of nitrogen fertilizer, showing fit of the Mitscherlich curve with the type curve $y = a + bx + cx^2$ proposed by Pfeiffer. Plots with intermittent water and with excess water and the plants shaded. (Pfeiffer, Blanck, and Flugel, 1912)

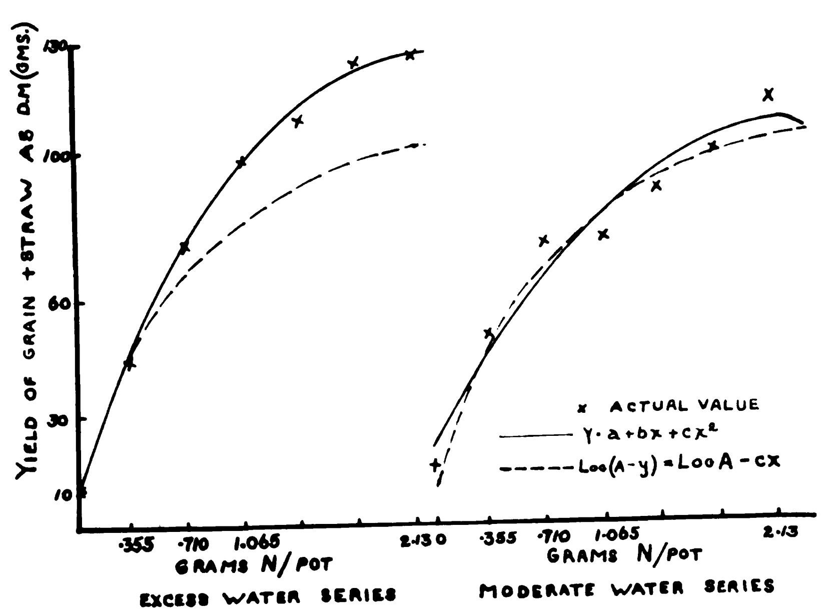

Figure 20. Yield of oats plotted against grams of nitrogen fertilizer showing the fit of the Mitscherlich curve and with the type curve $y = a + bx + cx^2$ proposed by Pfeiffer. Plots with excess water and moderate water. (Pfeiffer, Blanck, and Flugel, 1912)

fertilizer. This had been shown by Mayer (1869), Wagner (1883), and Wollny (1897, 1898) as well as by his own work (1909, 1911).

Mitscherlich's defense of his theory was investigated by Pfeiffer, Blanck, and Simmermacher (1915). These workers varied not only the rate of fertilizer application but also the amount of illumination in order to test the combined effects of two factors on the growth of plants. Their results indicated that the Mitscherlich equation was "most probably an accurate evaluation of the effect of growth factors on yield."

c. Criticisms by Briggs

A critical study of Mitscherlich's equation was carried out by Briggs in 1925. The basis of this study was stated in the form of two questions as follows: "Is the relation between y and x of the form

$$y = A(1 - e^{-cx})$$

That is, is the value of c a constant when calculated from this equation? Is the value of c the same for the same variable, irrespective of the plant, soil weather, etc?"

Mitscherlich maintained that these assumptions are valid because the values of y calculated on the basis of his equation, using a definite value for c, for each external factor agreed closely with the values found experimentally for different kinds of plants under various conditions. Briggs claimed that this type of data presented by Mitscherlich is not conclusive enough and to support this uses Mitscherlich's work (1912b) in which he attempted to find the amount of potassium available in a sample of soil. Using this data, shown in table 18, Briggs demonstrated that the equation

$$y = 50(1 - e^{-3.3x})$$

more closely agrees with experimental data than the equation

$$y = 50(1 - e^{-3x})$$

proposed by Mitscherlich. In a still more striking set of comparisons, Briggs showed that the closer fit to experimental data is given by the equation

$$y = A, \frac{x+b}{x+b+c}$$

where b = 0.03 and c = 0.0622.

While Briggs did not state that the Mitscherlich equation was not an accurate indication of the relationship between yield and applied fertilizers, he did state that Mitscherlich's work (1923a) indicated that the values, A = 50 and c = 3, were selected without sound mathematical justification. In this connection, he stated that "it seems that the guiding principle of assigning values to A was that c should be 3 and b such that the amount of potash in sand and soil deduced from any pair of equations should agree with that from any other pair. Not having the bias as to the immutability of c in the presence of limestone soil, but accepting the restriction as to b, we have applied other equations with the result that the potash in the sand and soil becomes 0.0088 gm. and 0.116 gm/kg respectively, as compared with Mitscherlich's figures of 0.01 and 0.08. It cannot be

Table 18. Yield of oats in mixtures of sand and soil, receiving varying amounts of potash. Data from Mitscherlich, 1912a and recalculated by Briggs 1925.

	A 6 Kilograms sand, no soil									
	Grain (a)			Straw (b)			Whole Plant (c)			
x	Observed	Calculated 1	Calculated 2	Observed	Calculated 1	Calculated 2	Observed	Calculated 1	Calculated 2	Calculated 3
0.0	5.5	5.7	6.1	10.9	11.3	10.3	16.3	17.0	16.1	17.1
0.1	12.3	12.2	12.7	22.2	21.2	21.3	34.4	33.4	33.7	35.5
0.25	16.0	16.6	16.6	27.3	27.5	27.9	42.4	44.1	44.1	43.1
0.60	18.2	18.8	18.4	29.6	30.7	30.8	47.8	49.5	48.7	47.9
1.50	19.4	19.0	18.5	31.3	31.0	31.0	50.8	50.0	50.0	50.5

Equations from which the calculated values were derived

1 A(a) $\log(19-y) = \log 19 - 3(x+0.052)$

1 A(b) $\log(31-y) = \log 31 - 3(x+0.066)$

1 A(c) $\log(50-y) = \log 50 - 3(x+0.060)$

1 B(c) $\log(51-y) = \log 51 - 3(x+0.095)$

1 C(c) $\log(57-y) = \log 57 - 3(x+0.130)$

1 D(c) $\log(55-y) = \log 55 - 3(x+0.200)$

Columns 2(A) values) 18.5, 31.0, 49.0, 53.0, 54.0, 55.0.

(c values) 3.3, 3.3, 3.3, 2.5, 3.3, 2.0.

(b values) 0.53, 0.53, 0.53, 0.106, 0.160, 0.268.

Columns 3(A value) 52.6, 52.6, 61.8, 60.2

(c values) 010622

(c values) 0.03, 0.055, 0.30, 0.13.

Table 18. (Concluded)

x	B 5.5 kg. soil, 5 kg. sand				C 5.0 kg. soil, .5 kg. sand				D 4.0 kg. sand 2.0 kg. soil			
	Whole Plant (c)				Whole Plant (c)				Whole Plant (c)			
	Observed	Calculated			Observed	Calculated			Observed	Calculated		
		1	2	3		1	2	3		1	2	3
0.0	20.8	24.5	24.2	24.7	36.0	33.8	36.1	34.7	41.0	41.2	39.0	40.7
0.1	37.3	37.7	36.8	37.6	47.2	45.4	45.0	46.0	43.3	48.1	44.9	47.4
0.25	50.6	46.3	46.2	43.7	51.9	52.9	51.0	51.9	53.0	52.5	49.9	51.7
0.60	49.3	50.6	52.1	48.7	54.3	56.6	53.7	56.6	54.4	54.8	54.9	55.4
1.50	49.3	51.0	53.0	50.8	54.2	57.0	54.0	59.5	55.3	55.0	55.0	55.0

claimed that the calculated values of y show a worse agreement with the observed than those calculated by Mitscherlich."

Briggs' work brings forth only one fact, namely that it is extremely difficult to apply any single equation to the relationship between the magnitudes of crop yields and the amounts of fertilizers used.

d. Other Criticisms of the "C" Factor

Various workers, at different times, have reported data indicating that the c value as applied by Mitscherlich is not constant in magnitude. Magistad (1938) conducted a critical comparison of the c value by growing oats in seven different soils. These tests were conducted simultaneously in Germany and also in the Hawaiian Islands. The results show that, if the c values are taken as 0.122 dz/hectar (153 lbs/acre) for nitrogen, and as 0.6 dz/hectar (753 lbs/acre) for P_2O_5, and as 0.93 dz/hectar (1170 lbs/acre) for K_2O, then the results obtained on the same soil and using the same crop are different in the two test areas and consequently the value of c is neither constant nor independent of soil, weather, crop etc.

Magistad, Farden, and Lambert (1932) earlier had shown that the value of c proposed by Mitscherlich was not constant under field conditions in Hawaii.

This work seems to indicate that there are restrictions in the extent of the area over which the value of c is constant.

Capó (1938) using Hegori Sorghum, fertilized with various amounts of nitrogen, phosphate, and potassium, also found that the c values as proposed by Mitscherlich were not constant, and he proposed values of 0.805 for K_2O, 0.5538 for P_2O_5, and 0.5968 for NH_3. Previously, Mitscherlich had proposed the values of 10.93 for K_2O, 10.6 for P_2O_5, and 10.10 for NH_3. Experimental data and the calculated results are given in table 19 together with the equations used. While this data shows close agreement between the calculated and observed results and an apparent lack of constancy of value of c for different crops and conditions, sufficient evidence is not available to permit definite conclusions as to the constancy of Mitscherlich's value c.

While the preceeding discussion indicates that there was disagreement as to the accuracy of the equation proposed by Mitscherlich (1909), particularly in regard to the constancy of c value, one must conclude, until it is proven otherwise, that the first approximation of the Law of Diminishing Returns is an accurate indications of the effect of a single factor on crop yields.

3. Application to Multiple Factors

a. Work of Baule

Mitscherlich's first approximation of the Law of Diminishing Returns

$$y = A(1 - e^{-c_1 x})$$

Table 19. Actual and calculated yields of Hegari Sorghum, expressed in pounds of green material per plot, when fertilized with various amounts of NH_3, P_2O_5, and K_2O. (Capó, 1938)

Pounds of fertilizer used (x)	Crop yields in pounds of green material					
	K_2O fertilizer		P_2O_5 fertilizer		K_2O fertilizer	
	Actual yield	Calculated yield 1	Actual yield	Calculated yield 2	Actual yield	Calculated yield 3
0	111.1	112.2	80.4	80.6	79.1	78.3
0.75	117.0	113.4	98.7	97.9	94.1	97.0
1.50	110.3	114.4	108.0	109.1	113.7	109.0
2.25	116.7	115.2	116.7	116.2	116.7	117.9

Equations:

1. $y = 120\ (1 - 0.80518^{12.61+x})$
2. $y = 129\ (1 - 0.55385^{1.66+x})$
3. $y = 136.6\ (1 - 0.5968^{1.65+x})$

or as it is more commonly stated

$$\log(A-y) = \log A - cx$$

cannot be used for stating the effects of two factors except by treating each graduation of one factor as a constant, the other factor being treated as the variable, and then reversing the procedure. In order to express the dependence of crop yield on the many variable factors operating under the actual conditions of crop production, a transformation and extension of this basic formula is necessary.

Baule (1918) recognized limitation of the Law of Diminishing Returns to only single factors affecting yield as it was proposed by Mitscherlich (1909). Baule consequently modified the expression while retaining its fundamental assumptions. He supposed that each of the factors influencing plant growth acted in accordance with Mitscherlich's assumption, and that the final yield was given by the expression,

$$y = A(1-e^{-c_1 x_1})(1-e^{-c_2 x_2})\ldots\ldots\ldots(1-e^{-c_n x_n})$$

He states that the percentage increase in the crop yield was a function of any growth factor. For instance, if one unit of a given fertilizer increased the yield 50 percent of the amount by which the attainable maximum exceeds the yield without fertilizer, then a second unit of fertilizer will increase the yield 50 percent of the remaining distance to the maximum, that is to say, two units of fertilizer will relieve 3/4 of the deficiency. A third unit raises the yield to 7/8 of its maximum, etc. The efficiency of the nutrient, or other factor, called by Baule the "Wirkungsmenge" and now known as the "Baule unit", is expressed by the relation

$$h = \frac{\log e^2}{c} = \frac{0.7}{c}$$

where c is Mitscherlich's constant and 0.7 divided by c is the "Baule unit". The Baule unit is defined as the amount of a factor needed to give half of maximum yield obtainable by increasing that factor while all others remain constant. Baule shows that there is a definite mathematical relationship between Mitscherlich's c value and the Baule unit. If the fertilizer increment added, (x), is such that

$$y = \tfrac{1}{2} A$$

then

$$1 - e^{-ch} = \tfrac{1}{2}$$

and h is equal to

$$\frac{\log e^2}{c} \text{ or } \frac{0.7}{c}$$

Baule was able to deduce mathematically that if two factors vary simultaneously, each produces its own effect on yield independently of the other. When oats were grown in sand and both the water and phosphate supply were varied, the relative effects of the water remained the same no matter how much phosphate was given. The original data are presented in table 20.

The theoretical curves expressing the yields are sigmoid if the Baule units of the two variable factors are approximately of the same order of magnitude, but if one is significantly greater, the curve

Table 20. Yield of oats in pot experiments fertilized with different amounts of phosphate and receiving different amounts of water. (Mitscherlich, 1918)

Units of $Ca_3(PO_4)_2$	Yield of oats		
	Water 1 unit a	Water 2 units b	Ratio b/a
0	6.4	11.0	1.72
1	14.6	25.6	1.75
2	22.6	36.6	1.62
4	29.7	53.1	1.79
8	41.3	70.6	1.71
16	50.8	77.5	1.53
32	55.7	88.5	1.59

remains logarithmic as is seen in figure 21.

Mitscherlich, working with oats (1918), sugar cane (1919), and mustard (1919a), confirmed the validity of Baule's procedure for calculating the effects of multiple factors of crop yields.

Spillman (1924) carried out several experiments in which more than one plant nutrient was varied, and found no indication of a sigmoid yield curve. He noted, however, that on the portion of the sigmoid curve beyond the point of inflection an excellent fit with theoretical calculations was obtained. This showed that for the greater applications of fertilizer, even in cases which involve the sigmoid curve, the simple formula is applicable. Spillman suggested that this may be due to the fact that Baule's interpretation may not apply to cases in which the two variables are both nutritional in nature, but will apply when one factor is a nutrient and the other is some such factor as soil moisture. That this interpretation is incorrect has been shown clearly by Bray (1948) working with corn, fertilized with various amounts of phosphate and potassium.

In conclusion, we may say that the Mitscherlich equation in its simplest form, presupposes the presence in excess of all nutrients except the one limiting growth. If two nutrients are simultaneously increased in constant proportions, the combined effect will be the product of their individual effects, as proposed by Baule (1918), and the curve relating the magnitude of the yields to amounts of fertilizer becomes sigmoid. The specific form of the curve varies according to the relative proportions of the factors acting on the yield.

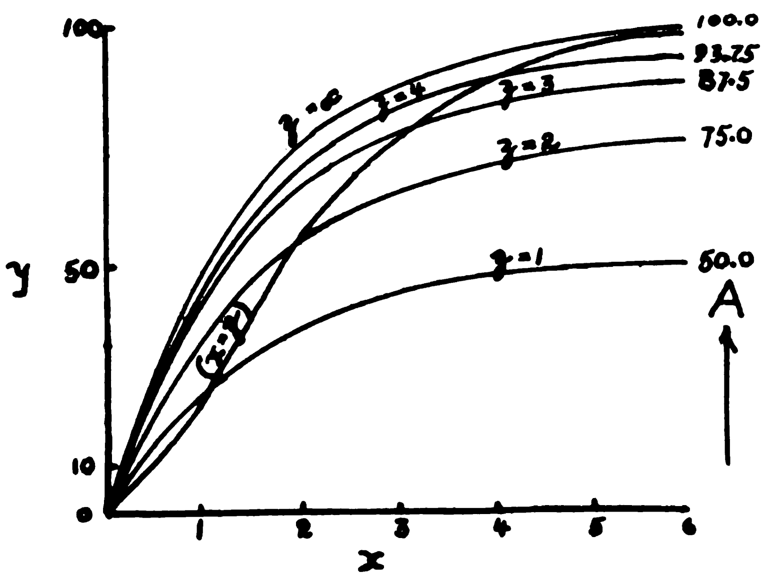

Figure 21. Baule's curves showing the yields (y) as percentages of the maximum possible yield when two factors x and z vary. When x = z the curve becomes sigmoid. (Baule, 1918)

b. Work of Balmukand

The effects on crop yields of the simultaneous variation in the supply of more than one nutrient have been represented mathematically by Balmukand (1928) in a manner somewhat different than did Baule. Balmukand considered that Mitscherlich's equation expressing yield-factor relationships, while satisfactorily expressing the response of a crop to variations of a single factor, does not adequately represent the effects of simultaneous variations of two or more factors. He points out that in Mitscherlich's equation, $y = A\ (1-e^{-cx})$, that if x is changed to x', that is to say x amount of fertilizer is increased x' amount, then y will be changed to y' independent of the value A.

Balmukand proposed the use of the "resistance formula" which requires that, instead of the ratio y'/y being independent of the influence of other factors when the value of one factor changes in intensity, the expression $\frac{1}{y} - \frac{1}{y'}$, or the difference of the reciprocals of the yields, should be independent of other factors. The validity of this assumption is supported by his data, presented in table 21, taken from field plots and pot-cultures with wheat. They show that, while the ratio y'/y in the case of the pot culture work varies from 4.9 to 24.0, the reciprocal difference $\frac{1}{y} - \frac{1}{y'}$ varies only from 0.26 to 0.29.

Balmukand therefore proposed the use of a formula of the resistance type

$$\frac{1}{y} = F(N) + F'(K) + F''(P) \cdots\cdots\cdots\cdots \quad C$$

where,

$$F(N) = \frac{an}{n+N}, \quad F'(K) = \frac{ak}{k+K}, \ etc.,$$

... the growth of wheat from the Broadwalk field and from pot cultures indicating that the ratio y'/y is dependent on A while $1/y - 1/y'$ is independent of this factor. (Balmukand, 1928).

Broadwalk Field Data

Fertilized used							Yield, Bushels per acre	$\frac{y'}{y}$	$\frac{1}{y} - \frac{1}{y'}$
Amount of K_2SO_4, lbs. per acre	Amount of Na_2SO_4, lbs. per acre	Amount of $MgSO_4 \cdot 7H_2O$ lbs. per acre	Amount of superphosphate (0-20-0), lbs. per acre	Amount of $(NH_4)_2SO_4$, lbs. per acre	Amount of NH_4Cl lbs. per acre				
---	---	---	---	---	---	y	12.296	1.589	0.03021
---	---	---	---	200	200	y'	19.504		
200	100	100	392	---	---	y	14.180	2.212	0.03765
200	100	100	392	200	200	y'	31.367		

Pot culture data

Milligrams of P_2O_5 per pot	Crop yields in grams per pot			
	Milligrams nitrogen per pot 15	1215	Ratio of yields obtained by the two levels of nitrogen $\frac{y'}{y}$	Reciprocal difference $\frac{1}{y} - \frac{1}{y'}$
5	3.06	15.00	4.902	0.2602
15	3.36	17.88	5.321	0.2417
45	3.03	29.60	9.769	0.2963
135	2.39	68.10	20.089	0.2784
405	3.60	86.40	24.000	0.2663

in which N, K, and P are the nutrients added to the soil, n, k, and p are the nutrients originally present in the soil, and a_n, a_k, and a_p are constants for the type of fertilizer used.

Balmukand reported the actual and calculated values obtained from an experiment with potatoes in support of the accuracy of his mathematical theory. His data given in table 22 show the yields of potatoes expected from theory and those actually obtained on plots receiving four different amounts of nitrogen, and four different amounts of potash, in all combinations. He concluded that the resistance formula fits the observed data closer than does the Mitscherlich formula, and that the parameters appropriate to each nutrient, being independent of the abundance of other nutrients, are capable of direct physical interpretation. While this approach by Balmukand is rather unique, we must conclude that it does not detract from the value of Mitscherlich's equation. The mathematical statement of Balmukand is sound, and probably states the effects of multiple factors on crop yields as well as does Baule's equation (1918), but the complexity of the mathematics used by Balmukand renders his resistance formula impractical for routine use.

c. Work of Gregory

The work of Gregory (1937) indicated that under certain circumstances the relationship of crop yields to the simultaneous increases in two plant nutrients, added in constant proportions, is represented

Table 22. Actual and calculated yields based on Balmukand's formula of Kerr's Pink potatoes expressed as tons per acre. Plots in Stackyard field, 1926. (Balmukand, 1928)

Pounds of ammonium sulphate added per acre	Yield of potatoes in tons per acre							
	Unfertilized		100 lbs. per acre of K_2SO_4		200 lbs. per acre of K_2SO_4		400 lbs. per acre of K_2SO_4	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
0	7.80	7.10	7.80	7.80	8.01	7.95	7.79	8.05
100	7.73	8.35	8.98	9.93	9.17	9.54	9.01	9.69
200	9.40	9.08	10.56	10.25	10.30	10.51	10.44	10.69
400	9.53	9.92	11.15	11.32	11.62	11.64	12.34	11.86

$k = 0.48 \pm 0.60$ $\qquad$ $n = 1.738 \pm 0.85$

$a_k = 0.0089 \pm 0.0129$ $\qquad$ $a_n = 0.0986 \pm 0.0616$

by a straight line. These results were obtained by adding nitrogen, potassium and phosphorus fertilizers to barley. When the yields were plotted against the amounts of the nu rients taken up by the plant, three types of relationships were established: 1) with an excess of all nutrients excepting the one being added in increasing amounts, the relationship of yield to the uptake of the nutrient is given by a curve of the typical Mitscherlich type; 2) when the yields are plotted against the uptake of one of the nutrients available in adequate amount, a curve showing an ever increasing yield is obtained; 3) when the supply of the two nutrients were increased in a certain fixed proportion, the curve relating the yield to the uptake of either nutrient becomes linear. These relationships are shown in figure 22. It was further shown in sand culture that the uptake by the plant of the nutrient in minimum was proportional to the amount of nutrient applied to the sand. These data appear in figure 23. Consequently, a rough approximation of the yield can be derived from the amounts of a nutrient absorbed by the crop.

4. Second Approximation

Experimental work in the field frequently shows that increasing increments of a fertilizer applied to the soil give regular and profitable increases in crop yield for the first few increments, but with further increments, the yield not only ceases to increase but may be depressed.

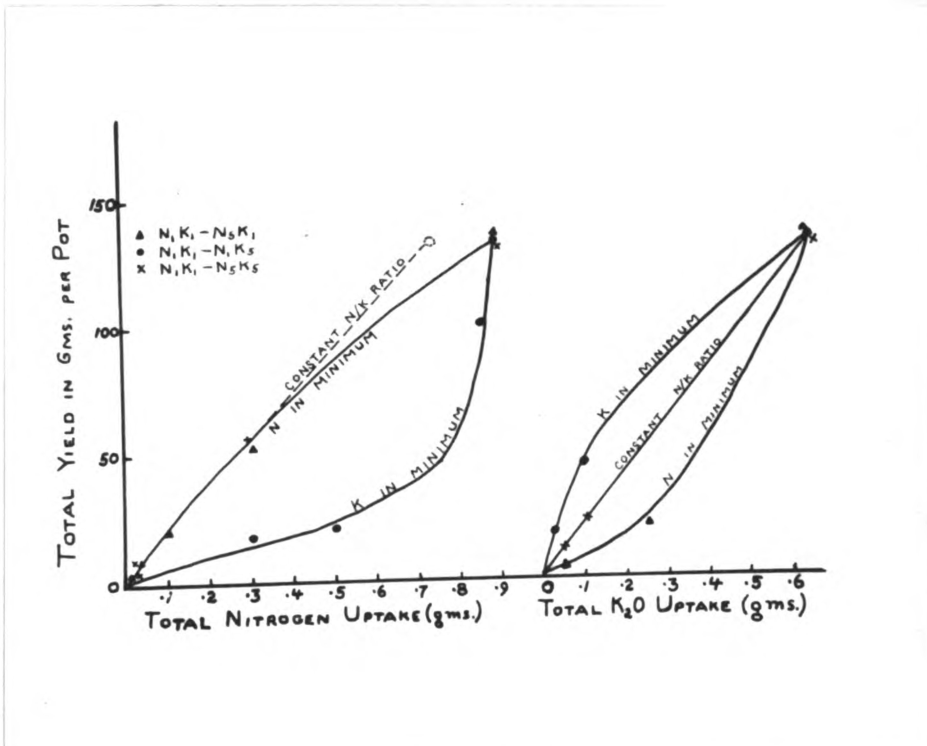

Figure 22. Relationships between total crop yields and amounts of nitrogen and potassium absorbed from the soil. One nutrient in minimum means that the others are present in excess. (Gregory, 1937)

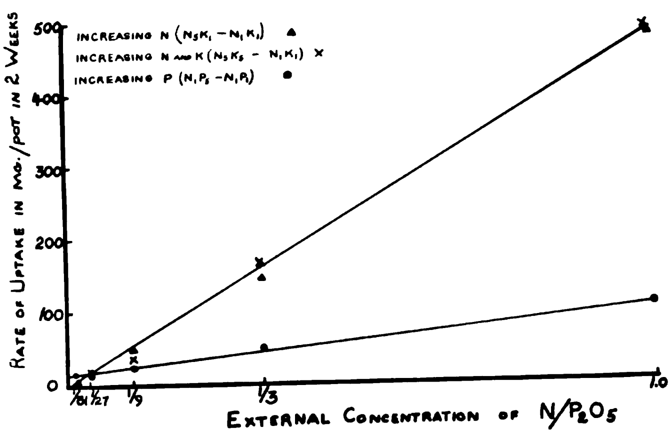

Figure 23. Relationships between the rate of uptake of a nutrient and its concentration in the soil. Experiments carried out with barley and harvested six weeks after germination. (Gregory, 1937)

Mitscherlich's (1909) first approximation of the Law of Diminishing Returns was based on experiments under conditions which pertained only to the first part of the yield curve. As long as these conditions existed, the law apparently expressed a true relationship between the amounts of fertilizer applied and the crop yields obtained. Other experimenters, notably Pfeiffer, Blanck and Flungel (1912), and Briggs (1925), operating under conditions which produced a depression of the yield, where c was not constant, and the logarithmic equation did not apply, assumed that Mitscherlich's concept was inherently erroneous.

Mitscherlich (1928) met the criticisms with an extensive new series of experiments in which the three principal plant nutrients, nitrogen, phosphate, and potassium, were employed in different combinations, and then proposed his second approximation of the law.

In the development of the second approximation, Mitscherlich (1928) noted that the yield curves from all the experiments in which depressions of yield occurred because of an unbalanced nutrition of the plants, exhibited two portions: an initial portion along which the increments of yield exhibited the normal course in accordance with the normal yield equation, and a subsequent portion which followed a different mathematical law.

The initial portion, of course, follows the curve given by

$$y = A(1-10^{-cx}) \text{ or } y = A(1-e^{-c_1 x})$$

which may be expressed as

$$\frac{dy}{dx} = c(A-y)$$

If both sides of this expression are divided by the factor y, then

$$\frac{1}{y}\frac{dy}{dx} = c_1\frac{(A-y)}{y} = c_1\frac{(10^{-cx})}{1-10^{-cx}}$$

where

$$A-y = A(10^{-cx}),$$

and

$$y = A(1-10^{-cx})$$

But y is proportional to the yield depression factor $-2k_1x$, and by adding this factor to the equation the second approximation of the Law of Diminishing Returns is obtained as follows:

$$\frac{1}{y}\frac{dy}{dx} = \left(c_1\frac{10^{-cx}}{1-10^{-cx}}\right) - 2k_1x$$

$$\int\frac{1}{y}\,dy = \int\left(c_1\frac{10^{-cx}}{1-10^{-cx}}\right)dx - \int(2k_1x)\,dx$$

$$= \log(1-10^{-cx}) - kx^2 + c$$

$$y = (1-10^{-cx})(10^{-kx^2})10^c$$

$$= A(1-10^{-cx})(10^{-kx^2}), \quad where\ A = 10^c$$

In this equation, A represents the maximum possible yield under normal conditions, y represents the yield actually obtained, x is the amount of nutrient used, c is the constant effect factor for this nutrient, and k is a depression factor that expresses the difference of the abnormal from the normal nutritional condition. Mitscherlich considered that, unlike c which retains its constancy with all plants under normal conditions, k varies with the circumstances responsible for the unbalanced depressive condition. The magnitude of k has to be determined for each specific case.

Mitscherlich presented the results of this extensive investigation in the series of curves reproduced in figure 24. These curves represent crop yields when nitrogen is added in increasing amounts until nutritionally toxic concentrations are attained which give the values for k indicated. These curves are calculated from the equation

$$y = 100(1-10^{-0.122x})10^{-kx^2}$$

and are supported by large numbers of experimental data.

The second approximation of the Law of Diminishing Returns, of course, has no practical value since the object of fertilizer studies is to determine only the additions which produce the optimum crop yield. For practical purposes, we need be concerned only with the first approximation of the Law of Diminishing Returns, particularly in the form proposed by Baule.

The addition of the depression factor to the expression serves to strengthen the Mitscherlich-Baule concept in that it shows that the logarithmic expression can be used, if necessary, to include the effects of any amount of fertilizers on crop yield.

5. Status at the Present Time

At the present time when the Law of Diminishing Returns is thought of at all, it is considered more in the light of the interpretation given to it by Baule (1918) than in the interpretation given it by either Mitscherlich (1909) or by Spillman (1924). Currently there are

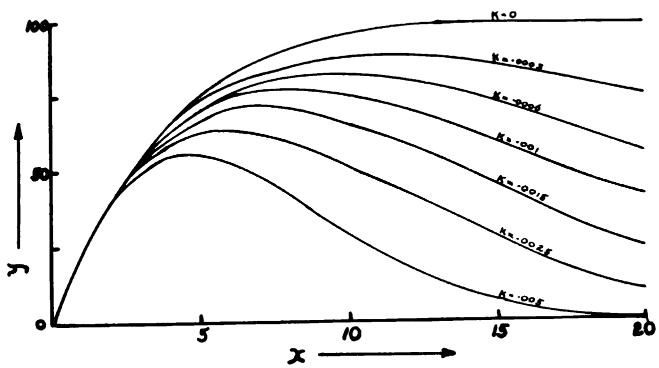

Figure 24. Yield depression curves derived from the equation $y = 100(1-10^{-.122x})10^{-kx^2}$. (Mitscherlich, 1928)

three degrees of opinion as to the value of these laws in agricultural research. There is a group which rigidly follows and applies the principles of the law as exemplified by Willcox (1930, 1937, 1941, 1943, 1944, 1945). A second group, chiefly represented by Bray (1948), has modified the approach with regard to the significance of the c value. Bray has referred to his modification as the mobility or elasticity concept of soil fertility. A third group has completely ignored the laws and does not make use of them in any way. The opinions of this last group probably resulted from the great advances made recently in the field of statistical analysis by Fisher (1934) at Rothamstead.

It is admitted that it is difficult to apply a rigid mathematical formula to experimental data of a biological nature because they are affected by many uncontrollable factors. It must also be admitted that only one mathematical expression has stood the test of time, while many others such as those proposed by Pfeiffer, Blanck, and Flungel (1912), Briggs (1925), and Balmukand (1928), have become submerged in the accumulating scientific literature.

There can be no doubt that the Mitscherlich-Baule expression is accurate in its statement of the relationship between crop yields and single factors. The weakness of the formula is that it is difficult to apply to more than one limiting factor, and unfortunately the problem of a single factor seldom occurs while the problem of multiple factors is constantly confronting students of plant nutrition.

Spillman (1933), so firmly believed in the authenticity of the logarithmic relationship between crop yields and fertilizer applied that he considered that any deviation of experimental data from those predicted by the law was due to experimental error. Macy (1936) made a critical study of the logarithmic relationship and concluded that both Liebig's and Mitscherlich's laws apply, but over different portions of the curve. He considered that when the amount of nutrient was low, the yield is directly proportional to the amount of nutrient in accordance with Liebig's law. During adjustment to a poverty level of nutrition, the necessary percentage of the nutrient in the plant increases with the sufficiency of that nutrient so that the response of the plant to the fertilizer decreases progressively as prophesied by Mitscherlich's law. Finally when a sufficiency of fertilizer is reached at a critical concentration and luxury consumption sets in, Liebig's law again holds because there is no appreciable further increase in yield. These relationships are shown in figure 25 which is based on data from Pfeiffer, Simmermacher, and Rippel (1919).

The work of Gregory (1937), Lagatu and Maume (1927-1933) and others has recently suggested the importance of considering the interactions of the various nutrients as well as their individual effects. Many research workers in the field of fertilizer utilization consequently lack an interest in the Law of Diminishing Returns in its original form.

In a series of publications appearing during the last 20 years, Wilcox has attempted to increase general interest in the usable aspects of the Law of Diminishing Returns. These publications (Willcox 1930,

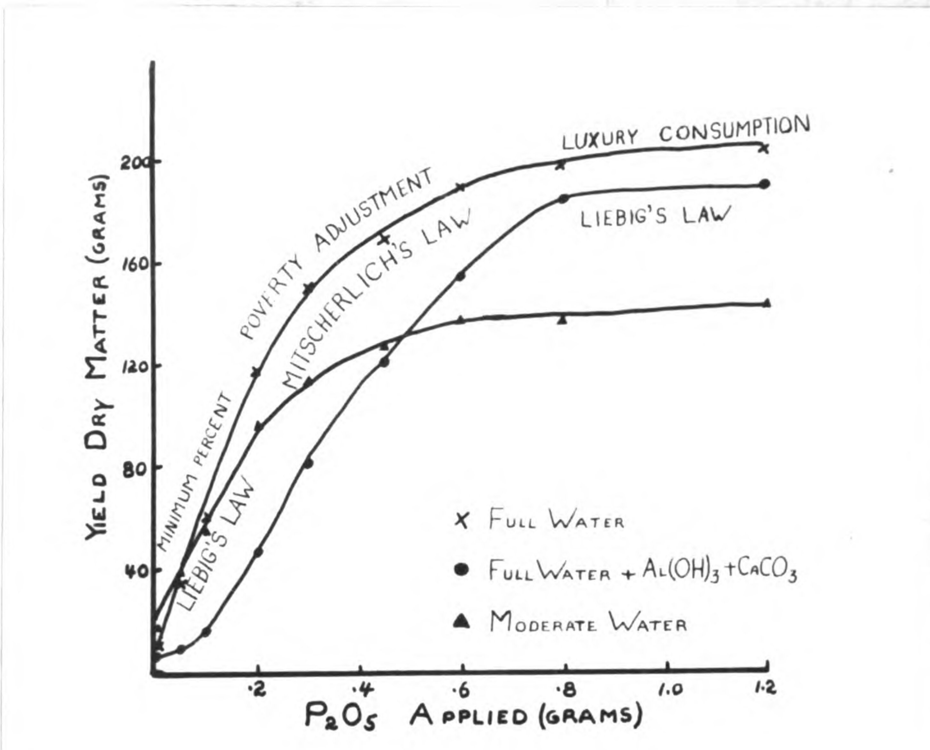

Figure 25. Yield curves for oats harvested at the milk stage and supplied with various amounts of phosphate fertilizer. Data from Pfeiffer, Simmermacher, and Rippel, 1919. Superimposed is the relationship between the Laws of Liebig and Mitscherlich proposed by Macy. (Macy, 1936)

1937, 1940, 1941, 1941a, 1941b, 1943, 1944, 1945, 1947, 1947a, 1949) are interpretations of data from field experimenta reported by other workers, and they attempt to show the usability of the Law of Diminishing Returns.

Two especially significant papers recently published are those of Mitscherlich (1947) and Gericke (1947) in which these authors report summaries of the results of an extensive soil fertility survey by field tests carried out in Germany in the 1930's. In this survey there were more than 27,000 replicated field trials, each with four or five levels of nitrogen, phosphate, or potash. The test covered a dozen kinds of staple crops on all kinds of soil and under a wide range of climatic and seasonal conditions. They found that when the results of all homologous tests with any kind of crop, and with any plant nutrient, were averaged, the yield data conformed to the requirements of the Mitscherlich equation $\text{Log}(A - y) = \text{Log } A - c(x + b)$. For example, the averaged data for 1642 tests with potatoes and phosphate fertilizer were as shown in table 23. These data fit the equation

$$\text{Log}(283 - y) = \text{Log } 283 - 0.6\ (x + 1.32)$$

within $\pm$ 0.8 percent for any one treatment. This indeed is a formidable set of data and should be carefully considered by any worker studying fertilizer requirements before he decides that the concept of Mitscherlich cannot be applied.

Table 23. Field tests in Germany with potatoes fertilized with varying amounts of phosphate. The calculated data were obtained by Mitscherlich's formula $\log(283 - y) = \log 283 - 0.6(x + 1.32)$. (Mitscherlich, 1947)

P_2O_5 metric quintals per hectar	Yields in metric quintals per hectar	
	Observed	Calculated
0.0	237	237
0.2	251	253
0.6	261	263
0.9	269	270
1.2	275	274

It appears that the science of fertilizer use is at a crossroad in its development, and each individual must weigh the evidence and decide for himself whether the Law of Dimishing Returns should serve as a valuable tool, or be discarded as the majority of workers have done. Irrespective of whether Willcox is correct, it is true that the Law of Diminishing Returns, particularly as modified by Baule (1918), has unique possibilities in its possible applications to field techniques for determining the fertilizer requirements of crops. Its value is becoming more widely appreciated at the present time through the efforts of Bray (1948) and Wynd (unpublished data).

The specific application of the Law of Diminishing Returns to techniques for determining the amounts of nutrients in soils and the determination of the fertilizer requirements of crops will be the subject of our next discussion.

V. APPLICATIONS OF THE LAW OF DIMINISHING RETURNS

A. Pot Cultures

1. Mitscherlich's Technique

Pot culture experiments have been conducted since the middle of the nineteenth century, but little quantitative value was attached to this type of experiment until Mitscherlich (1909, 1930) developed it as the basis of his studies on the effect of a single nutritional factor on plant growth. The technique devised by Mitscherlich essentially is a test for soil nutrients by which the nitrogen, phosphate and potassium requirements are determined simultaneously.

Mitscherlich suggested the use of enameled metal pots 20 centimeters tall and 20 centimeters in diameter, and possessing a central basal drainage hole. Each pot is placed on a metal stand in an enameled metal drainage saucer 24 centimeters in diameter. As the plants are grown out of doors, galvanized wire supports are used to protect the plants against wind damage. The experiments usually are conducted within a wire enclosure to avoid damage by birds. The construction and plans for the necessary equipment are given on pages 50-51 of Mitscherlich (1930).

The sample of soil to be tested should amount to 60 to 70 pounds, and should be obtained by collecting approximately 50 subsamples representing the upper 10 inches of the profile from systematically spaced sites in the field under investigation. The soil is screened to remove stones and to ensure thorough mixing of the added fertilizers.

The pots are brought to similar weights by adding washed gravel which is then mixed at the rate of 3 kilograms of soil to 6 kilograms of quartz sand. Calcium carbonate also is added at the rate of 1.5 grams per pot. Solutions of plant nutrients are added with a pipette as required to make up the experimental series and the entire mass is mixed thoroughly and transferred to the pot. The lower layer of 5 centimeters should be fairly well packed, but the remainder should be rather loose. For each soil tested, there should be a series of pots consisting of one pot without added nitrogen, three pots without added potash, three pots without added phosphate, and three pots with a complete fertilizer. The fertilizers used are added in solutions as follows:

1. 1.0 gram N as NH_4NO_3 in 20 milliliters of water,
2. 1.5 grams K_2O as K_2SO_4 in 50 milliliters of water,
3. 1.1 grams P_2O_5 as superphosphate in 50 milliliters of water,
4. 0.5 gram NaCl and 0.5 gram SO_4 in 5 milliliters of water.

Mitscherlich suggested using a variety of oats possessing stiff culms as the test plant in order to decrease error from wind damage. Damage from disease may be reduced by treating the seed with Upsulum. When dry, the oats are planted at a depth of 1.5 centimeters at the rate of 2 seeds in each of 25 equidistant holes in the soil. The pots are kept covered with the drainage saucers until the seedlings emerge. At the two-leaf stage, the plants are thinned to 25. The original weight of the pot is recorded and maintained by adding water on alternate days during the early stages of growth and daily thereafter until the crop is ripening. Less frequent watering is required as the plants approach maturity. Any drainage water should be added to the pot before watering and the soil brought to its water holding capacity.

The plants are cut at the soil level, the straw separated from the grain, and each dried at 100° C. in order to obtain the weight of the straw and grain as well as of the total yield.

The yields are interpreted from the Mitscherlich equation, $\text{Log } (A - y) = \text{Log } A - bc$, where c is the constant effect factor. For nitrogen, the c value is 0.122 dz./hectar, for P_2O_5 it is .6 dz./hectar, and for K_2O it is .93 dz./hectar.

The mean yield of the pots with complete fertilizer represents the maximum yield obtainable, and this is the A value in the equation. The yield from the cultures which received no phosphate nor potash represent the corresponding values of y.

Mitscherlich (1930, pages 23-26) presented tables from which the nutrient reserve in the unfertilized soil in pounds per acre may be found from the experimental data. These values are, of course, based on the assumption of a constant effect factor for each fertilizer component. It is also possible to use Mitscherlich's tables to calculate the percentage increase in yield which will result from the addition of a given amount of fertilizer.

Mitscherlich's technique had a wide acceptance from its inception, and it has been used extensively in Germany. Mitscherlich reports (1930) the foundation of the "Society of East Prussian Farmers and

Landowners" in 1923 to develop the application of his pot culture technique. Starting in 1923 with 23 soils, the procedure increased to include 2400 soils in 1929.

Only a few workers have used the method as outlined. Volk and Truog (1934), for example, used the method and the soils supplied by Mitscherlich to standardize their own rapid chemical soil analysis procedures. Magistad (1938) conducted a series of experiments with Hawaiian soils using the method and the mathematical constants as outlined by Mitscherlich. In this cooperative investigation, the soils were tested by Mitscherlich in Germany and by Magistad in Hawaii at the same time. The two sets of data are presented in table 24 and are shown graphically in figure 26. Considering that the techniques and soils were the same, the agreement between the two series is not good. Magistad noted that while the crop grew in 73 days in Germany it required from 82 to 172 days in Hawaii and that the grain represented 40 percent of the total crop in Germany but only 20 percent in Hawaii. The data indicated twice as much plant nutrients in the soil when tested in Hawaii as may be seen from table 25. This discrepancy probably was due to the higher temperatures in Hawaii making the nutrients more readily available, or to the longer growing period which permitted a longer feeding period by the crop.

Magistad concluded that oats were not suitable as an indicator crop in Hawaii and urged that care be taken in selecting a more appropriate indicator crop. There appeared to be a definite geographical limitation to the use of oats as an indicator crop in pot experiments.

Table 24. Yield of oats, expressed as percentages of the maximum yield, grown simultaneously in Hawaii and Germany using the Mitscherlich pot culture techniques. (Magistad, 1938)

Grams of nutrient per pot	Field 85		Field 14		Field 71		Field 109	
	Ger.	Haw.	Ger.	Haw.	Ger.	Haw.	Ger.	Haw.
Nitrogen variable								
0	7.5	18.1	17.7	22.3	5.1	16.1	5.3	10.1
.1	14.5	26.8	27.2	32.6	8.9	23.6	11.6	22.7
.25	26.0	39.7	37.3	42.3	19.5	34.5	23.9	44.1
.6	57.9	53.9	56.5	62.7	47.9	52.6	52.7	73.1
1.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Phosphorus variable								
0	7.7	9.7	10.1	32.6	6.2	38.6	7.8	62.3
.1	19.1	22.3	25.3	62.9	13.9	55.4	20.1	71.5
.25	37.7	59.3	47.7	87.0	31.1	81.1	48.2	88.3
.6	82.3	79.8	88.4	100.0	89.8	79.6	87.7	92.4
1.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Potassium variable								
0	78.7	106.0	89.2	106.8	71.1	84.4	73.3	92.4
.1	81.3	89.7	82.5	107.3	75.4	86.2	81.6	94.2
.25	82.4	94.9	89.9	107.7	83.2	88.7	82.0	91.7
.6	89.6	97.6	91.0	108.8	85.4	85.8	91.0	99.2
1.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Wt. complete grams	134.1	110.7	152.7	122.7	128.1	146.7	120.0	79.3

Table 25. Calculated b values or the amount of available plant food in dz/hectar of the sand-soil mixture of four soils tested by Mitscherlich in Germany and by Magistad in Hawaii. (Magistad, 1938)

Soil	Available nutrients in soil					
	Nitrogen		P_2O_5		K_2O	
	Germany	Hawaii	Germany	Hawaii	Germany	Hawaii
85	0.26	0.70	0.06	0.07	0.72	4.00
14	0.69	0.89	0.07	0.28	1.04	0.84
71	0.18	0.62	0.05	0.35	0.57	0.87
109	0.19	0.37	0.06	0.71	0.61	1.22
Mean of seven soils	0.30	0.60	0.08	0.35	0.74	1.85

Calculated from $b = \frac{\log A - \log(A - y)}{c^c}$

where $c = 0.122$ dz N/hectar

0.6 dz P_2O_5/hectar

0.93 dz K_2O/hectar

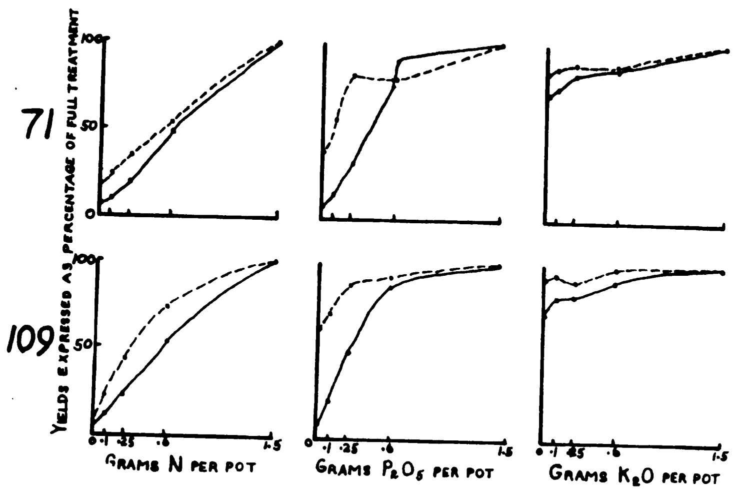

Figure 26. The relationship of yield of oats expressed as percentages of the maximum yield for various amounts of applied nitrogen, P_2O_5, and K_2O on four soils tested by the Mitscherlich pot technique in Germany and Hawaii. A. Soils 71 and 109. (Magistad, 1938)

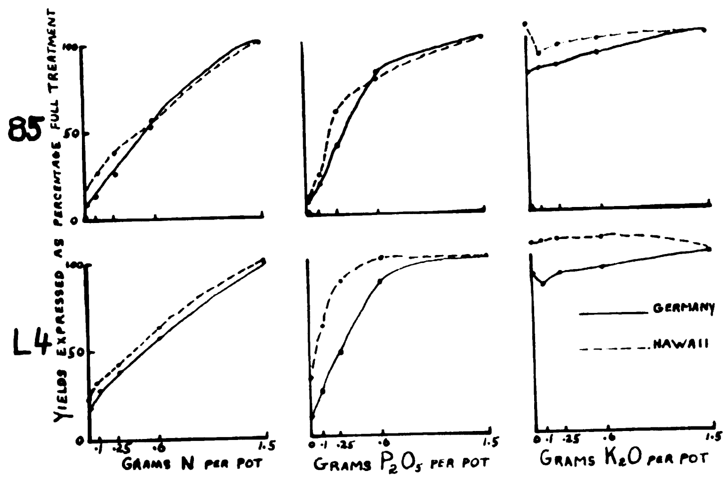

Figure 26. Continued.
B. Soils 85 and L4. (Magistad, 1938)

Better results were obtained by recalculating the c values.

Even as late as 1943 there was discussion of the possible value of these pot tests. Olsen and Shaw (1943) reported that the Mitscherlich pot tests gave a somewhat better differentiation of the available potassium than did the chemical tests of soils that were known to respond to added potassium.

The complex and expensive equipment required to conduct the Mitscherlich pot tests properly has discouraged its use and has encouraged the development of a number of simpler and less expensive techniques, even though they may not be as accurate.

2. Stephenson and Schuster Technique

The technique of conducting the pot test proposed by Stephenson and Schuster (1941) is a modification of the Mitscherlich method so that the availability of crop nutrients other than nitrogen, phosphate and potassium might be determined. The method is based on the vigorous extraction of nutrients by rapidly growing sunflower-plants on a small quantity of soil.

The plants are grown in the greenhouse in No. 1 tall, lacquered fruit cans containing 400 grams of air dried soil.

The quantity of soil required depends upon the number of plant nutrients being tested. The bulk sample should be composed of a sufficient number of subsamples to represent the field as a whole. When sieved, each can should receive a maximum of 400 grams of the thoroughly mixed, air dried soil. Nutrients are supplied from molar stock solutions of KH_2PO_4, K_2HPO_4, KCl, $CaH_2(PO_4)_2$, $Ca(NO_3)_2$, $MgSO_4 \cdot 7H_2O$, and $CaSO_4$, and from a 0.00567 molar solution of $B_2O_3 \cdot H_2O$. For the complete fertilizer treatment, all nutrients, properly diluted, are added and for other treatments, all nutrients excepting the one being tested are added. The nutrient ions are supplied at the following rates expressed as parts per million: N = 200, P = 217, K = 350, Mg = 168, S = 224, and B = 2.5. Lime, when used, is applied at the rate of 0.8 gram of calcium carbonate per can.

The sunflower seeds are planted, and after about nine days, the seedlings are thinned to five per can. The soils are kept at an optimum moisture content by adding distilled water at frequent intervals during the growth period of 6 to 10 weeks.

At the end of the growing period, the plants are cut off at the soil level and dried at 70° C. The average dry weight, obtained by any treatment is compared with that of the plants receiving the complete fertilizer treatment.

While Stephenson and Schuster (1941) have used their technique with good results on more than 20,000 soil samples, it would appear that it lacks the quantitative mathematical interpretation possible by the Mitscherlich method for there is no direct way of using the data to determine the actual fertilizer requirements of a crop.

3. Jenny Technique

Vandecaveye (1948) described a pot culture technique of Jenny using lettuce as the indicator crop. This crop is grown in pots in the greenhouse in the winter or outside in the summer. Lettuce is grown in six inch flower pots coated inside and out with two layers of asphaltum paint and with an additional layer of aluminum paint on the outside. Drainage water was collected in saucers treated in the same manner.

Each pot requires approximately 4 pounds of soil, and depending on the number of nutrients being tested, and the number of replicates used, the total amount of soil required is between 100 and 200 pounds. The sample being tested should be composited from a number of samples representing the upper 8 inches of the profile.

Jenny bases his fertilizer treatments on the following units, indicated as subscripts to the nutrient in question.

Nutrient	Milligrams per pot	Pounds per acre
N_1	$N = 80$	$N = 100$
P_1	$P_2O_5 = 80$	$P_2O_5 = 100$
K_1	$K_2O = 80$	$K_2O = 100$

He suggests that the most useful combinations of units are as follows:

$N_oP_oK_o$ $\quad$ $N_3P_oK_2$

$N_3P_3K_2$ $\quad$ $N_3P_3K_o$

$N_oP_3K_2$

Before the lettuce seedlings are transplanted, appropriate amounts of stock solutions are added to the pots and thoroughly mixed with the soil. Because in combination of units, $N_3P_3K_2$, has been shown over a period of years to give the highest yield and best growth of lettuce on all soils, Jenny considered that this rate of fertilizer application be considered optimum.

One lettuce plant is transplanted from a flat to the moist soil when about 4 weeks old, one plant to each pot. Distilled water is added regularly and any drainage water accumulating in the saucer is returned to the pot. After a six weeks growth period in the soil, the plants are harvested, dried at 70° C., and weighed.

The yields are recorded as percentages of the theoretical maximum. Jenny used these percentage values because they are less influenced by the environment than are the actual yields. Table 26 represents a tentative scale of nutrient deficiencies based on the percentages obtained. This technique requires much less time and equipment than does Mitscherlich's procedure.

4. Value of Pot Cultures

Pot cultures have been used in every phase of nutritional research and they still represent a very important technique. All such methods when used for the diagnosis of fertilizer requirements exhibit important weaknesses. The volume of soil accessible to the roots of the plants in the pot is much less than in the field, the mechanical disturbance of the soil in the preparation of the cultures changes the physical composition of the sample. Both of these factors affect the availability of the nutrients to the plant. Consequently, results obtained with pot cultures can be compared to those obtained under field conditions only with reservations. It has been reported by Stephenson and

Table 26. Tentative scale for estimating nutrient deficiencies by growing lettuce in pot culture by the method of Jenny. (see Vandecaveye, 1948)

Fertilizer added		Nutrient being tested	Yields as percentages of maximum		
For standards (100 percent yield)	For comparison		Definite deficiency	Probable deficiency	Uncertain deficiency
NPK NPKL NPKLS NPKLS ME	PK PKL PKLS PKLS ME	N	Less than 20	20 - 50	51 - 70
NPK NPKL NPKLS NPKLS ME	NK NKL NKLS NKLS ME	P	Less than 20	20 - 50	51 - 65
NPK NPKL NPKLS NPKLS ME	NP NPL NPLS NPLS ME	K	Less than 70	70— 75	76 - 80
NPKS NPKLS NPKLS ME	NPK NPKL NPKL ME	S	Less than 66	66 - 76	77 - 83
NPKL NPKLS NPKLS ME	NPK NPKS NPKS ME	Lime	Less than 55	55 - 73	74 - 80

N = nitrogen; P = P_2O_5, K = K_2O, L = $CaCO_3$, S = Sulphur, ME = Zn, B, Fe, Cu, Mg, Mo.

Schuster (1940) that in some cases the responses of plants observed in pots may be the reverse of those observed in the field.

Another questionable aspect of most pot culture techniques is the use of specific indicator plants. It was pointed out earlier in the present discussion that different species differed in their ability to absorb nutrients from the soil, and these differences certainly would limit the usability of data obtained from an arbitrarily selected species.

Another limitation to the use of pot culture techniques is the expense involved in both equipment and labor. While the experiments can be conducted with less expense than field trials, they are more costly than either chemical analysis of soil or of plant tissues.

The most accurate pot culture technique is that of Mitscherlich. Whereas other workers, such as Jenny (see Vandecaveye, 1948), and Stephenson and Schuster (1940), have attempted to simplify Mitscherlich's rather expensive and complicated procedure, their modifications have lessened the accuracy of the method. While it is agreed that pot culture techniques may yield valuable information as to the relative amounts of available plant nutrients in a soil, it should be recalled that they cannot be used for a quantitative approach to the fertilizer requirements of a crop. Mitscherlich's procedure, however, appears as an exception to this generality.

B. Field Techniques

1. Introduction

The Law of Diminishing Returns has been used most frequently for the interpretation of crop yields obtained under field conditions. Most of these applications were made during the first quarter of the present century in attempts to either prove or disprove the validity of applying the mathematical expression of the Law to the study of single factor relationships.

Within the last ten to fifteen years, however, some interesting work has been conducted with the Law of Diminishing Returns as the basis of interpretation by such workers as Magistad, Farden, and Lambert (1932), Farden and Magistad (1932), Capo (1938), Willcox (1941), Olsen and Shaw (1943), and Bray (1948). The work of Willcox (1941) and Magistad, Farden and Lambert (1932) was a direct application of the principle as laid down by Mitscherlich and Spillman, while other work, such as that of Bray (1948), is based on modifications of the concept. The discussion below is based primarily on the field techniques of these last three workers because it seems probable that their work will furnish the answer to the problem of the quantitative determination of fertilizer requirements.

2. Procedure of Magistad and Co-workers

a. Introduction

Since Magistad and his co-workers used the type of analysis put forth by Spillman (1924) in preference to that of Mitscherlich (1909),

it is necessary to examine the details of the Spillman technique as it was mentioned only briefly in the general discussion on the Law of Diminishing Returns.

Spillman's equation expressing the Law of Diminishing Returns is

$$Y = M - AR^x$$

where Y is the yield of the crop in tons per acre, M is the theoretically maximum crop yield, A is the yield increase due to the applications of large amounts of fertilizer, R is the ratio between successive increases in yield resulting from equal additions of fertilizer, and x is the number of units of fertilizer applied. Spillman (1924) showed that this equation was similar mathematically to that originally proposed by Mitscherlich (1909) and Baule (1918). As with the Mitscherlich equation, the Spillman equation does not apply when the nutrients are high enough to cause a depression in crop yield. An experiment conducted on pineapples by Magistad, Farden and Lambert (1932) will be taken as an example of this procedure.

The fertilizer was worked thoroughly into the upper three inches of soil. In December, 1927, 60 percent of the nitrogen, 57 percent of the P_2O_5, and all but fifty pounds of the K_2O were applied while the rest of the K_2O was applied in May and November, 1928. The fertilizer treatments and the crop yield data are given in table 27, and their mathematical analysis will be briefly described.

The major differences between the equation Log $(A - y) = \text{Log } A - bc$

Table 27. Fertilizer and yield data for the pineapple crops harvested in 1929. Treatments consisted of uniform amounts of nitrogen and of P_2O_5, and increasing amounts of K_2O. (Magistad, Farden and Lambert, 1932)

Plot	Fertilizer used pounds per acre			Total number of fruits	Total wt. of fruit (lbs.)	Aver. wt. of fruits (lbs.)	Tons of fruit per acre
	N	P_2O_5	K_2O				
A	287.5	150	125	3692	14131	3.83	16.21
B	287.5	150	200	3661	15068	4.12	17.27
C	287.5	150	300	3731	15855	4.25	18.17
D	287.5	150	400	3663	16211	4.43	18.58

and that proposed by Spillman, $Y = M - AR^x$, are the constants. Mitscherlich obtains his A constant directly from the yield of a plot receiving fertilizers for maximum yield and maintained that his c value was constant. Spillman, on the other hand, has three distinct constants M, A, and R, each of which must be determined for the particular experiment.

There are two methods by which these constants can be calculated, both of which will now be explained.

b. Calculation of Constants by the Method of Least Squares

The most probable values of M, A, and R are those which reduce to a minimum value the sum of the squares of the differences between the observed and the computed yields, or Y. That is

$$(Y = (M - AR^{x_1})^2 + (Y = (M - AR^{x_2})^2 \ldots\ldots\ldots\ldots \text{a minimum}$$

where

$x_1, x_2, x_3 \ldots\ldots\ldots\ldots x_n$ = unitsof fertilizer

$Y_1, Y_2, Y_3 \ldots\ldots\ldots\ldots Y_n$ = corresponding yields

The values of M, A, and R that reduce the equation, $Y = (M - AR^{x_1})^2$, to a minimum are obtained by differentiating the equation first with respect to M, then with respect to A, and finally with respect to R. When these differentiations are set to equal zero and then solved for M and A we have:

$$M = \frac{1}{n}(\Sigma Y + A \Sigma R^x)$$

$$A_1 = \frac{n \Sigma YR^x - \Sigma YR^x}{(\Sigma R^x)^2 - n \Sigma R^{2x}}$$

$$A_2 = \frac{n \Sigma (Y_x R^x - \Sigma Y \Sigma (_x R^x)}{\Sigma R^x \Sigma (_x R^x) - n \Sigma _x R^{2x}}$$

Having obtained these equations, the next step is to try a series of values of R and solve for A_1 and A_2. When these values of A_1 and A_2 are plotted against the R values and the curves drawn, the intersection of the curves will be the actual value of R and A.

c. Calculation of the Constants by the Simplified Procedure

A simpler procedure that may be followed to obtain the values of M, A, and R is to find the greatest common divisor of the amount of fertilizer applied per acre for each treatment and indicate this as "units" of fertilizer as has been done as shown below:

Lbs. K_2O used per acre	K_2O in units of 25 lbs. used per acre (x)	Yield in tons of fruit per acre (Y)
125	5	16.21
200	8	17.27
300	12	18.17
400	16	18.58

Then by assuming a value for R and solving the equations for A_1 and

A_2, it is found that for this data $A_1 = 6.4599$ and $A_2 = 6.4947$. Having obtained these values, the values of A_1 and A_2 are obtained for values of R above and below the original estimated R value. The results will be of the nature of that shown below.

R	A_1	A_2	Difference
.84	6.682	6.757	-.0752
.85	6.4599	6.4947	-.0348
.86	6.2813	6.2437	.0376

From these figures, it is apparent that the true value of R lies between 0.85 and 0.86. To find the actual value of R, the A_1 and A_2 values are plotted against R as has been done in Figure 27. The intersection of the two curves gives the true value of R, in this case 0.854, and the true value of A, in this case 6.38. Then from the equation

$$M = \frac{1}{n}\left(\Sigma Y + \Sigma A R^x\right)$$

it is possible to find M which in this case is 19.0 tons per acre. Using the three constants, the yield curve is given by the equation

$$Y = 19.1 - 6.38(0.854^x)$$

where x is the number of units of 25 pounds each of K_2O used per acre, or

$$Y = 19.1 - 6.38(0.538^x),$$

where x is the number of units of 100 pounds of K_2O per acre. The curve shown in figure 28 can now be used to indicate the units of x required to give optimum yield.

d. Determination of the Available Nutrient in the Soil

As with Mitscherlich's equation, the Spillman equation can be used after the constants have been calculated to determine the amount of a plant nutrient in the soil, that is, we can obtain a biological soil analysis. An examination of figure 28 shows that when no potash is applied, a yield of 12.72 tons is the equivalent of Y, and if the curve is projected to the abscissa we theoretically have no yield at all. This occurs when $x = 1.78$ which is equivalent to Mitscherlich's b value. Thus in this case, when $Y = 0$, then $x = 178$ pounds per acre, or the theoretical amount of available K_2O in the soil.

e. Determination of the Profitable Amount of Fertilizer

Farden and Magistad (1932) used the equation described above to determine the economic limit of fertilization. They considered both the cost of the fertilizers and the economic value of the crops they produced. They reasoned that if one ton of pineapples sold for V dollars and if x units of K produced Y tons of pineapples per acre, then the total value per acre would be VY dollars. If one unit of K costs K dollars and the N and P used cost L dollars, then the total cost of the fertilizer would be $(L+Kx)$ dollars. The profit from the crop would be

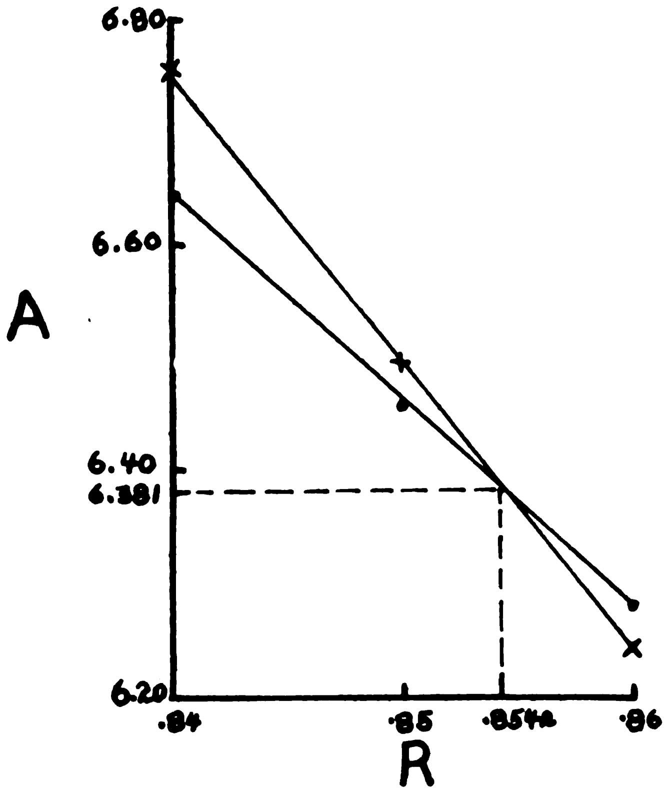

Figure 27. Graphic method for the determination of the true values of A and R in the equation $Y = M - AR^x$. (Magistad, Farden, and Lambert, 1932)

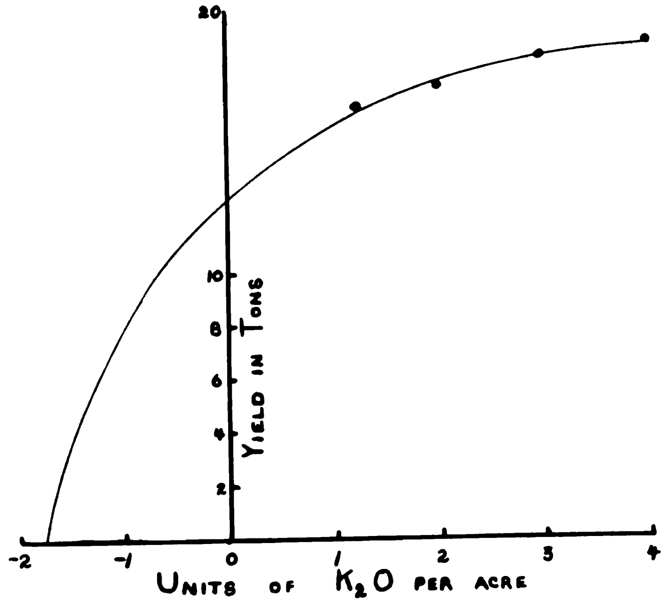

Figure 28. Fit of the observed values of crop yield in tons per acre to the yields calculated from the equation, $Y = 19.1 - 6.38(0.538)^x$. (Magistad, Farden, and Lambert, 1932)

$$P = VY - (L + Kx)$$

but $Y = (M - AR^x)$, hence

$$P = V(M - AR^x) - (L + Kx)$$

If one ton of pineapples sold for 25 dollars, and if 287.5 pounds of nitrogen and 150 pounds of P_2O_5 cost 45 dollars, one unit of K_2O cost 6.30 dollars, then

$$P = 25\ (19.1 - 6.38\ (.538^x)\} - (45 + 6.3x)$$

If various values of x are substituted, the curve reproduced in figure 29 is obtained. The most economical rate of fertilizer application is represented by the peak of the curve. This point may also be found by the following mathematical manipulation:

$$\frac{dP}{dx} = -.2303 VA \log R \cdot R^x - K$$

when $\frac{dP}{dx} = 0$

$$R^x = \frac{K}{-.2303 VA \log R}$$

$$x = \frac{1}{\log R}\left(\log K - \log(-.2303 VA \log R)\right)$$

The value of x is found to equal 4.45 units of K_2O and from the curve in figure 29 the maximum profit is found to be 394.76 dollars.

f. Discussion

There is a serious disadvantage to the Magistad technique which was frequently found in the earlier attempts to apply the Law of Diminishing Returns. His technique is based on the actual crop yields per

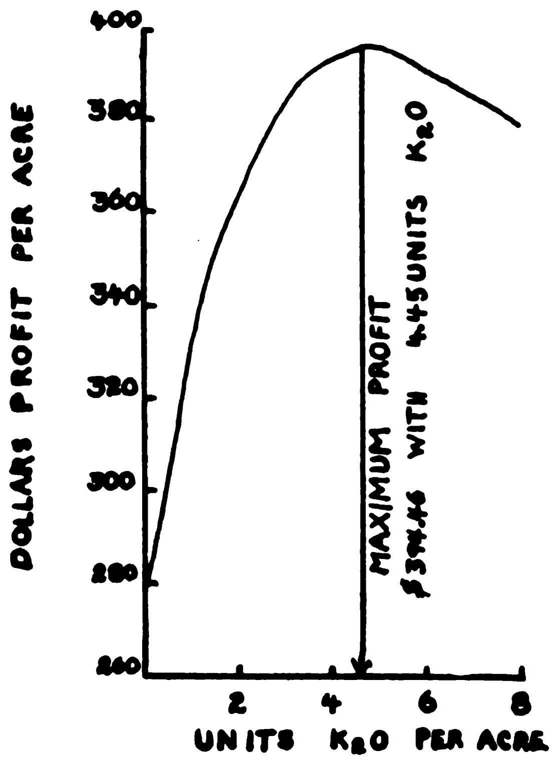

Figure 29. Graphical method for determining the rate of fertilizer application which will give maximum financial profit. (Farden and Magistad, 1932)

acre and consequently the result is subject to serious meterological effects. If this source of error was corrected using the percentage of the maximum yield rather than the absolute yield, the disadvantage of the technique would be greatly diminished. However, the procedure possesses a valuable feature not often considered because it takes into consideration the economics of the fertilizer problem. It permits the calculation not only of the amount of fertilizer that will give the greatest yield but also of the amount which produces the greatest financial profit.

As the complexity of the mathematics involved in the Magistad technique does not allow easy interpretations of field trials, the practical value of the technique for general fertilizer investigations is small. Consequently, other techniques for applying the Law of Diminishing Returns are more useful.

3. Procedure of Roger Bray

a. Introduction

The bases of Roger Bray's technique of applying the Law of Diminishing Returns is his own (1948) mobility or elasticity concept of plant nutrients, and the percentage yield concept of Baule (1918). Bray's mobility concept assumes that "the available soil nutrients have a variable availability which depends upon the mobility of the nutrients in the soil and on the nature of the plant. Those with little mobility like potassium, phosphorus, calcium and magnesium tend to follow the Baule (1918) percentage yield concept. The mobile nutrients, like nitrate nitrogen and water, tend to follow the Liebig idea of a limiting nutrient." The Baule percentage yield concept stated that the final yield is the product of all the factors involved in yield. If each nutrient level is expressedin terms of its ability to produce a certain percentage of the maximum yield attainable with a sufficiency of the nutrient the final yield is the product of the various percentage yields.

Bray studied the problem of the determination of fertilizer requirements of crops and stated the problem in the form of two questions: "1. How deficient is the soil to a given nutrient; that is, how much will it respond to addition of adequate amounts of the nutrient? 2. How much fertilizer is needed to give this response; that is, what is an adequate amount?" While any well designed field plot experiment answers both of these questions, the application of the results obtained by a field plot experiment to soils and conditions other than those under which the trial was conducted is the real problem which must be solved.

Bray (1948) studied problems of potassium and phosphorus availability in the soils of Illinois and attempted to answer the questions he posed. The first question was answered simply by comparing the crop yield from a plot which had received adequate amounts of all plant nutrients with the yield from a plot treated similarly but with one nutrient omitted. The second question was answered by the

application of a series of increments of one nutrient across plots fully fertilized with all other nutrients.

Bray pointed out that the fault of the majority of field experiments based on single-increment studies is that the increment applied represents a practical amount. This of course will neither indicate the extent of the deficiency nor the amount of fertilizer needed to overcome the deficiency. Bray avoided this fault by a modification of the Mitscherlich-Baule theorem so that the constancy of c value need not be assumed under all conditions, and he succeeded in developing a technique by which the quantity of fertilizer required for a given crop could be determined by a simple chemical analysis of the soil. His analytical methods have been standardized against field trials over a wide area and over a number of years by using the Mitscherlich logarithmic equation as the basis of comparison.

b. Correlation of the Potassium Test with Percentage Yield

The correlation of the chemical test for available potassium with the percentage yield illustrates the general procedure used by Bray. It is important to emphasize that the chemical methods used by Bray were precise laboratory techniques for determining the total exchangeable and water soluble forms of potassium and the chemosorbed and easily acid-soluble forms of phosphorus.

Bray established a series of 23 test plots on soils which were fully fertilized except for potassium. One plot of each set was supplied with adequate amounts of lime, phosphorus, and potassium (LPK)

while the second plot received lime and phosphorus only (LP). Green manure legume crops were grown in rotation to supply nitrogen. Before fertilization, each of the plots were thoroughly sampled to a depth of 6.6 inches.

The yields of corn obtained on the LP plot were expressed as percentages of the yield from the LPK plot which was considered to have given a maximum, or 100 percent, yield. For example, a soil which contained 158 pounds of available potassium per acre of 2,000,000 pounds of soil, the crop yields were 79.3 bushels of corn on the LP plots and 89 bushels on the LPK plots. Thus the yield from the LP yield is 89 percent of that from the LPK plot.

The percentages of the maximum yield obtained from the 23 plots are plotted against the amounts of available potassium expressed in pounds per acre in figure 30 together with the yield curve obtained by the equation, $\text{Log}(A-y) = \text{Log}A - c_1b_1$, where A is the 100 percent yield from the LPK plot, y is the yield from the LP plot, c_1 is the proportionality constant, and b_1 is the amount of potassium found by chemical test on the LP plot.

While Bray (1948) used the logarithmic equation developed by Mitscherlich, he did not agree with Mitscherlich(1909), Baule (1918), and Wilcox (1937) that the c value is constant under widely different conditions. He therefore approached the problem in a slightly different way. He averaged the proportionality constants for a large number of field tests carriedout over an area. If the individual values of the proportionality constants were not significantly different, he

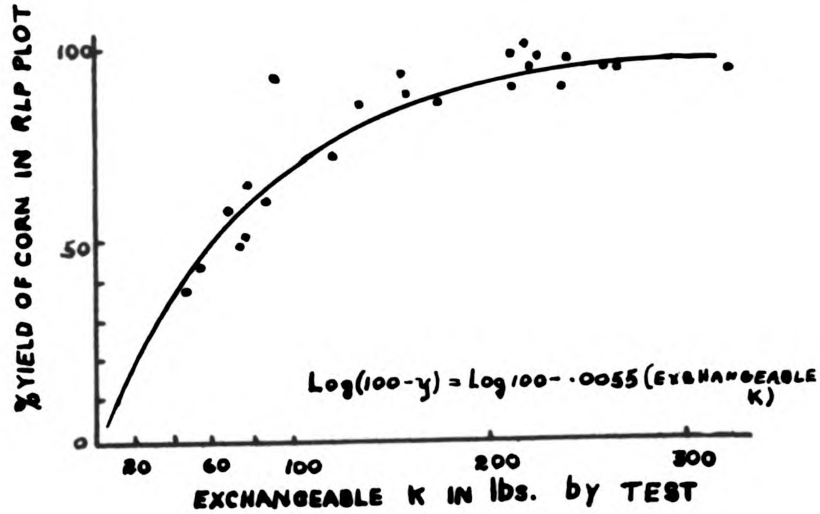

Figure 30. Relationships between exchangeable potassium and corn yields on untreated plots receiving no K_2O. (Bray, 1948)

considered that the average value would serve as a constant over the particular area.

In his study of available potassium, Bray averaged the values of c_1 and by taking this averaged value he constructed the curve shown in figure 28. As the standard error of estimate for this curve was 5 percent, Bray considered that it was possible to use the same interpretation of his chemical test for available potassium for all soils and conditions represented in his study. Using this curve, Bray prepared table 28 to predict the percentage increase in yield obtainable when adequate potash fertilizer was used for the different crops.

Bray realized that in nature there is seldom a single factor governing plant growth and he therefore assumed that any practical procedure must be capable of determining the requirements for more than one fertilizer at the same time. He accomplished this by applying Baule's percentage yield concept as described in the succeeding section.

d. Application of the Baule Percentage Yield Concept to Several Nutrients at the Same Time

The Baule percentage yield concept states that if there is enough available potassium in the soil to give 80 percent of the maximum yield obtainable with adequate potassium, and enough phosphorus to give 70 percent of the maximum yield obtainable with adequate phosphorus, then the yield obtained will be

$$\text{Yield} = \left(\frac{80}{100}\right)\left(\frac{70}{100}\right) = 56 \text{ percent}$$

Table 28. Relationship between the percentage of maximum yield without added potash and chemical test for available potassium. (Bray, 1948)

Available potassium (b_1)	Yields without added potash		
	Corn (c_1 0.0065)	Soybeans (c_1 0.0077)	Wheat or oats (c_1 0.012)
Lbs. per acre	Percent	Percent	Percent
40	45	51	66
60	60	65	80
80	70	76	90
100	78	83	94
130	85	90	97
150	90	93	98
200	95	97	--
300	98	--	--

This means that if all factors affecting growth were optimum excepting potassium and phosphorus, and if these nutrients are present in the percentages of the optimum concentration as indicated, the crop yield would be 56 percent of that obtainable if adequate potassium and phosphorus were present. Bray (1944, 1944a) used the data from the potassium experiments shown in table 28, and a further set of data for experiments with phosphorus in his application of this concept to crop yields in Illinois. The agreement which he found between the calculated and the observed yields are shown in figure 31. Unlike Baule (1918) and Mitscherlich (1909), Bray does not consider that the percentage yield concept can be applied to such mobile nutrients as nitrogen and water and therefore restricts the application of this concept to phosphorus and potassium.

d. Correlation of Soil Analyses with Fertilizer Requirements

Bray defines fertilizer requirement as the amount necessary to obtain 98 percent of the maximum yield obtained if the nutrient under consideration were present in an optimum amount. The value 98 percent is taken rather than 100 because of the increasing requirement as the theoretical maximum is approached. As the fertilizer requirement changes from year to year, with the kind of fertilizer used and with the method of application, it is necessary that new field trials be conducted for each fertilizer and for each method of its application. It is assumed, of course, that the fertilizer requirement follows the

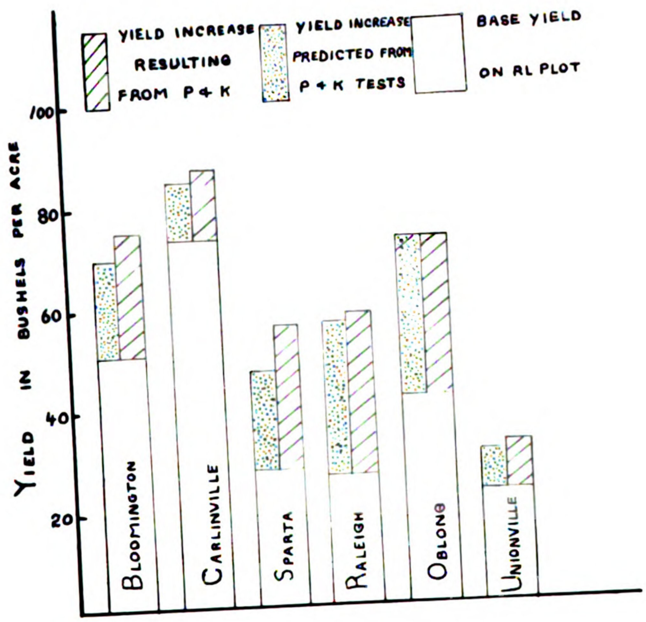

Figure 31. Comparison of actual and calculated increases in corn yields from field plots. Calculated yields are derived from Baule's percentage yield concept, and are based on chemical analysis of the soil. (Bray, 1944a)

Law of Diminishing Returns. If it is further assumed that the amount of a nutrient found by field test required to produce the maximum crop yield on a particular plot is accurate, then the application of the Law of Diminishing Returns permits the determination of the complete range of fertilizer requirements, as exemplified for the potassium tests presented in table 29.

Bray conducted his experiments on a series of fields over the State of Illinois, but he pointed out that the same information could be obtained by an adequate field plot design. For example, if for a given crop, an average c value in the equation $\text{Log}\,(A-y) = \text{Log}\,A - c_1 b_1$ is found to apply over a wide range of soil conditions, then a chemical analysis of the soil can be used to estimate the fertilizer requirements of the crop throughout the area.

Experimental procedure requires that one plot be adequately fertilized with all nutrients except one, and an adjacent plot be treated with the optimum amount of the fertilizer being studied. The experiment should be repeated over as wide a range of soil types and conditions as practical. If it is found that the c_1 value is constant, then the method is applicable to the area in question.

To establish the more exact fertilizer requirement for each chemical analysis of the soil, a study of the rates of fertilizer application is required. Such a study may be conducted as a separate experiment or it may be conducted simultaneously by applying a further modification of the Mitscherlich formula:

$$\text{Log}\,(A-y) = \text{Log}\,A - (c_1 b_1 + cx)$$

Table 29. Approximate potassium requirements, calculated as potassium chloride, for individual crops based on chemical analysis of the soil. (Bray, 1948)

Available potassium by test b_1	Potash requirements for different crops in terms of muriate of potash (0-0-50)		
	Corn and clovers (c 0.0094)	Soybeans (c 0.015)	Wheat and oats (c 0.020)
lbs. per acre	lbs. per acre	lbs. per acre	lbs. per acre
40	153	94	62
60	136	84	50
80	124	72	36
100	110	62	24
130	92	46	--
150	74	36	--
200	40	--	--

where c is the constant for the fertilizer used and x the increment or amount of fertilizer added.

It is advisable to establish a central plot deficient in the fertilizer being studied and four surrounding plots receiving increasing increments of the nutrient under investigation. The greatest fertilizer application should be adequate to support the maximum yield in so far as this nutrient is concerned. It is important to point out that it is essential to have as little soil variation as possible between the deficient plot and the various increments and consequently the plots should not be randomized.

e. Discussion

The contributions of Roger Bray constitute the most interesting advances of the present day in the study of the fertilizer requirements of crops. His studies have been conducted with corn, wheat, oats, and soybeans in Illinois, and other workers are applying his techniques to other crops in many areas. The writer is applying the technique to the tomato fields of Prince Edward County in Ontario, and to the potato fields near Guelph, Ontario. While there is no doubt that Bray's procedure may be applied in areas of relatively uniform soil conditions such as are found in Illinois, there is the possibility that difficulty will be encountered in areas where wider variation of soil types are found. If this possibility proves to be unlikely, or of small significance, Bray's contribution to agricultural science is of a high order.

One point that might be questioned in Bray's concepts is his belief that those nutrients regarded as "mobile" cannot be investigated by Baule's percentage yield concept. As was pointed out earlier, the amounts of nitrogen and water applied by numerous workers was part of the basis upon which the logarithmic yield equation was developed. Appleton and Wynd (1951) found that in 1948, the relation between yield and applied irrigation water followed the relationship expected from Bray's mobility concept, while in the following year this relationship followed Baule's percentage yield concept. Whether or not Bray's concept of the special problem of the "mobile" nutrients is correct, his contribution to the problems of potassium and phosphate fertilizer use is of unquestionable value.

4. Procedure of Willcox

a. Introduction

One of the less generally understood techniques of interpreting field experiments in order to show the maximum crop yield obtainable with the use of fertilizer, is that proposed by Willcox (1930-1949). This is probably due to the general lack of understanding of the Law of Diminishing Returns. Perhaps the application of this law has been hindered by the very mass of evidence which Mitscherlich (1947), Gerlich (1947) and Willcox have presented to support it. Only Bray and Willcox have made significant attempts to apply the Law of Diminishing Returns in recent years.

The greatest difference between the work of Bray and Willcox is the adherence of Willcox to the idea of the constancy of the effect factor as expressed in Baule units and as used in the equation

$$\text{Log }(A-y) = \text{Log } A - .301\ x$$

where x is in Baule units. Willcox has proposed techniques which may be used by the research workers for their interpretation of the data obtained from field trials (1940, 1941, 1941a, 1941b, 1944, 1945, 1945a, 1947, 1947a) and also a simpler "farmers" technique (undated circulars a and b).

b. Procedure for Research Workers

(1.) Preparation of the Standard or Universal Yield Diagram

Willcox (1947a) described the construction of a universal yield diagram which is the basis of the interpretation of his yield data. He plotted on a standard sheet of graph paper the units of yield per acre as the ordinates and the Baule units of fertilizer as the abscissa. He then drew the curves from A = 10 to A = 24 inclusive, using his published co-ordinates (1947a), and obtained a diagram similar in form to that shown in figure 32. After these whole number curves have been drawn the intermediate curves may be drawn by interpolation. Willcox refers to this diagram as the universal yield diagram because it presents the crop yields obtained up to 8 Baule units of a plant nutrient, beyond which no increase in yield could be expected.

As field plot experiments frequently use less than 1 Baule unit

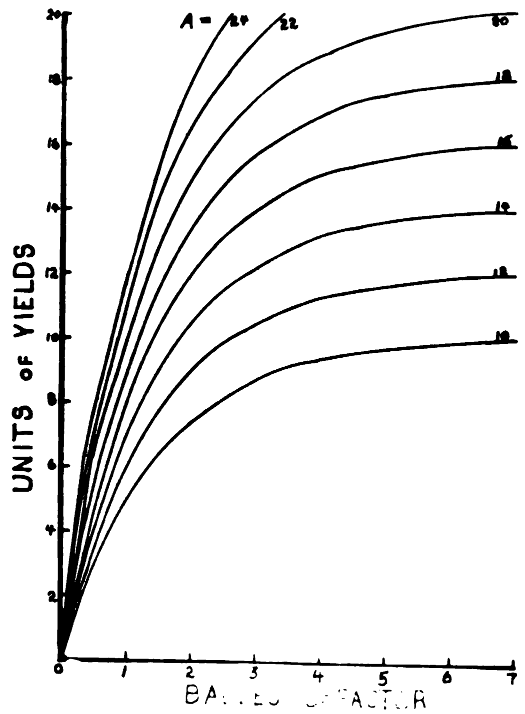

Figure 32. General agrobiologic yield diagram from 0 to 7 Baule units. (Willcox, 1947a)

per acre of each of the major plant nutrients, it is inconvenient to plot yields obtained by these small additions on this general diagram. Willcox suggested that the investigator provide himself with two standard yield diagrams, one for very small increments of fertilizer (from 0 to 2 Baule units), and one for intermediate quantities (from 0 to 4 Baule units). This permits a spreading of the curves which facilitates the convenient use of the diagram. Table 30 permits the determination of the co-ordinates for a diagram of any scale. This table shows the percentage of the maximum yield obtainable from a given number of Baule units of any nutritional factor. For example, if the coordinates for A = 25 are being computed, the head of the first column in table 30 indicates that 0.1 Baule will produce 6.7 percent of the maximum yield. For curve A = 25, 1.65 or 0.067 x 25 is the point where this curve crosses the ordinate at 0.1. This procedure repeated gives the complete series of curves necessary for the universal yield diagram.

It should be pointed out that the magnitudes of the Baule units are as follows:

Nitrogen = 223 pounds per acre

P_2O_5 = 45 pounds per acre

K_2O = 82 pounds per acre

(2.) Interpretation of Field Plot Data

The use of the general or universal yield diagram described above has been described by Willcox in a series of papers beginning in 1941

Table 30. Data for computing a Universal Yield Diagram on any scale. (Willcox, 1947a)

Baule units	% of A	Baule units	% of A	Baule units	% of A	Baule units	% of A	Baule units	% of A
0.1	6.70	1.0	50.0	1.9	73.21	2.8	85.64	3.7	92.31
0.2	12.94	1.1	53.35	2.0	75.00	2.9	86.60	3.8	92.82
0.3	18.77	1.2	56.47	2.1	76.67	3.0	87.50	3.9	93.30
0.4	24.21	1.3	59.39	2.2	78.24	3.1	88.34	4.0	93.75
0.5	29.29	1.4	62.11	2.3	79.69	3.2	89.12	5.0	96.88
0.6	34.02	1.5	64.64	2.4	81.05	3.3	89.85	6.0	98.44
0.7	38.44	1.6	67.01	2.5	82.32	3.4	90.53	7.0	99.22
0.8	42.57	1.7	69.22	2.6	83.51	3.5	91.16	8.0	99.61
0.9	46.41	1.8	71.28	2.7	84.61	3.6	91.75	9.0	99.80

and continuing to the present day. The majority of these papers are examples of the applications to the data published by other workers. Willcox himself hasnot conducted experimental work under field conditions. It will serve our purpose to explain his technique of interpretation by referring to one of his applications (1943) based on data published by Olsen and Shaw (1943).

In this study, as seen in figure 33, the universal yield diagram consists of a series of Mitscherlich yield curves numbered from 6 to 17, calculated from corresponding values of A by the Mitscherlich-Baule equation,

$$\log (A-y) = \log A - 0.301\ x$$

The first step in the procedure was to obtain the total yield in pounds of dry matter. Since this gives large values, it is convenient to convert these into units which will fit on the diagram. The most convenient unit is 800 pounds. The yield data from the Olsen and Shaw experiment, when so converted, are shown in table 31.

It was necessary to convert the amount of fertilizer added to Baule units. Olsen and Shaw (1943) used 0, 10, 20 and 40 pounds of K_2O per acre and as 1 Baule unit is equivalent to 82 poundsof K_2O per acre, the number of Baule units added were 0.122, 0.244, and 0.488, as indicated in table 31.

The interpretation is given below, using the experiment on Miami silt loam in 1939, and represented by B in figure 33. A sheet of transparent paper was placed over the universal yield diagram. A line was drawn over the horizontal line corresponding to the ordinate 9 on

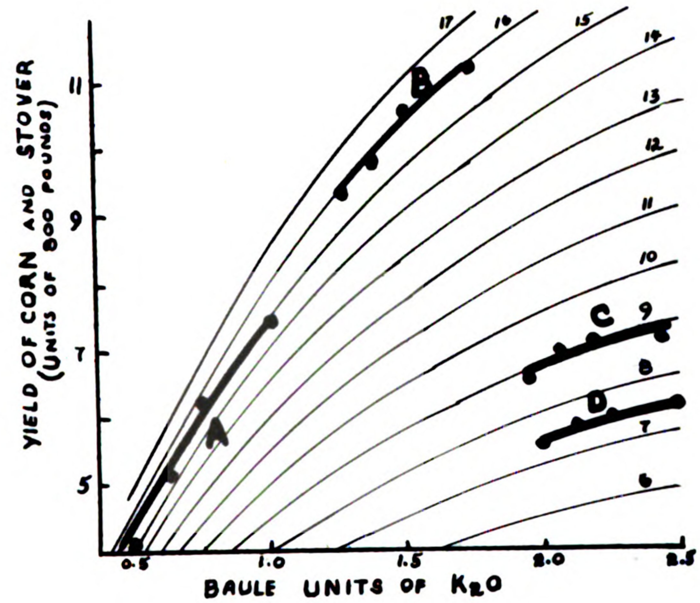

Figure 33. Four of Olsen and Shaw's (1943) field experiments plotted on the universal yield diagram,

A. Clermont silt loam (1940)
B. Miami silt loam (1939)
C. Muskingum silty clay (1940)
D. Wooster silt loam (1940)
(Willcox, 1943)

Table 31. Data for the yields of corn from Olsen and Shaw (1943) as calculated for interpretation on the universal yield diagram by Willcox. (Willcox, 1943)

Soil	Yields pounds	Yields units	Baule units K_2O	Soil	Yields pounds	Yields units	Baule units K_2O
A	3229	4.04	0	B	7437	9.30	0
	4137	5.17	0.122		7874	9.84	0.122
	4976	6.22	0.244		8452	10.56	0.244
	5874	7.34	0.488		8935	11.17	0.488
C	5260	6.57	0	D	4444	5.55	0
	5579	6.97	0.122		4665	5.83	0.122
	5625	7.03	0.244		4800	6.00	0.244
	5676	7.09	0.488		4850	6.06	0.488

A Clermont Silt Loam

B Miami Silt Loam

C Muskingum Silty Clay

D Wooster Silt Clay

the diagram, and on a vertical line above this, a point is indicated at the value of 9.30. A second point is drawn at 0.122 Baule units to the right corresponding to ordinate 9.84, a third at 0.244 Baule units to the right of the first dot corresponding to ordinate 10.56, and a fourth point at 0.488 Baule units to the right corresponding to the ordinate 11.17. The transparent paper is then removed to the right or left until the four dots aline themselves, as close as possible to one of the curves. The value of A is given by the number of the curve which shows the closest fit. In this case, the curve is number 16 and A is equivalent to 12,800 or 16 x 800 pounds per acre yield of corn plus stover. This is the maximum yield that could be obtained on this field by using potash in such a year as 1939. The amount of potash in the untreated soil is determined by finding the Baule units of K_2O in the check plot, which produced 9.30 units of yield, and then multiplying by 82 to convert the value to pounds per acre of K_2O. The same procedure will give curves of similar type for the other data in table 31.

Besides the maximum yield (A) and the amount of a nutrient in the soil (b), there are other important conclusions that can be drawn from the use of this universal diagram. For example, a curve located as is the one for Miami silt loam indicates that the soil contained a good supply of nutrients other than potassium, but that potassium was the limiting factor. From the slope of the curve, one could expect that larger additions of potassium fertilizer would further increase the yield in this case. The equation,

$$\log (16-y) = \log 16 - 0.301 x$$

can be used to determine what increase in yield could be expected with the addition of the optimum amount of potash. For example, if Olsen and Shaw had added 40 pounds more of K_2O, they would have added a total of 2.251 Baule units. The equation above shows that this further addition would have resulted in an increase of 1176 pounds of corn plus stover. The same situation is apparent from the data obtained on Clermont silt loam (A). On the other hand, the Muskingum silty clay (C), and the Wooster silt loam (D) contain identical amounts of potash, and when yield data from these two soils are plotted, the curves in the diagram indicate that the factors causing the different yields are other than the amount of available potassium. This conclusion follows because the yield curves fall on the flat portion of the Mitscherlich curve.

These examples cited by Willcox in which all points fall on the curve are, of course, exceptions and Willcox has found it necessary to study the instances in which this is not the case. In the "scatter" type of distribution discussed by Willcox (1944, 1944a) the points cannot be made to fit smoothly on any of the normal yield curves, but arrange themselves in the form of an inverted crescent in some cases, as illustrated in figure 32, and in other cases they oscillate from one side of the standard curve to the other. Willcox maintained that, except in the most extreme cases, the aberrant points of this

type line up along the curve with sufficient closeness to make identification of the most probable value of A fairly certain. In the case of the experiment with sugar cane, the results shown in figure 34 indicate that the aberrancy probably was due to small differences in the individual plots and represented experimental error over which the investigator had little or no control.

A second type of aberrancy is shown in figure 35 and is referred to as the "near-end" depression, and was discussed by Willcox (1944, 1944a, undated c). This particular type of aberrancy is marked by an abnormal spread between the yields obtained from the untreated plots and those obtained by the first increment of fertilizer, even though the yields obtained by the addition of the higher increments conform to the normal yield curve. Willcox does not consider that he had a certain explanation of this effect, but he suggested that it may have been due to one of two causes; either from the depression of the third and fourth points due to the heavy application of potash, or to the influence of the potash on some unknown soil condition which established a level of fertility qualitatively different from that existing in the untreated plot.

A third type of aberrancy is referred to by Willcox as a "far-end" depression which he discussed in a series of papers (1941a, 1944, 1945, 1945a, 1947). This effect, due to the depressive action of some factor on growth, was expressed by Mitscherlich (1928) mathematically in the equation

$$y = A\,(1 - 10^{-cx})\,10^{-kx^2}$$

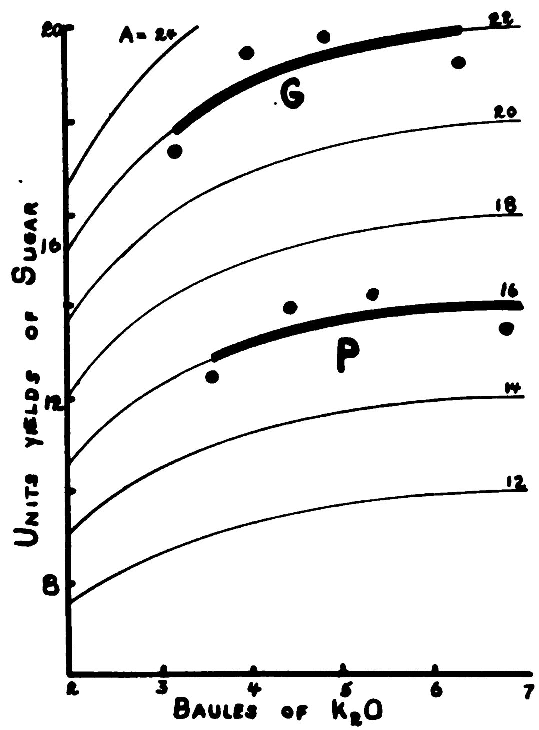

Figure 34. The scatter type of aberration in a sugar cane-potash experiment conducted in a good(G) season and in a poor (P) season. (Willcox, 1944a)

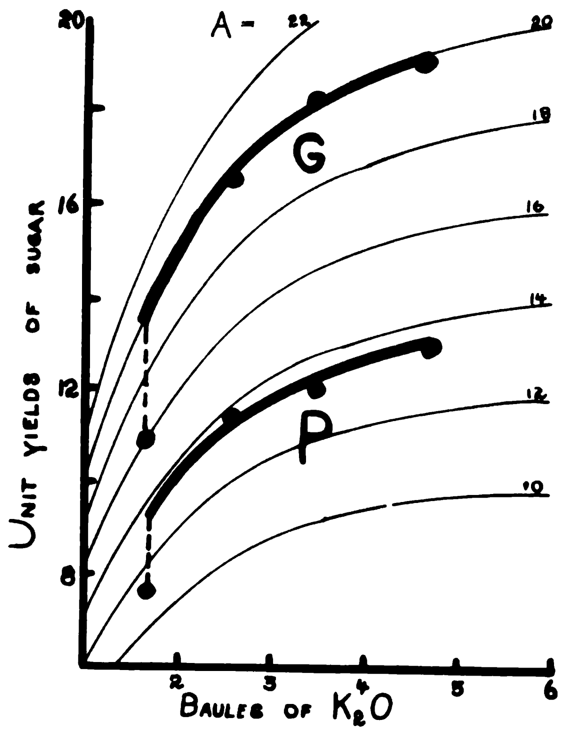

Figure 35. The "near-end" type of aberration in sugar cane treated with potash in a good (G) season and in a poor (P) season. (Willcox, 1944a)

An example of this effect is given in figure 36. This curve was prepared by Willcox (1944) from Prescott's data on corn yields and it shows that the yield is depressed by the higher applications of nitrogen. Willcox maintained that this type of aberration generally is due to the lack of "agrobiological balance" between the increased amount of the nutrient being studied and the amounts of other factors which are being kept at a constant optimum. It would seem that in such instances, some other factor must be corrected before the maximum benefit could be obtained from the fertilizer.

c. Procedure for Farmers

Willcox (undated a, b) recognizes the necessity of a simple method whereby a farmer could determine how much plant nutrient is contained in an untreated soil and how much fertilizer could be profitably used.

He proposed that the farmer select a strip 484 by 105 feet on the field to be tested, and plant a crop such as corn. The test strip will therefore be comprised of 35 rows wide. If all three major fertilizer components are to be investigated, alternate groups of five rows should be fertilized with K_2O, P_2O_5, and nitrogen at a given rate, as indicated in figure 37. The crop should be given the normal care and at harvest the farmer would measure or weigh the crop of grain from the middle strip and from the two adjacent untreated strips. The yields from the untreated strips would be averaged for comparison with the yield from the treated strip.

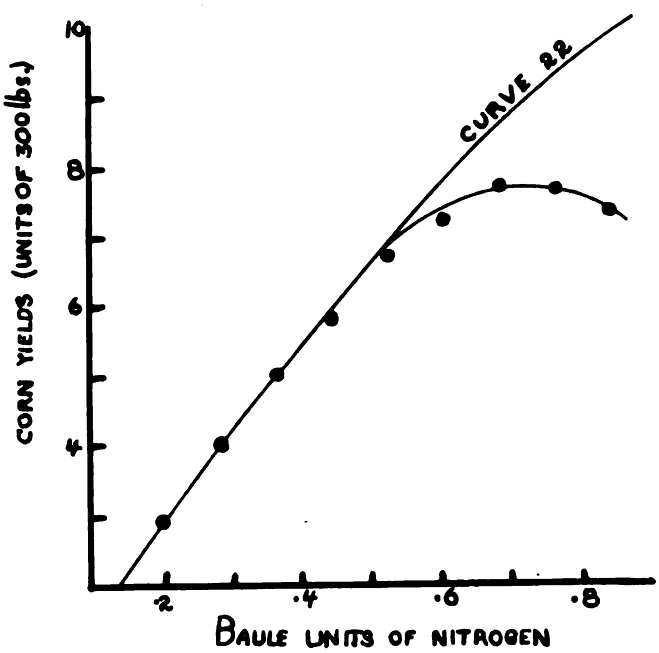

Figure 36. "Far-end" depression found in Prescott's field test with corn. (Willcox, 1944)

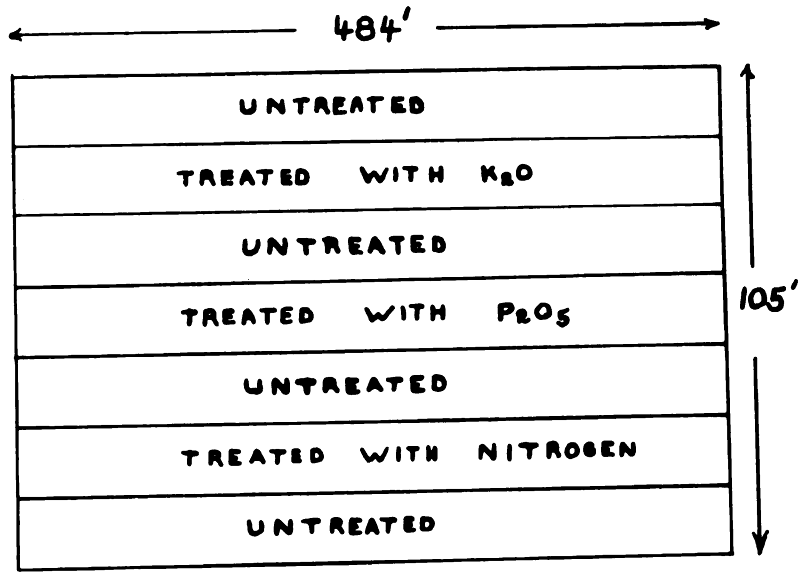

Figure 37. Plot design for three one-plot fertilizer tests with corn. (Willcox, a)

The data the farmer will have at his disposal in the case of a study of the effects of potash on corn would be as follows:

Amount of K_2O used	38 pounds per acre
Yield from treated strip	72.3 bushels per acre
Yield from untreated strip	51.0 bushels per acre
Percent increase in yield due to the added potash	41.76 percent

By referring to figure 38 or to other appropriate curves, (Willcox b), and by locating the percentage increase in yield on this graph, the farmer would find that there were 58.5 pounds of K_2O in the untreated soil.

Then by referring to figure 39, or to other appropriate curves, he would detect what percent of the maximum yield this value represented. In the present instance, the value is 40 percent which means that the 51 bushels obtained are only 40 percent of the yield obtainable by adding an adequate amount of potash.

Finally, by calculating the cost of the fertilizer, and the value of the increased yield, the farmer could determine from figure 39 how much fertilizer he could apply economically.

d. Discussion

Unfortunately the techniques proposed by Willcox enjoy no general recognition as to their value and importance. The greatest disagreement concerning their value has centered around the question of whether or not the effect factor c in the Mitscherlich equation is a constant

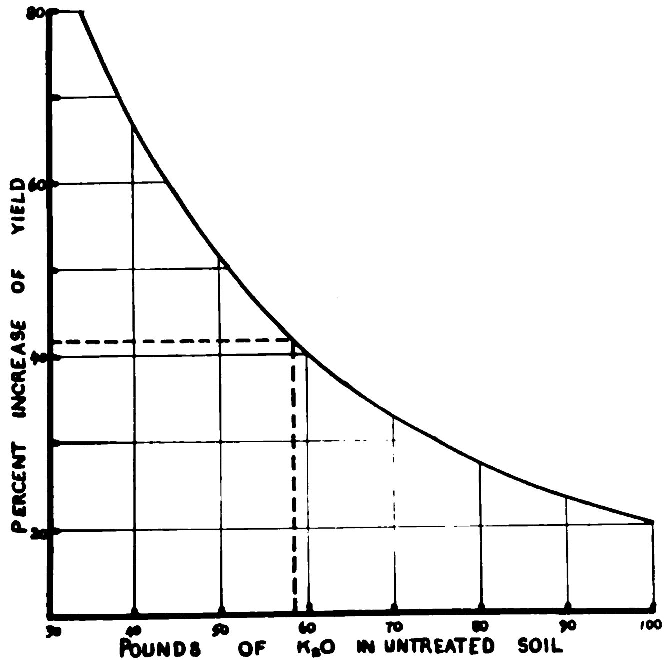

Figure 38. Diagram for use with the one-plot technique to show the amounts of K_2O in the soil. (Willcox, a)

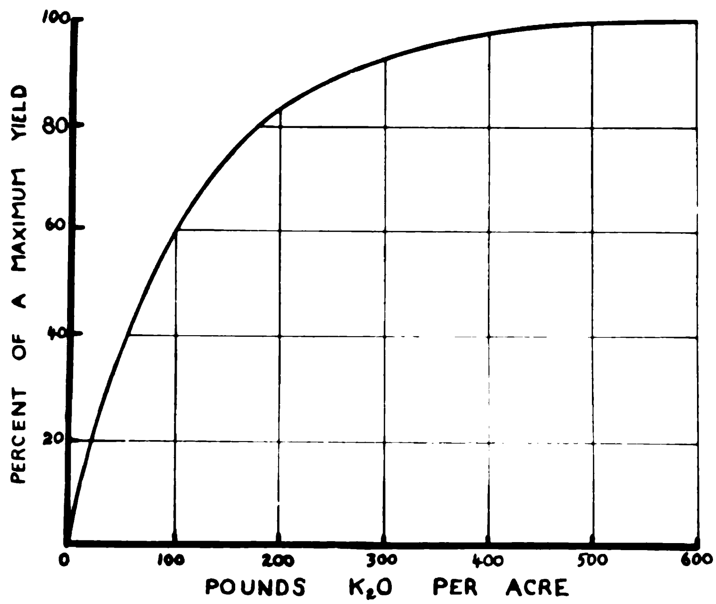

Figure 39. Diagram for use with the one-plot technique to show the amount of K_2O fertilizer which may be profitably applied to a crop. (Willcox, a)

under the widely different conditions under which Willcox used it. He quoted one worker's comments on this point as follows: "It is unthinkable that one equation will be equally applicable to all growth factors unless that equation is so general or lacking in precision as to be of very little value..........Surely, not all growth factors work in the same way." (Willcox c). The criticism of "unthinkability" is not evidence and in no way could such a subjective reaction discredit the work of Willcox. It is by demonstration, not by "unthinkability," that Willcox's proposals should be investigated.

The mass of information on a large number of crops growing under widely different conditions accumulated by Willcox does not necessarily prove that his concepts are correct, but it does justify careful consideration of his work. Until actual experiment adequately disproves the work of Willcox, his concepts must be regarded as a significant contribution to the science of fertilizer use.

It is the opinion of the writer that the techniques described above should not be delegated to the minor role they now possess, but should be applied more generally in an effort to show by actual data if they can replace current methods based on statistical treatment. If these techniques can be shown to apply, they will be of extreme importance for they put the interpretation of chemical analyses of soil on a firm foundation and permit individual farmers to conduct and evaluate their own field trials.

VI. USE OF SEEDLINGS FOR DETERMINING NUTRIENT DEFICIENCIES OF CROPS

A. Introduction

The various methods for using seedlings for the determination of plant nutrients in soils, developed during the last twenty-five years, were attempts to replace chemical analyses and pot culture procedures. The theory of the seedling techniques was that the plant itself was the ideal extracting reagent. The theory implies that chemical methods were restricted in their value because no extracting agent had been found which would give a true indication of the amounts of available nutrients under all conditions. The seedling techniques were developed as a supplement to pot studies and field plot tests because of the need for a simpler and more rapid laboratory method. Furthermore, these procedures were thought necessary to supply more detailed information concerning the fertility of the individual fields, for of course, it is economically impractical to use field plots or pot experiments for this purpose. The most acceptable procedure utilizing seedlings was that developed by Neubauer and Schneider (1923) and Neubauer (1923, 1925, 1929) and later modified by Thornton (1931, 1931a, 1932, 1932a, 1935, 1935a), Pettinger and Thornton (1934), and McGeorge (1939, 1942, 1946, 1946a).

B. Neubauer Procedure

1. Technique

The principle of the Neubauer method is the intensive absorption of nutrients by a large number of rye seedlings grown on a small quantity of soil. These plants, growing under optimum conditions, form extensive root systems and exhaust the soil of the available plant nutrients in a comparatively short time. Chemical analysis of the tops and roots of the seedlings discloses the amounts of nutrients removed from the soil.

The conduction of Neubauer's test requires meticulous care, and the details of manipulation must be standardized so that comparative data may be obtained. Failure to adequately standardize the conditions probably is the major cause of the failures to obtain satisfactory data that have been reported in the literature.

It is necessary to obtain a representative sample of soil at the depth desired, to air dry it under uniform conditions, and to mix it thoroughly by passing it through a 2-millimeter sieve. A moisture content must be determined so that samples equivalent to 100 grams of air dry soil may be taken from the bulk soil sample.

One hundred grams of the soil are mixed with 50 grams of quartz sand which has been washed with hydrochloric acid and distilled water and then dried at 150° C. The mixture is placed in a glass dish 11 centimeters in diameter and 7 centimeters deep and moistened with 20 milliliters of distilled water. A further 150 grams of dry quartz sand are distributed evenly over the surface and moistened with an additional 20 milliliters of distilled water.

Rye seeds exhibiting a high percentage germination, selected for size, and weighing approximately 4 grams per 100 seeds, should be used.

Neubauer used the Petkuser variety of rye furnished by the Stastliche, Landwirtschaftliche, Versuchsanstalt, Dresden-A, Stubel Allee 2, Germany. The rye seeds are disinfected by soaking 1.5 hours in a solution of Uspulum. Using a standard pegboard, Neubauer punched 100 uniformly spaced, concentric holes in the soil in each container and planted one seed in each hole with the germ end placed at the bottom. To facilitate subsequent watering, a glass tube is forced into the soil in the center of the container.

A further 100 grams of quartz sand, moistened with 20 milliliters of water, are spread over the surface and the weight of each dish then recorded. Three dishes are prepared as above for each soil being studied and three blanks are used containing only quartz sand.

The cultures are maintained at constant temperature, preferably 20° C. $\pm 1^\circ$ C, and protected from direct sunlight. Each day, the cultures are brought to their original weights by adding distilled water. The seedlings are harvested at the end of 17 days and dried.

The roots and tops of the dried seedlings are ashed, and the total amounts of K_2O and P_2O_5 which they contain are determined. The amounts of P_2O_5 and K_2O found in the seedlings grown in the blank cultures are subtracted from these amounts in order to obtain the amounts of the nutrients removed from the soil. These corrected values are recorded as the number of milligrams of P_2O_5 and K_2O removed from 100 grams of dry soil, and they are assumed to represent the total available amounts of the nutrients in the soil.

Neubauer used the data from a large number of tests, and determined the limits below which deficiencies occurred. The "limit values" given by him in three publications exhibit a certain variability as indicated below.

Neubauer Limit values, below which sub-maximum yields occur, expressed as milligrams per 100 grams of dry soil

Nutrient	Year	Barley	Oats	Rye	Wheat	Turnips	Potatoes	Sugar Beets
P_2O_5	1923	6	6	8	8	--	9	10
	1925	7	7	8	8	14	10	12
	1929	6	6	5	5	7	6	6
K_2O	1923	14	17	17	15	--	37	33
	1925	18	18	14	14	45	39	37
	1929	24	21	17	20	39	37	25

2. Discussion

The seedling technique using rye described by Neubauer and Schneider (1923) has been used extensively, and has been critically investigated, particularly by the German investigators. There is a vast number of papers in the German literature concerning the application of the method, particularly in relation to its correlation with results obtained in the field.

A number of aspects of Neubauer's procedure have been thoroughly reviewed by Stewart (1932). Neubauer and Schneider (1923) maintained that the seedlings grown in the dark absorbed the same amount of nutrients as did those growing in the light. Gunther (1926) and Thornton

(1935) agreed with Neubauer, but other workers, especially Wiessman (1925) did not agree, for they believed that the stronger the illumination, the greater its influence on nutrient absorption, and seedling growth. Thornton (1935) reported that his data indicated that "no significant differences could be observed for plants growing simultaneously in different light intensities or for plants growing at different seasons of the year, or under different weather conditions." Thornton states, however, that a strong, evenly diffused light is best and direct sunlight is undesirable.

Probably the most important single factor influencing the results is the temperature, and this has been studied both by Neubauer (1923) and by Thornton (1935). Both workers showed that a lower temperature retards the development of the seedlings while a higher temperature materially shortens the growth period. In either case, nutrient absorption is hindered. Thornton reported that differences as great as 50 percent are produced when the temperature varies from 12° C. to 23° C.

As mentioned above, accurate data are obtained only if strict attention is paid to the standardization of the growing conditions.

C. Thornton Procedure

The seedling technique followed by Thornton (1931, 1931a, 1932, 1932a, 1935, 1935a) is essentially the same as that proposed by Neubauer (1923) except for some minor modifications necessary because of lack of laboratory space and equipment. Further modifications have been made in the choice of seed and the disinfectant used to sterilize them. Thornton used Rosen rye supplied by the Farm Bureau Services, Inc. at Lansing, Michigan, and he treated the seed with Semesan Jr. instead of with Upsulum. Thornton apparently rigidly followed the essential details of the original Neubauer technique and merely altered those aspects which rendered it more readily followed under the conditions under which he was working.

D. McGeorge Procedure

The McGeorge technique of conducting seedling tests is another minor modification of Neubauer's original procedure. McGeorge (1942, 1946, 1946a) modified the Neubauer technique to include the determination of the minor nutritional elements. The details of the procedure and the calculation of the data for the minor elements are presented in the cited publication (1946).

E. Discussion

A number of factors somewhat decrease the value of the seedling methods for the estimation of the amount of available nutrients in the soil. One of these is the need for special equipment that is seldom found in plant nutritional laboratories.

It is apparent that even slight differences in technique bring about large differences in results. Ames and Gerdel (1927, 1927a, 1928), for instance, used larger containers and a larger amount of soil for the growth of their wheat seedlings. When they failed to find a correlation with other methods, they concluded that the seedling technique did not indicate the amount of potassium in the soils they studied. It would appear, however, that the failure to obtain agreement with other procedures was probably due to the larger amount of soil which Gerdel used. If correlation is obtained with 100 rye seedlings in 100 grams of soil and not with 100 wheat seedlings in 200 grams of soil, the lack of correlation was probably due to the failure of the wheat seedlings to exhaust the available nutrients from the soil.

On the other hand, workers who have followed carefully the details of the technique and have adequately standardized it, have found close agreement between the results obtained by the seedling method and other biological and chemical methods. Stewart, Sackett, Robertson, and Kezer (1932), Thornton (1935), McGeorge (1946), and Lea and Midgley (1934) all have reported satisfactory agreement of their data with those obtained by other methods.

Thornton (1935) probably has done more than any other worker to broaden the application of the seedling method to the study of soil fertility. He has studied such problems as fertilizer fixation by the soils, fertilizer availability, and the influence of one nutrient on the absorption of another. Lea and Midgley (1934), using a combination of the Neubauer method and chemical procedures, were able to prepare a soil map of the Champlain Valley in the State of Vermont. Volk and Truog (1934), Stewart, Sackett, Robertson and Kezer (1932), and others used the seedling method to standardize other biological techniques and also chemical procedures.

It is questionable whether the seedling technique can be used for the determination of the actual amounts of fertilizer required by crops for their optimum growth. Neubauer and Scheider (1923) have attempted to do so, but they had to make a number of broad assumptions. For instance, they assumed that the seedlings removed all of the available nutrients from the soil. However, plants in the field can remove only a fraction of this total supply. These authors referred to this fraction as the "utilization coefficient", and published the following statement of their probable magnitudes:

Neubauer Utilization Coefficients
expressed as percentages

Nutrient	Barley	Wheat	Potatoes	Roots	Legumes
K_2O	12	15	25	33	20
P_2O_5	20	33	33	33	33

Using these utilization coefficients, Neubauer calculated the "limit values" already noted. He assumed that only a fraction of the applied fertilizers could be used by the plant during a single season. For example, only 60 percent of any potash fertilizer was supposed to be effective or available, and the phosphate fertilizers were considered to be utilized to the extent of 20 percent by barley and 33 percent by other crops. Using these assumptions, he calculated the required quantities of P_2O_5 and K_2O needed by the crops under field conditions to counteract the deficiencies shown by the seedling test.

It is apparent that these estimated quantities of phosphate and potash fertilizers are based on two assumptions; the so-called "limit values" and Neubauer's assumption that 1 milligram of a nutrient per 100 grams of dry soil is equivalent to 23 pounds per acre. It is further apparent that since these are subject to error, accurate estimations from seedling tests of crop requirements are difficult.

Any comparison of the results obtained by seedling tests with those obtained from field experiments must be done on the basis of the "limit values." In fact, every field experiment is, in a way, a determination of the limit value for a particular crop and soil, and it does not appear that enough attention has been paid to the relationship between limit value and soil type.

The seedling methods for the determination of the amounts of available nutrients in soil are more closely related to pot cultures than to other methods. In conclusion, it can be stated that both methods serve only to differentiate the more extreme limits of the nutrient content of soils or the fertilizer requirements of crops.

VII. USE OF MICRO-ORGANISMS FOR THE DETERMINING OF FERTILIZER REQUIREMENTS

A. Introduction

The use of micro-organisms to determine the amounts of plant nutrients in soil, like the use of seedlings, is another method designed to replace chemical analyses of the soil and to overcome the expenditure of time and labor involved in field plot trials and pot-culture techniques. The principal methods which utilize bacteria or fungi as the test organisms are the *Azotobacter* method (Niklas and Hirschberger, 1924; Truffaut and Bezssonoff, 1927; Winogradsky, 1927, 1928), the *Aspergillus niger* method (Benecke and Söding, 1927; Niklas, Poschenrieder, and Trischler, 1930, 1930a; Mehlich, Truog, and Fred, 1933), and the *Cunninghamella* method (Mehlich, Fred, and Truog, 1934, 1935). These methods have been modified by many workers, but as the methods are of doubtful value as practical procedures, the following discussion is limited to the presentation of their principal aspects.

B. *Azotobacter*

1. Technique

As early as 1924, Niklas and Hirschberger described a method for rapidly determining available phosphorus in soils by using *Azotobacter*, and in 1927 Truffaut and Bezssonoff also reported their use of *Azotobacter* as a measure of the availability of various phosphates in the soil. Winogradsky (1925) demonstrated that colonies of *Azotobacter* grew luxuriantly on soil plaques if they contain suitable carbohydrate as an energy source, and the necessary mineral nutrients. Two years later, Winogradsky and Ziemiecka (1927) reported a close similarity between the nutritional factors limiting the growth of Azotobacter and those limiting the growth of plants although he did not at that time use this similarity for determining plant nutrients in soils. In this connection he stated: "The method is intended for the study of nutrient fixation in soil, a study which is scarcely yet underway. It is clear, however, that the reactions of microbes which are so sensitive to mineral nutritional factors can serve to indicate the latter in the soil and to do so with a sensitivity greater than possible with chemical methods. Azotobacter has already played this role as an indicator of the lime requirements, but the procedure used by the earlier investigators could not give results as precise as those obtained by the method of spontaneous cultures." This latter method has been described in detail by Winogradsky (1927, 1928), and has been modified and placed on a more practical basis by Sackett and Stewart (1931). The details of the technique used by Sackett and Stewart will be described.

A sample of the soil representing the upper 6 to 8 inches of the profile is air-dried and passed through a 20-mesh sieve to remove stones and to ensure thorough mixing of the sample. If the pH value of the soil is less than 6.8, calcium carbonate should be mixed with the soil in an amount from 8 to 10 percent of the weight of the soil. Four 50-gram samples of the soil are weighed out and 5 percent of its weight of cornstarch is added. Sandy soils, being low in the anaerobic organisms necessary for conversion of starch into forms available to *Azotobacter*, must receive 1 milliliter of 50 percent sucrose solution. Nutrient solutions are added as follows:

1. No additions, used as the control.
2. 5 milliliters of 3 percent K_2SO_4, used for potassium deficiency.
3. 5 milliliters of 6 percent $Na_2HPO_4 \cdot 12H_2O$, used for phosphorus deficiency.
4. 5 milliliters of 3 percent K_2HPO_4, used for potassium and phosphorus deficiencies.

The suggestion is made that from 5 to 10 grams of Kaolin be added to improve the texture of sandy soils and 10 grams of coarse silica be added to improve the aeration of clay soils.

Enough distilled water is added to give the soil the consistency of moulding clay and this material is pressed into a Petri dish and the surface is smoothed with a microscope slide. Since *Azotobacter* is affected greatly by moisture variations, care should be taken to make certain that equivalent amounts of moisture are present in each culture. One milliliter of a suspension of *Azotobacter* grown for 72 hours on mannite-agar is added to the center of each culture. This suspension is prepared by washing the growth from the agar with 0.85 percent sodium chloride solution and diluting to 100 milliliters. The inoculated soil cultures are allowed to stand in a moist atmosphere for 72 hours at 30° C. and the bacterial growth observed.

Sackett and Stewart (1931) base their interpretation of the experimental data on the number, size and pigmentation of the colonies appearing on the soil plaques. They recommend that the growth of the Azotobacter colonies be compared and the cultures classified as follows:

Class 1. Very deficient soil. No, or few to numerous, extremely small pinpoint colonies on unfertilized plaques. Few to numerous medium to large, distinct and vigorous colonies on fertilized plaques.

Class 2. Moderately deficient soils. Few to numerous, but small and weak, colonies with little or no pigment on unfertilized plaques. Few to numerous, distinct and vigorous colonies on the fertilized plaque.

Class 3. Slightly deficient soil. Number of colonies on unfertilized plaque as numerous as on the fertilized plaques, but smaller and less luxuriant. Few to numerous, distinct and vigorous colonies on the fertilized plaque.

Class 4. Not deficient soils. Colonies on both fertilized and unfertilized plaques equal in number and development.

2. Discussion

There is no doubt that the Azotobacter technique is a useful tool for the diagnosis of soil deficiencies although some difference of opinion is evident as shown by table 31 prepared by Halverson and Hoge (1942).

The technique is qualitative only, and its quantitative application

...verson and Hoge (1942).

Investigator	Publication year	Source of Soil	Appraisal by the Investigator
Winogradsky	1925, 1927	France	Devised the soil plaque technique
Niklas and Hirschberger	1924	Germany	Recommended the test but did not approve its use for phosphate
Ziemiecka	1932	Rothamstead	Reported that the test was of value
Guittonneau	1929	France	Tests confirmed by field tests and chemical methods.
Walker and Sullivan	1929	Iowa	Tests gave encouraging results
Itano and Arakawa	1930	Japan	Tests not applicable to rice fields
Sackett and Stewart	1931	Colorado	Recommended the test highly
Stewart, Sackett, Robertson and Kezer	1932	Colorado	Reported that the test was superior to the Neubauer test
Dahlberg and Brown	1932	Western States	Test gave accurate results
Jones	1932	Ontario	Test gave reasonably good correlations with crop returns. Reported the minimum requirements for Azotobacter higher than for plants
Greene	1932	Arizona	Correlations with field conditions in only 12 percent of the cases
Young	1933	Iowa	Test of little value for phosphate deficiencies
Fuller	1934, 1935	Massachusetts	Test indicated all soils tested were phosphate deficient despite the fact that all produced good crops

would be difficult. No conclusions could be drawn other than general opinions concerning what fertilizer treatments _might_ improve crop yields. Stewart, Sackett, Robertson, and Kezer (1932) claimed that it is sufficiently quantitative for practical purposes and that it could be used to indicate the amounts of fertilizer necessary to obtain maximum crop yields, especially in the case of phosphorus. These workers, as well as Dahlberg and Brown (1932), have used the method in extensive surveys of different soils and concluded that the procedure was as accurate as either chemical analyses or seedling methods for determining nutrient deficiencies. The failure of the method, however, to give truly quantitative data has delegated it to the role of a general survey method, as also are the seedling techniques.

C. _Aspergillus niger_

1. Introduction

Vandecaveye (1948) credits Butkewitsch, in Russia, as the first to suggest the use of _Aspergillus niger_ as a biological indicator of the amounts of available plant nutrients in soils. Benecke and Söding (1928), however, first developed the method, and used it to determine the available phorphorus and potassium in soils. They compared growth of the mycelium in liquid cultures to which a definite amount of phosphorus and potassium and a small amount of soil had been added. The first extensive use of the method was by Niklas, Poschenrieder and Trischler (1930, 1930a), Niklas and Poschenrieder (1932, 1936). The procedure was modified later by Mehlich, Truog and Fred (1933) and by Mulder (1939-1940).

2. Niklas Procedure

The original method devised by Niklas and his co-workers was an adaptation of the earlier methods which permitted the quantitative determination of available phosphorus, potassium and to some extent also the magnesium in the soils. They added 2.5 grams of air dry soil to 30 milliliters of a nutrient solution inoculated with a suspension of _Aspergillus niger_ spores. The culture flask was incubated at 35° C. for four days, and the mycelium was then washed, and dried at 50° to 60° C. for 12 hours and then at 60° to 90° C. for 1 hour. They cited the minimum weights for the dried mycelial pads below which a deficiency of the particular nutrient being studied occurred. Niklas used the method to test a large number of soils in Germany and found a close agreement between the results of the _Aspergillus_ technique and those of Neubauer obtained from the growth of rye seedlings.

3. Mehlich Procedure

A critical study of the Niklas procedure induced Mehlich, Truog and Fred (1933) to add $CaHPO_4.2H_2O$ to the medium as a buffer. When this was done, they obtained a better growth of the mycelium, particularly if the soil was strongly acid. They further obtained more consistent data if the mycelial mats were dried at 70° to 90° C. for 12 to 14 hours and at 105° C. for 2 hours, and then analysed the dried material for the nutrients being studied.

In order to permit quantitative determinations and accurate comparisons, Mehlich, Truog and Fred (1933) calculated the data on the basis of the amount of K_2O taken up by the mycelium from 100 grams of dry soil. Their results indicated that *Aspergillus niger* removed considerably more of the nutrient from the soil than did Neubauer's rye seedlings. As a result of their comparisons of their data with those obtained by the Neubauer method, and with those obtained by chemical analysis, they proposed general standards for the estimation of K_2O deficiencies in soil, either by the weight of the dry mycelial pads or by the amount of K_2O removed by the mycelium from 100 grams of dry soil. These standards were as follows:

Weight of four pads	K_2O absorbed by *A. niger* from 100 grams of soil	Degree of potassium deficiency in the soil
Less than 1.40 grams	Less than 15 mgs.	Very deficient, potash required
1.40 to 2.00 grams	15 to 20 mgs.	Moderately to slightly deficient, small applications of K might be beneficial
200 grams or more	20 mgs. or more	Not deficient. No need for potassium

4. Mulder Procedure

The technique developed by Mulder (1939, 1940) has a limited application as it is adapted to the determination only of copper or or magnesium deficiencies in soil. It differs from the procedures of Niklas and of Mehlich in that the color of the mycelium and spores serves as the quantitative index, rather than the weight or chemical analysis of the mycelium. The method is based on the addition of a given quantity of soil to the nutrient solution and the comparison of the color of the mycelium with that evidenced by mycelia grown in the same manner, but without the addition of soil, and with the addition of definite increments of copper or magnesium. Gradation from the colorless mycelium grown in the deficient cultures to a dense black when adequate amounts of the two elements are present is considered to be sufficiently predictable to permit interpretation. The method apparently is reliable as the results are easily reproducible.

5. Discussion

The Aspergillus methods have the same faults as those inherent in use of Azotobacter in that they are mainly only qualitative, although, as has been noted, some attempt has been made to place them on a quantitative basis. The procedure has been used on various occasions by Truog and Fred (1933) to check the results of chemical analyses and those obtained by the Neubauer, Niklas, and Mehlich procedures. It seems apparent that the data should correlate with those obtained by chemical methods. Smith, Brown and Schlots (1932) report a comparison of the Niklas method with the Truog method in which 0.002 N sulphuric acid was used as the extracting solution. The results of this comparative study are shown in figure 40. It may be seen that the two methods are qualitatively, but only roughly quantitatively, similar, and consequently the *Aspergillus* technique may not be used to indicate the amounts of fertilizer required, although it will show if the soil is low enough in phosphorus or potassium to warrant further investigation.

D. Cunninghamella

1. Technique

The *Cunninghamella* plaque method was developed by Mehlich, Fred, and Truog (1934, 1935) for the quantitative determination of available phosphorus in soils. The procedure depends on the growth of a species of *Cunninghamella* on the surface of moist soil supplied with nutrient solutions. It is the only microbiological technique having a mathematical foundation.

The culture dishes used were devised by Mehlich, Fred, and Truog (1935) and shown diagramatically in figure 41. This dish, replacing the Petri dish used in earlier work (1934) permitted a more uniform growth of the fungus and has given good results with a large number of tests.

The soil was screened through a 20-mesh sieve. A ten-gram sample was mixed with just enough nutrient solution of the composition given below to saturate the soil with moisture.

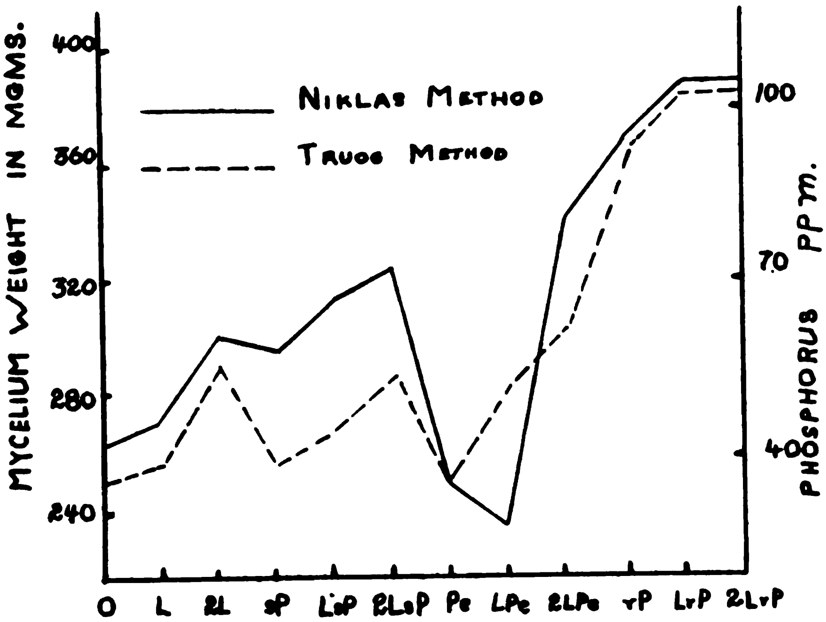

Figure 40. Available phosphorus six weeks after the soil was placed in the greenhouse. Soil was treated and kept at 22-24 percent moisture throughout the six week period.

L-3 tons lime per acre.
2L-6 tons lime per acre.
rP- 1000 lbs. 300 mesh rock phosphate per acre.
sP-120 lbs. 20 percent superphosphate per acre.
Pe- 780 lbs. of 300 mesh rock phosphate per acre.
(Smith, Brown, and Schlots, 1932)

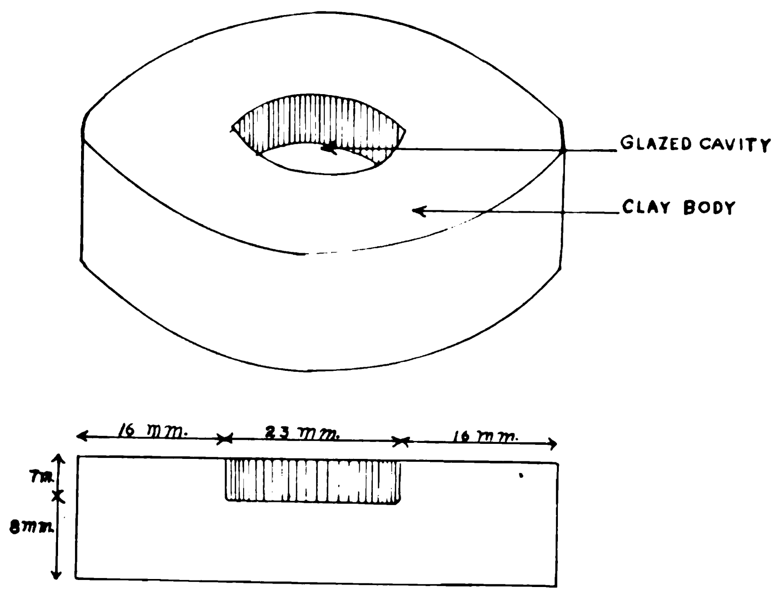

Figure 41. Diagram of the plague used in the Cunninghamella method of soil analysis. (Mehlich, Fred, and Truog, 1935)

Stock solution of salts		Nutrient solution	
K Cl	20.0 gms.	Glucose	25.0 gms.
$MgSO_4.7H_2O$	10.0 gms.	NH_4NO_3	2.5 gms.
$FeSO_4.7H_2O$	0.2 gms.	Peptone	0.5 gms.
$ZnSO_4.7H_2O$	0.2 gms.	Stock solution of salts	5.0 mls.
H_2O	1000 mls.	H_2O	1000 mls.

The nutrient solution must be prepared fresh every 48 hours in order to avoid contamination with micro-organisms. The saturated soil is packed into the plaque and the plaque placed in a pan of distilled water which is loosely covered to allow aeration.

The stock fungus culture was grown on malt extract agar containing 2.5 percent malt extract and 2 percent agar in water. A transfer was made from the stock culture at least once every four months to prevent deterioration. The soil was inoculated with a rich spore suspension prepared by adding 2 milliliters of nutrient solution to a test tube culture of the fungus which was from one to four weeks old. One drop of this suspension was placed on the soil plaque with a wire loop.

Two species of the fungus have been recommended. Cunninghamella elegans has been used most frequently, but for adequate growth on calcareous soils it is necessary that they be neutralized. Cunninghamella blakesleeana is recommended when unneutralized calcareous soils are used.

The inoculated soil is incubated at 20 to 29° C. for a period of

48 hours when testing mineral soils, and for 72 hours when testing organic mucks or peats.

2. Interpretation

Mehlich, Fred, and Truog (1934) carried out tests using soils of known crop response to phosphate fertilizers and found that the diameter of the *Cunninghamella* colony was a reliable index of the relationship between the phosphorus in the soil and crop yields. They used a modification of the Mitscherlich (1909) equation and calculated curves showing crop yields on the basis of increasing increments of phosphate fertilizer. The constant c factor was calculated from the equation

$$c = \frac{\operatorname{Log}(A-y_1) - \log(A-y_2)}{x_2 - x_1}$$

where A is the maximum growth of the fungus, y_1, y_2, etc. are the yields obtained from x_1 and x_2 amounts of added phosphate fertilizer. The constant was then used to calculate the amount of available phosphorus in the soil from the relationship,

$$b = \frac{\log A - \log(A-y)}{c}$$

where y is the growth obtained without the addition of phosphate.

Ten soils were studied in order to calculate the values used in the construction of the curve shown in figure 42. Mehlich, Fred, and Truog believed that the curve obtained in this manner eliminated the unsatisfactory relationship between the diameter of fungal colony and the phosphorus present in the soil.

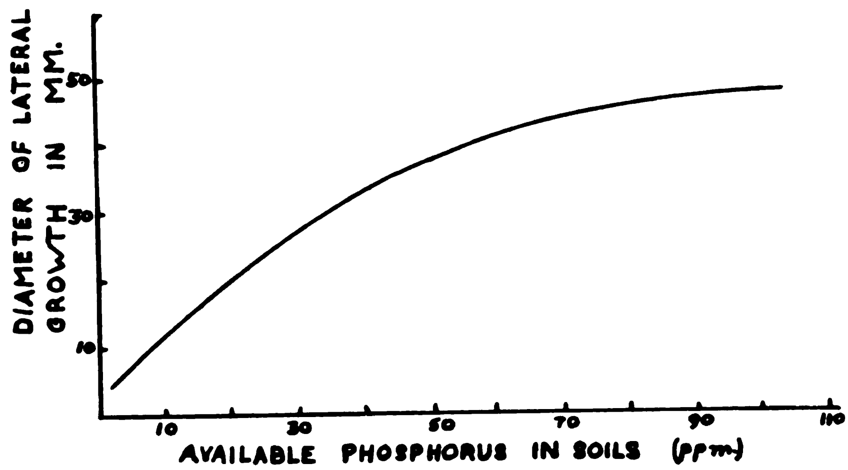

Figure 42. Relationship between the lateral growth of Cunninghamella and the amounts of available phosphorus in soils. (Mehlich, Fred, and Truog, 1934)

The standards used for the interpretation of the data were based on a comparative study of the results with those from field plot tests. Standards must be prepared for both *C. elegans* and *C. blakesleeana* as tests have shown that in the case of the former, sufficiency of the fertilizer is indicated when growth of the colony reaches a diameter of 22 millimeters, while in the latter, sufficiency is indicated by a growth of 16 millimeters in diameter. The standards for *C. elegans* are as follows:

Diameter of colonies	Degree of phosphorus deficiency	Need for phosphate fertilizer
Less than 10 mm.	Very deficient	Great
11-15 mm.	Moderately deficient	Moderate
16-21 mm.	Slightly deficient	Slight
More than 22 mm.	Not deficient	None

3. Discussion

The *Cunninghamella* plaque technique has been checked against the *Aspergillus* method, the Mitscherlich method, a chemical method, and field plot tests by Mehlich, Fred, and Truog (1934) using a large number of soils from widely separated areas and good correlations were obtained. Mooers (1938) studied 129 soils from nine soil types and concluded that the *Cunninghamella* and the *Aspergillus niger* tests were more reliable for the determination of available potassium than was the

Neubauer seedling test.

The *Cunninghamella* technique, like other methods using micro-organisms, will not permit the determination of the actual amounts of fertilizer needed for optimum crop yields.

E. Value of the Microbiological Methods

The microbiological methods are in the same class as the seedling methods as they yield data which are primarily qualitative rather than quantitative in so far as the determination of the fertilizer requirements of soils are concerned. Like the seedling tests, they can be used to determine the relative amounts of the various nutrients in the soil, and for this purpose they are probably reliable. Unfortunately they are not adaptable to the determination of nitrogen, and in order to get accurate tests for potassium and phosphorus it may be necessary to use two separate techniques.

As far as quantitative evaluations of soil fertility are concerned, the microbiological procedures contribute but little to the determination of soil fertility and fertilizer requirements because more reliable and more mathematically and chemically sound procedures are available. The use of micro-organisms to estimate how much nutrient a crop will remove from the soil has the same sources of error as many types of chemical analyses because there is no inherent relationship to crops which might serve as bases for correlations. To base an estimation of the fertilizer requirements for maximum crop yields on the amounts of nutrients available to micro-organisms is a rather hazardous procedure.

VIII. FOLIAR DIAGNOSIS

A. Introduction

An increasingly popular procedure for determining the nutritional status of crops is based on simplified chemical analysis of the leaves. This foliar diagnosis method was developed through the efforts of Lagatu and Maume at Montpellier, and of Thomas and Mack in Pennsylvania. The percentage composition of the dried plant material, or the number of milligram equivalents, was regarded by these workers as indicative of the nutritional status of crops grown on experimentsl field plots. The procedure implies that such data are adequate for the diagnosis of the nutritional requirements of plants of unknown history and therefore may be used to determine fertilizer requirements.

Beginning in 1924, Lagatu and Maume have published a series of papers describing the techniques and applications of foliar diagnosis. General accounts of the work have been published by Lagatu and Maume (1934, 1937, and 1937a). The original work was carried out on the grape vine by Lagatu and Maume (1924, 1924a, 1925, 1926, 1927, 1927a, 1927b, 1927c, 1927d, 1927e, 1928, 1928a, 1929, 1930b, 1930c, 1937c, 1938, 1938a), but later they also applied the method in their studies of the potato (1929, 1930, 1930a, 1930e, 1931, 1932a, 1932c, 1933, 1933a, 1934a, 1934b, 1934c, 1935a, 1935b, 1935c, 1937a), and later Maume and Dulac (1934a, 1935, 1935a, 1936, 1937, 1937a, 1938) and Maume and Bouat (1937, 1937a), applied the procedure to wheat. Lagatu and Maume (1935, 1935d, 1935e, 1936e) also studied the nutritional status of tobacco by foliar diagnosis. This impressive list of published papers proceeding from the work of Lagatu and Maume and their co-workers indicates the extensive amount of work that has been done with this technique.

Thomas (1929, 1930, 1932, 1934) and his associates in Pennsylvania adopted the procedure and applied it in a modified form to various vegetable and fruit crops. The general descriptions of the procedure, including the modifications they made, may be found in the papers of Thomas (1939, 1945), and of Thomas and Mack (1940, 1944). Thomas and his collaborators applied their techniques to corn (Thomas 1938, 1939, 1939a, 1939b, 1939c, 1943; Thomas and Mack 1943, 1944a), potatoes (Thomas 1936, 1937, 1938a, 1938b; Thomas and Mack, 1938, 1939c, 1939d), tomatoes (Thomas and Mack 1940a, 1941, 1941a), beans (Thomas, Mack, and Cotton 1942), and peaches (Thomas, Mack, and Fagan 1948).

Foliar diagnosis was used not only for interpreting the results of fertilizer experiments, but also for elucidating the effects of disease, cultivation, irrigation, pruning, and varying weather conditions. Lagatu and Maume (1935d, 1938a) appear to have used the method on occasion to indicate the causes of deficiency disorders of unknown origin.

The foliar diagnosis school has adopted the view that the "nutrition" of the plant is governed not only by the nutrients in the soil or even by those absorbed by the plant, but by the whole complex of environmental factors. Therefore, the chemical compositions of the leaves and the changes they undergo during the growing season provide the most direct information obtainable concerning the "nutrition" of the plant. By definition, then, data obtained by foliar diagnosis cannot be readily used for the determination of fertilizer requirements for optimum yields.

Foliar diagnosis methods are of such great value for the study of the nutritional status of crops that their details will be discussed in detail in the succeeding paragraphs.

B. Theory of Foliar Diagnosis

1. Experimental Basis

Foliar diagnosis developed from the belief expressed by Lagatu and Maume (1934) that "the first condition necessary to guide us to the proper nutrition of the plant is to know, at a given time, what the nutritional status is." Agricultural science has shown that the mineral composition of a particular species is not a fixed characteristic but varies over a wide range according to the conditions influencing the plant growth (see Section II). Lagatu and Maume realized the complexity of the problem of crop production and believed that the logical procedure was to divide soil fertility investigations into separate lines of attack, namely, physiological studies of the plant designed to determine the requirements for maximum growth, and chemical and physical studies of the soil designed to determine how a particular soil can be improved so that better crop yields will be obtained. It was through the first of these avenues that Lagatu and Maume attacked the general problem of crop production.

The experimental facts upon which foliar diagnosis are based have been set forth by Lagatu and Maume and by Thomas and Mack in the publications already cited.

It was assumed that two morphologically homologous leaves or leaves of the same physiological or metabolic age from plants of the same species and variety are the seat of identical physiological processes, at least when the environment of the two is identical. Conversely, the physiological processes are different when the environment is different (James, 1931; James and Penston, 1933; Lagatu and Maume, 1930, 1932c, 1933; Thomas, 1937; Thomas and Mack, 1939).

The chemical composition of leaves of the same physiological age from plants growing on homogeneous soil, but which have received different fertilizer treatments, differs. The addition of any fertilizer effectively influencing the response of the plant always is associated with an increase of the nutrient in question in the dried foliage (Lagatu and Maume, 1930, 1932c; Thomas, 1937; Thomas and Mack, 1939). Further, the change in the composition of the leaves is related to the yields of the plants (Lagatu and Maume, 1932c; Gregory, 1938; Vinet and Lemesle, 1930; Thomas and Mack, 1937).

The magnitude of the variations in the composition of the leaves is relatively large and is easily determined. The leaf is, therefore, a highly sensitive index of the factors affecting the nutrition of the plant, (Lagatu and Maume, 1928, 1930, 1930a, 1934a; Maume and Dulac, 1935a; Thomas and Mack, 1939a, 1941, 1943).

The proponents of foliar diagnosis base their procedures on the above facts. Data will be presented later to show whether or not these "facts" are based on sound experiment.

2. Definition of Terms

Since the idea of foliar diagnosis is based on the work of two men, Lagatu and Maume, it is necessary to define the terms used by them in order that their work may be discussed.

Lagatu and Maume (1930) define foliar diagnosis as the determination of the chemical composition of the leaf with respect to the dominant nutritive mineral "elements", nitrogen, P_2O_5, and K_2O, at the time of sampling. The foliar diagnosis for any given season consists of a sequence of chemical determinations indicating the sequence of the nutritional status during the growth period.

The composition is expressed as percentages of the components in the dry matter of the leaf without reference to the weight of the dry matter at each sampling or to the number of leaves sampled from each plant. All of the work by Lagatu and Maume, and therefore the whole concept, is based on this method of expression of the composition of the plant. Bartholomew, Watts, and Janssen (1933) in a study of the method criticized the use of the percentage composition of the dry matter and proposed the use of the milligrams of the indicative components in the total amount of dry matter. But Thomas (1937) opposed this saying that "this is a misunderstanding for its (percent of dry matter) use is incorporated in the definition of foliar diagnosis" and in 1939 he insisted that there can be "no other basis of expressing results than in the percentage of the dry weight of the leaf."

Lagatu and Maume (1930) defined the Intensity of Nutrition as the sum of the percentages of the "elements", $N+P_2O_5+K_2O$, in the dried foliage at the time of sampling.

The Quality of Nutrition is the ratio of the percentages of the three "elements" in the NPK unit at the time of sampling. This definition of the quality of nutrition is the essential difference between the procedures developed at Montpellier and in Pennsylvania. According to Lagatu and Maume (1930) this term is calculated on the basis of the ratios of the percentages of the nutrients in the NPK unit in the dry matter of the leaf. On the other hand, Thomas (1937) and Thomas and Mack (1939) express this magnitude in milliequivalents because the chemical reactions taking place in the leaf are involved in this relationship, but in the case of the magnitude of the intensity of nutrition, consideration is given only to the presence of the "elements" in the leaf whatever their role may be!

The Quantitative Index, as defined by Thomas and Mack (1939), expresses the effect of one fertilizer "element" at the moment of sampling. It is the ratio of the amount of the "element" contained in the leaf of the plant growing on the experimentally fertilized plot to the amount in the leaf of a plant growing on an unfertilized plot.

3. NPK Unit

The NPK unit is the expression of the quantity or intensity of nutrition of the selected leaves and consists of the sum of the percentages of the major nutrients, $N + P_2O_5 + K_2O$, in the dried plant material.

If x is the percentage of N, y the percentage of P_2O_5, and z the percentage of K_2O, then

$$x + y + z = s \qquad (1)$$

Dividing equation 1 by s we get,

$$\frac{x}{s} + \frac{y}{s} + \frac{z}{s} = 1 \qquad (2)$$

where x/s, y/s, and z/s represent the proportion of the NPK unit contributed by N, P_2O_5, and K_2O respectively.

If we let $x/s = x^1$, $y/s = y^1$, and $z/s = z^1$ then by substituting in equation 2 we have

$$x^1 + y^1 + z^1 = 1 \qquad (3)$$

This expresses the proportions of nitrogen, phosphoric acid and potash that are present in a unit quantity of the leaf, and consequently, it is an expression giving the physiological ratios between N, P_2O_5, and K_2O in the "laboratory" of the leaf (Lagatu and Maume, 1934) independent of the total quantity of these nutrients in the dry matter. The NPK unit (s) is therefore a magnitude expressing the intensity of nutrition at the moment of sampling the leaf. On the other hand, the composition of s gives the ratios of the major nutrients in the NPK unit or the "quality" of nutrition. It is apparent that these two magnitudes quantity and quality are distinct but are related in the physiological processes of the leaf.

The schematic diagram proposed by Lagatu and Maume (1934) is an attempt to show more clearly what is implied in the NPK unit. If F in figure 43 represents a leaf into which nitrogen, P_2O_5, and K_2O are migrating together with other materials (R), a portion of each will remain in an unelaborated form. The method of foliar diagnosis may be represented by a vessel, F_1, of capacity 100 connected through the outlet tube M to the vessel F.

If we now imagine a third vessel, F_2, of unit capacity to be connected with F_1, to be completely filled, and into which N, P_2O_5, and K_2O are entering in the same proportion as they are present in F and F_1, then if the N, P_2O_5, and K_2O separate into layers as shown, the sum of the quantities x_1, y_1, and z_1 of nitrogen, phosphoric acid and potash respectively, in F_2 is always equal to 1 and this vessel may be regarded as the indicator of x_1, y_1, and z_1 when $x_1 = x/s$, $y_1 = y/s$, and $z_1 = z/s$.

Lagatu and Maume (1930, 1934, etc.) used the percentages of the components for calculating the NPK unit, but Thomas and Mack, on the other hand, considered that the "quality factor", that is the proportions of the elements found in the leaf at the various sampling dates, is more suitably expressed as milliequivalents because this factor

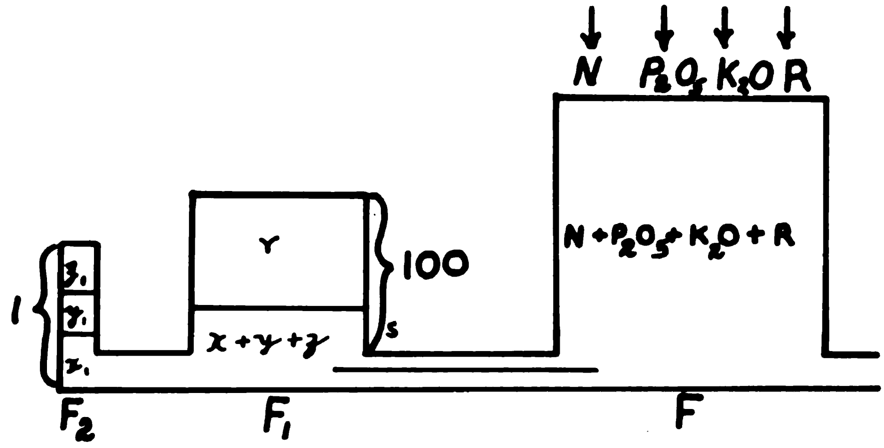

Figure 43. Diagrammatic representation of the concept of foliar diagnosis. (Lagatu and Maume, 1934)

(quality factor) must be dependent and related to the chemical reactions involved.

Thus if Mx is the percentage of nitrogen, My the percentage of P_2O_5, and Mz the percentage of K_2O in the leaf, then these quantities may be expressed in milliequivalents by

$$Ex = \frac{1000\ Mx}{N} \quad ; \quad Ey = \frac{1000\ My}{1/6\ P_2O_5} \quad ; \quad \text{and } Ez = \frac{1000\ Mz}{1/2\ K_2O}$$

If we divide the expression $S = Ex + Ey + Ez$ by S, then

$$\frac{Ex}{S} + \frac{Ey}{S} + \frac{Ez}{S} = 1$$

where Ex/S, Ey/S and Ez/S represent fractions indicating in milligram equivalents the proportional parts of N, P_2O_5, and K_2O in a unit quantity of the three major nutrients.

If $\frac{Ex}{S}$ be denoted by x', then $Ex = x'S$,

If $\frac{Ey}{S}$ be denoted by y', then $Ey = y'S$, and

If $\frac{Ez}{S}$ be denoted by z', then $Ez = z'S$,

and $x' + y' + z' = 1.$

This equation is multiplied by 100 to avoid fractions and

$$100x' + 100y' + 100z' = 100,$$

and $X + Y + Z = 100$

where $100x' = X$, $100y' = Y$, and $100z' = Z$. Finally, $X + Y + Z$ is the NPK unit.

Lagatu and Maume presented their data in two forms which have been adopted by Thomas and his collaborators.

In the first of these methods, the percentages of each of the elements are plotted as ordinates with the sampling dates as the abscissa. This type of graph shows the increase or decrease of an element with the advancing age of the leaf. An example of this type of graph is reproduced in figure 45.

The second method is the three component system based on the trilinear diagram. The geometrical theory upon which this system is based is explained by Thomas and Mack (1940). An example of a simplified trilinear coordinate diagram showing the changes in the NPK unit through the period of sampling is shown in figure 44. In this figure point 1 indicates that nitrogen comprises 71.8 percent, P_2O_5 20.1 percent, and K_2O 8.08 percent of the NPK unit and point 2 indicates that nitrogen comprises 51.5 percent, P_2O_5 43.8 percent, and K_2O 4.6 percent. This method is a derivitive of the first and shows the changes in composition with respect to nitrogen, P_2O_5, and K_2O, resulting from any particular treatment.

C. Technique of Application

The effects of applications of the major fertilizer components on the composition of the grape vine throughout the season were studied by Lagatu and Maume in a series of papers (1924, 1924a, 1925). Their work indicated that the absolute and relative amounts of the three major bases CaO, MgO and K_2O as well as of nitrogen and P_2O_5 remained in the same order in leaves sampled at monthly intervals from May 18

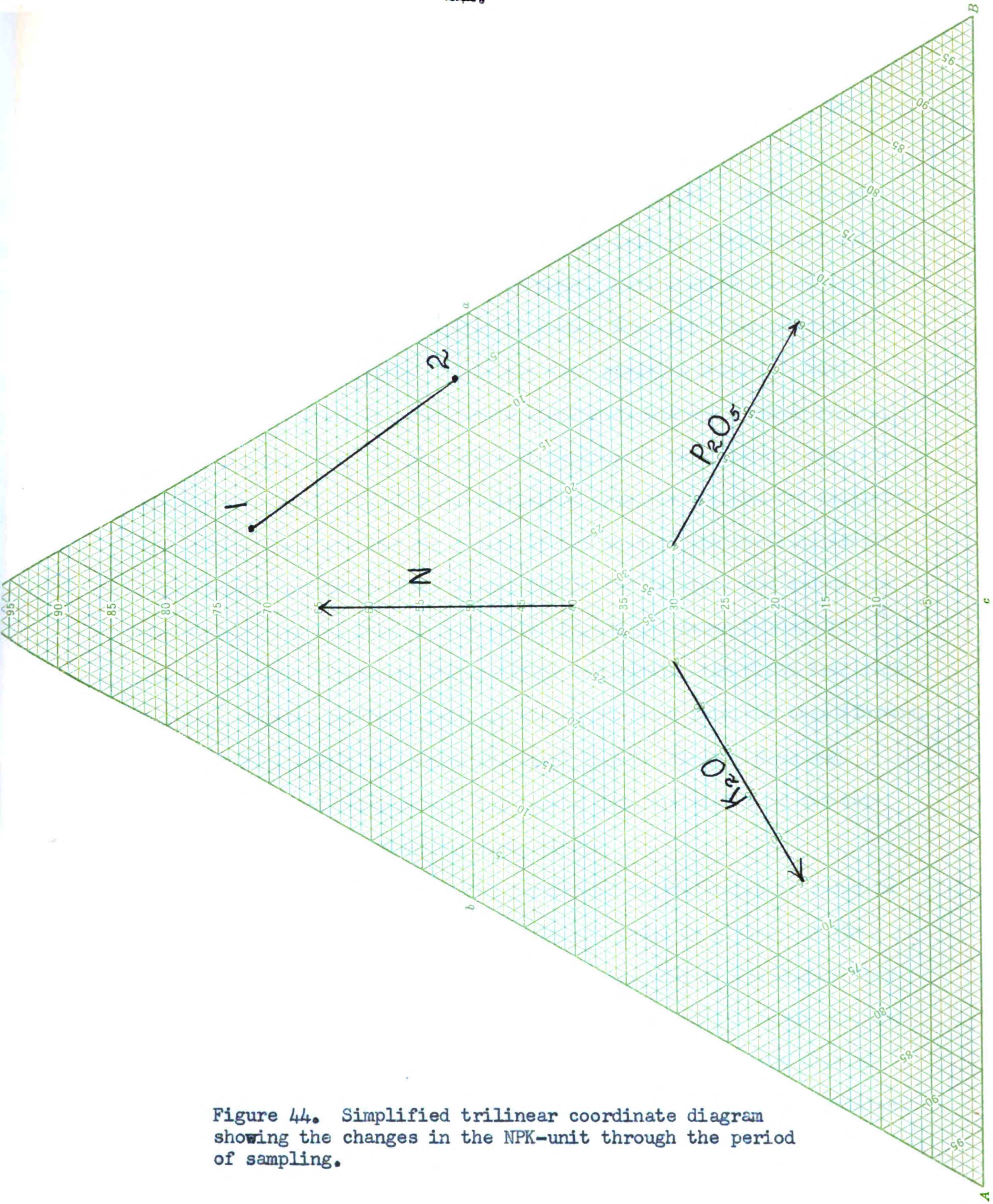

Figure 44. Simplified trilinear coordinate diagram showing the changes in the NPK-unit through the period of sampling.

to September 18. They concluded that such data could servo for recognizing the insufficiency or excess of a nutrient.

In a continuation of the study they explored the possibility of selecting a specific organ which would represent the "intensity" and "quality" of nutrition. In one experiment, they studied the effect of pruning three of the nine bunches of grapes from the vines growing under identical conditions. From the data shown in table they concluded that there was remarkable similarity between similar organs, leaves in this case. On the basis of studies of this nature, the proponents of foliar diagnosis have selected specific tissues for analysis. For grapes, they use the two leaves at the base of the fruiting structure (Lagatu and Maume 1930; Thomas 1937); for tobacco, the second and third leaf from the base of the stalk (Lagatu and Maume 1936e); for wheat, the tops (Maume and Dulac 1934); for tomato, the fifth leaf from the base (Thomas and Mack 1943) or the fourth leaf from the base (Thomas, Mack and Rahn 1944); for beans the basal leaves (Thomas, Mack and Cotton 1942); and for peaches, the leaves from the middle of the terminal growth (Thomas, Mack and Fagan 1948).

The necessity of the precise definition of the sample is shown in the work of Lagatu and Maume (1930d) on the grape in a study of the effect of different fertilizations. Using the variety Grand-Noir they sampled at various periods during the growing season the first two, the fourth and fifth, and the seventh and eighth leaves from the base of the fruiting stalk of plants from an unfertilized and from a fertilized plot. The percentages of CaO and nitrogen in the dry matter are given in figure 45. While each type of sample follows the same relative course of changes through the season, the older leaf represents the composition which will be the endpoint of the younger leaf. This also has been shown to be true for tobacco by Lagatu and Maume (1935, 1935e).

Experimental work by Thomas and Mack (1937) on potatoes will be described as an example of the details of the application of the foliar diagnosis procedure. This work was carried out on material from the vegetable Fertility plots at the Pennsylvania Agricultural Experimental Station during the year 1935. The plot treatments and yields are given in table 33 and the table 34 gives the compositions of the fourth and fifth leaves expressed in percentage of nitrogen, P_2O_5, and K_2O in the dried foliage, as milligram equivalents, and as fractions of the NPK unit.

To illustrate the mathematics involved in the calculation of the points of the NPK unit, data from the nitrogen plot sampled July 7 will be taken as an example. The chemical analyses show that $N = 5.12$ percent, $P_2O_5 = 0.404$ percent, and $K_2O = 3.97$ percent of the dry leaf material. The intensity of nutrition is therefore $5.12 + 0.404 + 3.97 = 9.494$ for the leaves sampled on July 7.

To evaluate the NPK unit the percentage values are converted into milligram equivalents of nitrogen, P_2O_5 and K_2O as follows:

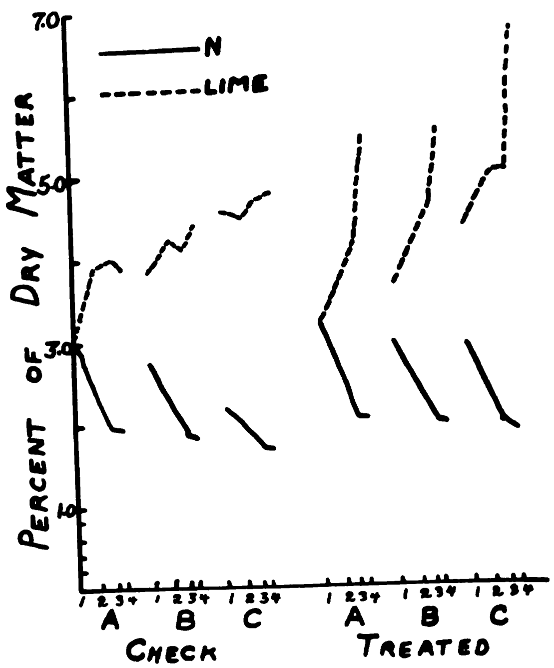

Figure 45. The percentages of nitrogen, and CaO in the dry matter of the first and second leaf from the base of the fruiting stalk (C), the fourth and fifth leaf (B), and the seventh and eighth leaf (A) on a plot treated with nitrogen fertilizer and an untreated plot at four dates during the growing season. (1. June 13, 2. July 10, 3. July 27, and 4. August 7) Plants studied were the grape. (Lagatu and Maume, 1930d)

Table 33. Plot numbers, fertilizer treatments, and yields of potatoes at the Pennsylvania Agriculture Experiment Station 1935. (Thomas, 1937)

Plot number	Fertilizer applied	Fertilizer applied per plot in lbs.	N, P_2O_5 and K_2O equivalents, in pounds	Symbol used	Yields per plot in lbs.
2	$NaNO_3$	4.0	0.6	N	109
3	Superphosphate	6.25	1.0	P	114
4	Potassium chloride	1.666	0.8	K	155
6	$NaNO_3$ Superphosphate	4.0 6.25	0.6 1.0	NP	124
7	$NaNO_3$ KCl	4.0 1.666	0.6 0.8	NK	163
8	Superphosphate KCl	6.25 1.666	1.0 0.8	PK	148
10	$NaNO_3$ Superphosphate KCl	4.0 6.25 1.666	0.6 1.0 0.8	NPK	162

in terms of $\bar{N}$, P_2O_5, and K_2O in the dried foliage, milligram equivalents and the NPK unit. (Thomas, 1937)

Treat-ment	Date	Composition of dried foliage			Milliequivalents				Composition of NPK unit		
		N M_x	P_2O_5 M_y	K_2O M_z	N E_x	P_2O_5 E_y	K_2O E_z	$E_x E_y E_z$ S	X $100E_x/S$	Y $100E_y/S$	Z $100E_z/S$
		%	%	%	me	me	me	me	%	%	%
N	1	5.12	0.404	3.97	365.6	17.1	84.6	467.3	78.2	3.66	18.09
	2	4.42	0.534	2.54	315.4	22.6	54.1	392.1	80.4	5.76	13.79
	3	4.15	0.472	2.01	296.3	19.9	42.8	359.0	82.5	5.56	11.9
	4	3.64	0.344	1.77	259.9	14.6	37.7	312.2	83.3	4.66	12.08
P	1	4.57	0.550	2.41	326.3	25.0	51.3	402.6	81.0	6.22	12.15
	2	3.46	0.521	1.64	247.0	22.0	34.9	303.9	81.3	7.25	11.5
	3	3.24	0.520	1.29	331.3	21.9	27.5	380.7	82.4	7.83	9.79
	4	2.79	0.450	1.11	199.2	19.0	26.6	244.8	82.4	7.87	9.77
K	1	4.61	4.24	6.32	329.1	17.9	134.6	481.6	68.3	3.73	27.84
	2	3.87	0.540	5.22	276.3	22.8	111.1	510.2	67.5	5.59	27.08
	3	3.39	0.464	4.91	242.0	19.6	104.7	366.3	66.7	5.36	28.58
	4	2.78	0.370	5.00	198.5	15.7	106.5	320.8	61.9	4.88	33.22
NP	1	5.14	0.590	2.53	367.0	24.9	53.91	445.8	82.3	5.59	12.09
	2	3.87	0.532	1.52	276.3	22.5	32.3	331.1	83.4	6.79	9.77
	3	3.71	0.486	1.19	264.9	20.6	25.2	310.7	85.3	6.62	8.13
	4	3.36	0.390	1.09	239.9	16.7	23.1	279.7	85.8	5.99	8.26
NK	1	5.10	0.4646	6.63	364.1	19.6	141.1	524.8	69.4	3.74	26.89
	2	4.03	0.510	5.55	287.7	21.6	118.3	427.6	67.3	5.04	27.66
	3	3.78	0.452	5.28	269.9	19.2	112.4	402.5	67.2	4.76	28.01
	4	3.26	0.352	5.46	232.7	14.9	116.2	363.8	63.9	4.09	31.94
PK	1	4.55	0.620	6.76	324.9	26.4	143.9	495.2	65.6	5.33	29.07
	2	3.36	0.510	5.32	239.9	21.6	113.3	374.8	64.0	5.76	30.24
	3	3.08	0.490	5.32	219.9	20.9	113.9	354.7	61.9	5.91	32.11
	4	2.82	0.430	5.04	201.4	18.2	107.3	326.9	61.6	5.57	32.83
NPK	1	4.98	0.584	6.59	355.6	24.7	140.3	520.6	68.3	4.74	26.96
	2	3.88	0.509	4.34	277.0	21.5	92.4	390.9	70.9	5.51	23.63
	3	3.62	0.482	4.70	258.5	20.3	100.0	378.8	68.2	5.38	26.42
	4	3.14	0.392	4.35	224.2	16.5	92.6	333.3	67.2	4.97	27.78

1 July 7; 2 July 29; 3 August 9; 4 August 24

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$E_x = 1000 \times .0714 \times 5.12 = 365.568$ ME of N

$E_z = 1000 \times .0213 \times 3.97 = 84.561$ ME of K_2O

$E_y = 1000 \times .0423 \times .404 = 17.089$ ME of P_2O_5

$S = 365.568 + 17.089 + 84.561 = 467.218$

Therefore

$$X = 100 \frac{E_x}{S} = 78.244$$

$$Y = 100 \frac{E_Y}{S} = 3.658$$

$$Z = 100 \frac{E_z}{S} = 18.098$$

$$S = \text{Total} = 100$$

These values are the three points required for plotting on the trilinear coordinate graph.

The relationships between the percentage composition of the dried leaves, and the increasing age, in plants growing on variously treated plots are shown in the data plotted in figure 46. This type of presentation shows, for instance, that the nitrogen fertilized plots actually contained more nitrogen than the untreated plots, and that the percentages of nitrogen in the tissue decreased with the advancing age of tissue. It shows that both the phosphorus and potassium content was increased by the added P_2O_5 and K_2O fertilizers respectively. It is apparent that the periodic analysis of the leaves from plants growing on plots treated with different fertilizers reflected the effects of these fertilizers. The positions and forms of the curves indicate the changes in the nutritional status of the plants with respect to

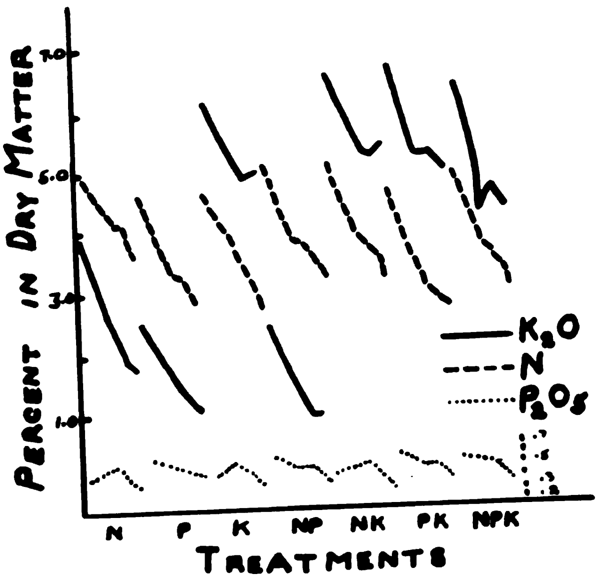


Figure 46. Periodic analysis of the fourth and fifth leaves of potato plants growing on differentially fertilized plots. The percentages of the major nutrients in the dried tissues are plotted as the ordinate and the dates of sampling as the abscissa. (Thomas, 1937)

nitrogen, phosphorus and potassium. The steepness of a curve representing a particular nutrient indicates the relative demand for the nutrient in relation to its supply.

Thomas (1937) warns that the interpretation of the NPK unit can be confused by three things: an increase or decrease in the intensity of nutrition; a change in the composition of the NPK unit; or a change in both simultaneously. The intensities of nutrition from the data of Thomas (1937) are shown in figure 47 for plants growing on variously fertilized plots. The intensities are the ordinate and the dates of sampling are the abscissa. This presentation indicates the differences due to the age of plant and the fertilizer treatments. The quality of the nutrition, as shown in the trilinear coordinates (figure 48), indicates how the NPK unit is displaced from one sampling period to another.

It is also of value to consider the relationship between the intensity of nutrition ($N+P_2O_5+K_2O$) and the mean NPK unit in relation to the yield. These values are presented in table 35. Data presented in this form indicate the ratio of the elements in the leaves which is favorable to optimum crop yields and which, as will be shown later, has been used by Lagatu and Maume (1937) to estimate fertilizer requirements.

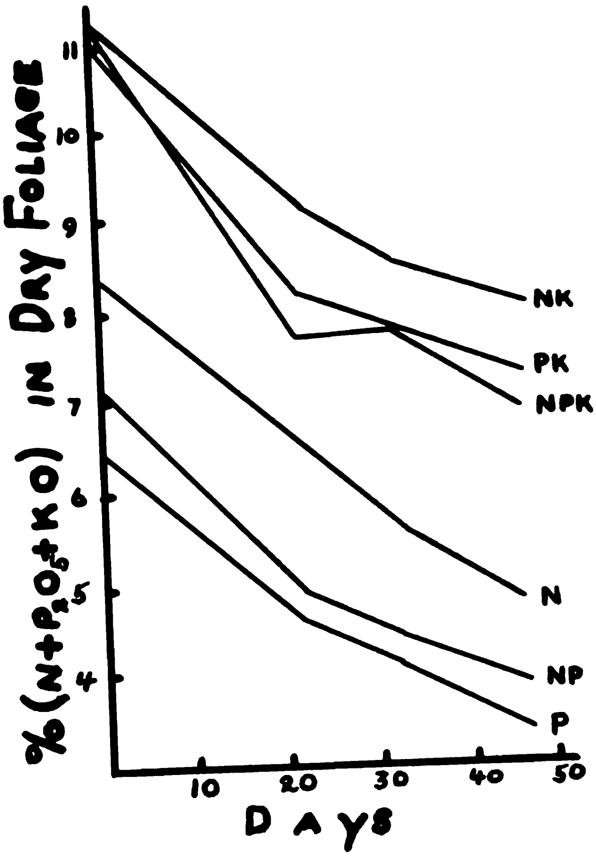


Figure 47. Relationships of the intensities of nutrition of plants growing on differentially fertilized plots. (Thomas, 1937)

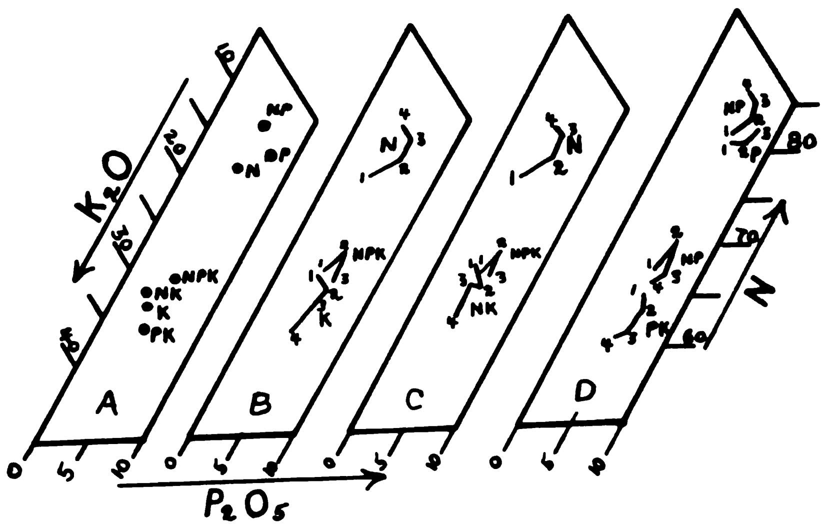


Figure 48. A, relative position of the NPK-unit.
B, course of nutrition of the nitrogen, NPK, and potassium plots. C, Course of nutrition of the nitrogen, NK, and NPK plots. D, course of nutrition of the PK, NP, NPK, and phosphorus plots. (Thomas, 1937)

Table 35. Relation of the mean NPK unit and the mean intensity of nutrition at four sampling dates to the yield of potatoes on variously fertilized plots. (Thomas, 1937)

Treatment	Mean NPK unit (mean of four samplings)			Intensity of nutrition (Mean of four samplings)	Yields lbs. per plot	Relative yield NK = 100%	Class order
	Percent N	Percent P_2O_5	Percent K_2O				
NK	66.92	4.28	28.61	10.24	163	100	1
NPK	68.65	5.15	26.19	9.39	162	99.38	
K	65.91	4.88	29.20	9.48	155	95.09	2
PK	63.29	5.64	31.06	9.56	148	90.80	
NP	84.18	6.25	9.56	6.10	124	76.07	
P	81.76	7.29	10.95	5.65	114	69.99	3
N	81.12	4.91	13.97	7.34	109	66.93	

D. Applications

1. Effects of Disease

In a few instances, foliar diagnosis has been used for the study of the effects of various diseases on the nutritional state of plants. For example, Lagatu and Maume (1935b) working with tobacco showed the effect of the wild fire disease. The results indicated that the diseased plants contained relatively higher percentages of nitrogen and lower percentages of potash in the NPK unit than did the healthy plants. It was further noted that apparently healthy plants growing in the diseased plantation were intermediate between the two others. It was concluded that these plants, although not showing symptoms of the disease, were approaching the diseased condition. Foliar diagnosis, therefore, was thought to detect plant diseases at an early stage of their development.

Thomas, Mack and Fagan (1948) also used foliar diagnosis to study the effect of bacterial leaf spot on the nutrition of peach trees.

2. Effects of Cultural Methods

Foliar diagnosis has been used to study the effects of cultural practices on the nutritional status of the crop. For instance, Lagatu and Maume (1935c) studied the effect of farmyard manure on the availability of potash to potatoes. They were able to show that luxury consumption of potash occurred, but the crop was able to utilize the excess potassium without incurring decreased yield. Lagatu and Maume

(1930c) also studied the influence of the time of application of fertilizer, of pruning of the bushes (1933, 1933b), and of tillage (1934b) on the nutritional status of the crop. This latter work showed that the tillage practices increased the intensity of nutrition and caused a variation in the nutritional balance, or "quality" of nutrition, in the crop as indicated in figure 49. The effect of tillage was a rather sharp increase in the absorption of phosphorus and a subsequent increase of P_2O_5 in the NPK unit. Both of these effects are evident in figure 49. Thomas and his collaborators at Pennsylvania also used the foliar diagnosis technique to study the effect of cultural treatments on the nutritional status of crops. This work included the study of the effect of applications of manure on tomatoes (Thomas and Mack 1940a), and the effect of irrigation practices on beans (Thomas, Mack, and Cotton 1942), and the effect of different methods of fertilizer placement on corn (Thomas, Mack, and Rahn 1944).

Foliar diagnosis is an effective technique for the study of the effects of cultural practices on the nutritional status of a crop, and while it has been applied only to the major nutrients, theoretically it should be possible to apply the technique also to the minor elements, for certainly they influence the nutritional status of the plant.

3. Effects of Nutrient Carriers

Although field plot experiments have been used almost since their inception to study the effect of different forms of nutrients on crop

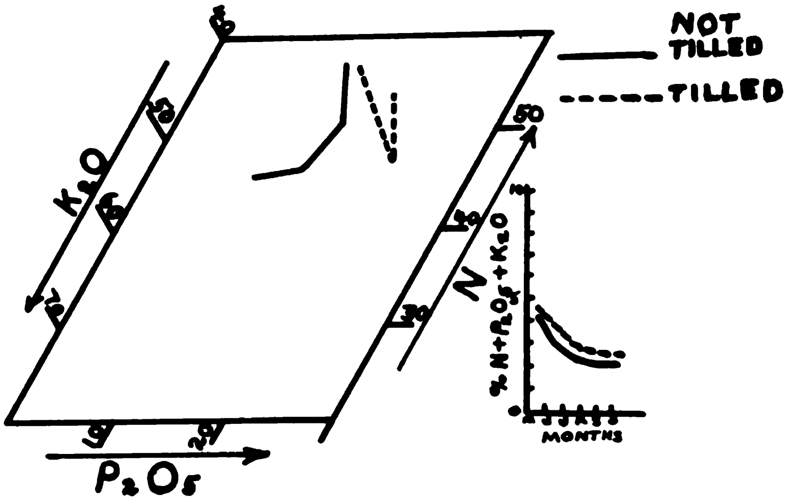


Figure 49. Effect of tillage on the intensity of nutrition and the NPK-unit in the grape at Montpellier during 1933. (Lagatu and Maume, 1934b)

yield, it was not until Lagatu and Maume became interested in 1927 that attention was paid to the effects of the different forms of a fertilizer on the nutritional status of the plant. These authors (1927, 1927d, 1927e) reported the effect of the forms of the major nutrients on the grape vine. Lagatu and Maume (1936f) later reported the effect of "scories de dephosphoration", superphosphate, and basiphosphate on the NPK unit, that is on the "quality" of nutrition of tobacco. Their data showed that the three forms of phosphate exerted different effects on the nutrition of the tobacco plants. There was not a very great effect on the phosphorus content itself, but there was a significant effect on the nitrogen content for the nitrogen fraction of the NPK unit was greatest with basiphosphate, lowest with "scories" and intermediate with superphosphate.

Thomas and Mack (1939c) used foliar diagnosis to study the effects of different forms of nitrogen on the nutrient content of corn, and later they (1944a) continued the study with potatoes. In the last study, the nitrogen carriers were $Ca(NO_3)_2$, $NaNO_3$, and $(NH_4)_2SO_4$ or tankage, and, as with the work reported by Lagatu and Maume on phosphorus carriers, the different carriers of nitrogen did not significantly affect the percentages of nitrogen in the dry matter as may be seen in figure 50. The greatest effect was on the relative amount of potash in the NPK unit as may be seen in figure 51. This figure shows that as the percentage of K_2O in the NPK unit decreased, the yield of the crop increased. These results show that the field plot trials used

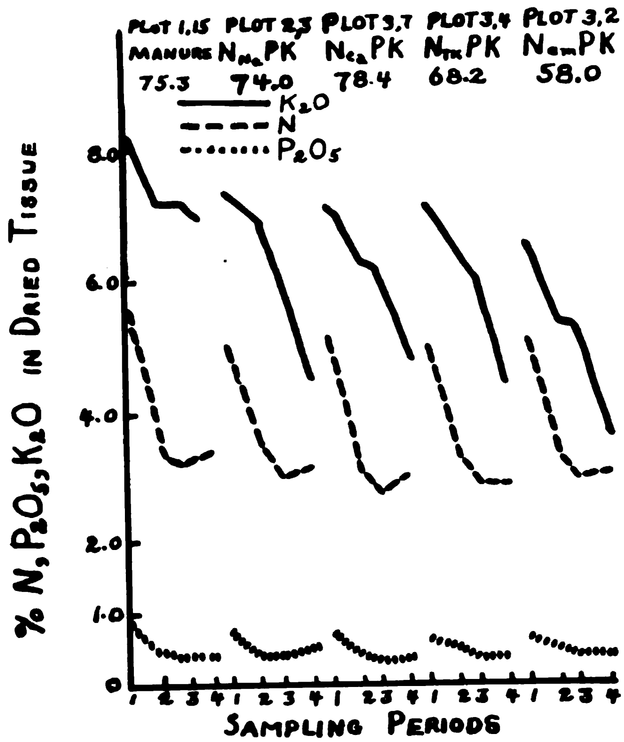
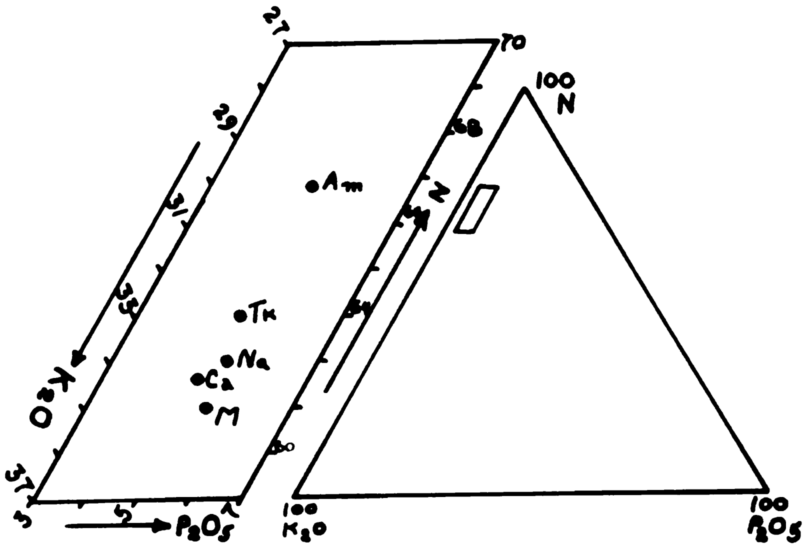


Figure 50. Percentages of nitrogen, P_2O_5, and K_2O in dried foliage of potatoes from differentially fertilized plots on four sampling dates: 1-July 6, 2-July 26, 3-August 6, and 4-August 26. (Thomas and Mack, 1944a)



Symbol	Treatment	Intensity	Yield
Ca	$N_{Ca}PK$	10.23	78.4
M	Manure	11.88	75.3
Na	$N_{Na}PK$	10.57	74.0
Tk	$N_{Tk}PK$	9.77	68.2
Am	$N_{Am}PK$	9.36	58.0

Figure 51. Intensities of nutrition (table) and NPK equilibrium (graph) in potato leaves during the growth period from plots receiving different fertilizer treatments. The symbols N_{ca}, N_{na}, N_{tk}, and N_{am} designate respectively calcium nitrate, sodium nitrate, tankage, and ammonium sulphate as nitrogen carriers. (Thomas and Mack, 1944a)

previously to study the effect of different carriers of a nutrient on yield may have been misleading since in both of the experiments described the carrier had little effect on the absorption of the nutrient itself but influenced the crop yield by affecting the absorption of other nutrients.

4. Effects of Climate

The effects of climate on the nutrition of plants have been studied by foliar diagnosis and data from the work of Lagatu and Maume (1928) obtained with the grape vine, and from the work of Thomas and Mack (1941) obtained with tomatoes, have been presented in Section II. Maume and Dulac (1935, 1935a) carried out an extensive study of the effects of climate on the nutritional status of wheat. Lagatu and Maume studied the effects of climate on potatoes (1930a, 1934a, 1935b) and on grape (1936c), and Thomas and Mack (1943) studied its effect on the nutritional status of the corn plant. Lagatu and Maume (1934a) state that it is difficult to determine the fertilizer requirements of crops if the climate varies greatly from season to season, and any recommendations made under such varying conditions are purely speculative.

5. Fertilizer Requirements

a. Introduction

While the preceeding paragraphs have described the use of foliar diagnosis to study the effects of disease, cultural practices, irrigation, pruning, weather, and nutrient carriers, the technique may be used as a tool for the interpretation of the results of fertilizer field plot trials, although but few examples of this practical application have been published. Many believe that data obtained by foliar diagnosis do not supply information concerning how the mineral composition of the crop may be altered to give higher yields. However, in the works of Lagatu and Maume and of Thomas there is evidence that foliar diagnosis may be used for determining the amount and kinds of fertilizer required by crops, and two examples of this application will be described.

b. Montpellier Technique

Lagatu and Maume (1936c) studied the foliar diagnosis of the grape over the period from 1921 to 1928 under twenty different fertilizer treatments and found that the optimum composition of the NPK unit for high yield is $N = 41$, $P_2O_5 = 8$, and $K_2O = 51$ in all stages of growth. Similarly, for the potato they report (1937c) that these values should be $N = 38$, $P_2O_5 = 4$, and $K_2O = 58$. Using these ratios they believed that recommendations for the correction of fertilizer deficiencies could be made. Such a recommendation of course, is essentially one for correcting nutritional state of the soil through several seasons, although the authors have admitted that this was difficult.

A mathematical method for interpreting the data in order to permit recommendation of fertilizer applications was described (1937). If x, y, and z represent the percentages of N, P_2O_5, and K_2O in the dry matter from the fertilized plot; and if a, b, and c represent the percentages of N, P_2O_5, and K_2O obtained from the unfertilized plot; and if a_o, b_o, c_o represent the percentages of N, P_2O_5, and K_2O in the plants representing the optimum nutritional levels, then

$$x + y + z = s$$

$$a + b + c = t$$

$$a_o + b_o + c_o = t_o$$

Carrying out the calculations as described previously,

$$X + Y + Z = 100$$

$$A + B + C = 100$$

$$A_o + B_o + C_o = 100$$

These equations are used to show the improvement resulting from fertilization and also how close this improvement has come to the ideal NPK unit.

The effect of the fertilizer on the composition of the NPK unit is measured by the ratios

$$\frac{\text{Realized difference}}{\text{Desired difference}} \text{ of } \frac{X-A}{A_o-A}, \text{ of } \frac{Y-B}{B_o-B}, \text{ of } \frac{Z-C}{C_o-C}$$

These fractions are interpreted in the following manner:

If the fertilizer has improved the nutritional status of the crop, each of the three ratios will be greater than 1. A negative ratio indicates that a nutritional factor has exerted a detrimental effect on the composition of the NPK unit.

Positive ratios, but less than 1, indicate the fraction of the desired nutritional state attained by use of the fertilizer. Positive ratios greater than 1 indicate to what extent the fertilizer used was an overestimation of that required.

The effects of the fertilizer treatments on the intensity of nutrition are measured by the ratio, $\frac{s - t}{t_o - t}$, and are interpreted in the same manner as the effects of the fertilizer on the NPK unit.

The data used to explain the technique of interpretation (1937c) were obtained from an experiment with the grape variety Vigne de Mauguio carried on from 1929 to 1934. Potassium was added annually as potassium chloride at a rate to give 600 kilograms of K_2O per hectar. This treatment increased the crop yields during the six years of the experiment. The data presented in tables 36 and 37 permit comparisons between the fertilized and unfertilized plots. Comparing the C and Z values with the optimum unit, 41:8:51, it is evident that in 1929 the percentage of K_2O in the foliage and in the NPK unit was lower than desired in both the unfertilized and fertilized crops. The addition of 600 kilograms per hectar of K_2O only partially corrected this deficiency, as all values were positive for the $\frac{Z - C}{C_o - C}$ ratios, during the first year. But the percentage of the K_2O in the tissue was only 10 percent of what it should have been. As the treatment was continued during successive seasons, the nutritional status improved to such an extent that in 1934 (table 34) the percentage of potassium in the NPK unit was approximately 75 percent of the optimum value and the percentage of phosphorus level in the NPK unit had decreased to a point

Table 36. Analyses of leaves of the grape, Vigne de Maugio, grown in 1929 on unfertilized and K_2O fertilized plots, expressed as percentages of dry matter and in NPK units showing the extent to which the fertilized crop approached the optimum nutritional status for high yields. (Lagatu and Maume, 1937c)

Date of crop	Percent N	Percent P_2O_5	Percent K_2O	Intensity of nutrition	Composition of NPK unit		
	Plot D Check plot no treatment						
	a	b	c	t	A	B	C
May 10	4.49	1.22	2.04	7.75	57.94	15.54	26.32
June 15	2.27	0.78	0.82	4.37	63.39	17.85	18.76
July 15	2.33	0.47	0.61	3.41	68.33	13.78	17.89
Sept. 9	1.86	0.33	0.36	2.55	72.94	12.94	14.12
	Plot E Treated plot 600 Kgs./hectar of K_2O as KCl						
	x	y	z	s	X	Y	Z
May 10	4.54	1.08	2.27	7.89	57.54	13.69	28.77
June 15	2.91	0.68	1.12	4.71	61.78	14.44	23.78
July 15	2.57	0.46	0.77	3.80	67.83	12.11	20.66
Sept. 9	1.85	0.29	0.46	2.60	71.15	11.16	17.69

	X-A	Y-B	Z-C	s-t	A_o-A	B_o-B	C_o-C	t_o-t
May 10	-0.40	-2.05	+2.45	+0.14	-16.97	-7.74	+24.68	-0.12
June 15	-1.61	-3.41	+5.03	+0.34	-22.39	-9.85	+32.24	-3.26
July 15	-0.70	-1.67	+2.37	+0.39	-27.33	-5.98	+33.11	+4.22
Sept. 9	-1.79	-1.78	+3.57	+0.06	-31.94	-4.95	+36.88	+5.08

	$\frac{X-A}{A_o-A}$	$\frac{Y-B}{B_o-B}$	$\frac{Z-C}{C_o-C}$	$\frac{s-t}{t_o-t}$
May 10	+0.024	+0.265	+0.099	-1.17
June 15	+0.072	+0.346	+0.156	+0.10
July 15	+0.026	+0.289	+0.072	+0.09
Sept. 9	+0.056	+0.360	+0.097	+0.01

Values required for optimum nutritional status

$A_o:B_o:C_o::41:8:51$

$t_o = 7.63$

Table 37. Analyses of the leaves of the grape, Vigne de Maugio, grown in 1934 on unfertilized and K_2O fertilized plots, expressed as percentages of dry matter and in NPK units showing the extent to which the treated plot approached the optimum nutritional status for high yields. (Lagatu and Maume, 1937c)

Date of crop	Percent N	Percent P_2O_5	Percent K_2O	Intensity of nutrition	Composition of NPK unit		
	Plot D. Check plot no treatment						
	a	b	c	t	A	B	C
May 11	4.59	1.10	1.72	7.41	61.94	14.85	23.21
June 14	2.07	0.77	0.99	3.81	54.05	20.10	25.85
July 17	1.72	0.56	0.99	3.27	52.60	17.13	30.27
Sept. 5	1.59	0.42	0.88	2.89	55.02	14.53	30.45
	Plot E. Annual treatment since 1929 of 600 kgs./hectar of K_2O as KCl						
	x	y	z	s	X	Y	Z
May 11	4.80	1.12	2.02	7.49	60.45	14.11	25.44
June 14	2.16	0.51	1.84	4.51	47.89	11.31	40.80
July 17	1.70	0.38	1.80	3.88	43.82	9.79	46.39
Sept. 5	1.56	0.43	1.71	3.70	42.16	11.62	46.22

	X-A	Y-B	Z-C	s-t	A_o-A	B_o-B	C_o-C	t_o-t
May 11	-1.49	-0.74	+ 2.23	+0.08	-20.94	- 6.85	+27.79	+0.22
June 14	-6.16	-8.79	+14.95	+0.70	-13.05	-12.10	+25.15	+3.82
July 17	-8.78	-7.34	+16.12	+0.61	-11.60	- 9.13	+20.73	+4.36
Sept. 5	-12.86	-2.91	+15.77	+1.41	-14.02	- 6.53	+20.55	+4.74

	$\frac{X-A}{A_o-A}$	$\frac{Y-B}{B_o-B}$	$\frac{Z-C}{C_o-C}$	$\frac{s-t}{t_o-t}$
May 11	+0.071	+0.108	+0.080	+0.36
June 14	+0.437	+0.726	+0.641	+0.18
July 17	+0.757	+0.804	+0.778	+0.14
Sept. 5	+0.917	+0.446	+0.767	+0.30

Values required for optimum nutritional status

$A_o:B_o:C_o::41:8:51$

$t_o = 7.63$

close to the optimum value. It appears from these experiments that foliar diagnosis may be used to determine the fertilizer requirements and that the investigator also may estimate how close he has come to producing the optimum nutritional status, and consequently yield, of the crop.

C. Pennsylvania Technique

Thomas and Mack (1939) have suggested a technique of interpreting data obtained by foliar diagnosis by which fertilizer recommendations can be estimated intelligently. It is essential, of course, that the fertilizer be known to influence the "intensity" of the "quality" of the nutrition of the crop. The technique is based on the assumption that there are no differences existing in the influence of external factors other than those related to the fertilizer treatments.

The data in table 38, obtained from a fertilizer experiment with corn, may be used to illustrate Thomas and Mack's application. The data obtained from plot 28 show how the composition of the crop can approach that found in the tissue of the highest yielding plot (22). The data show that the percentage of nitrogen in the NPK unit from plot 28 was lower than that from plot 22 throughout the season. The mean value of the percentage of P_2O_5 in the NPK unit from plot 28 was somewhat lower than from plot 22. The proportion of K_2O in the NPK unit from plot 28 is greater than from plot 22. The intensity of nutrition of the crop from plot 28 is always higher than from the highest yielding plot at every sampling. It is apparent from these

Table 38. The intensities, mean NPK units, and yields of corn from the three highest yielding fertilizer treatments. (Thomas and Mack, 1939).

Plot	Treatment	Mean intensity	Composition of NPK unit (Mean)			Yields in lbs.	
			Percent N	Percent P_2O_5	Percent K_2O	Corn	Stover
22	manure and lime	5.88	73.88	8.23	18.39	770.8	408.9
28	(3N)PK 9:6:12.5	6.73	70.51	7.32	22.17	727.7	372.6
26	NPK	6.54	69.57	7.58	22.84	705.4	359.5
1	check	4.62	73.67	5.74	20.59	165.4	117.1
23	lime	4.11	81.90	9.80	8.29	452.2	222.1
5	NP 6:12:0	----	-----	----	----	376.7	195.9

data that the intensity must be reduced from 6.73 to 5.9, the percentage of nitrogen in the NPK unit must be increased from 70.5 to 73.4, the percentage of P_2O_5 must be increased from 7.3 to 8.2, and the percentage of K_2O reduced from 22.2 to 18.4.

One must find some means of estimating the amount of the reduction of K_2O required to decrease the proportion of K_2O in the NPK unit from 22.2 to 18.4. A preliminary estimate may be obtained by comparing the data from plots 26 and 5 in table 38. It is seen that a reduction of 12.5 pounds of K_2O reduced the K_2O in the NPK unit by 15.6 percent. Since it is necessary to reduce the K_2O in the NPK unit by 3.8 units, therefore

$$\frac{15.6}{3.8} = \frac{12.5}{x}$$

$$x = \frac{(3.8)\ (12.5)}{15.6} = 3.04 \text{ pounds per plot}$$

The new fertilizer which should have been used is 9:6:9.5, that is, on the basis of the foliar diagnosis, optimum yields would be obtained by using a fertilizer containing 9 pounds of N, 6 pounds of P_2O_5, and 9.5 pounds of K_2O per plot.

Comparison of the data from plots 1 and 23 suggest another possibility, for here it is noted that liming caused a decrease in the intensity of nutrition, an increase in the N and P_2O_5 in NPK unit, and a decrease in the K_2O in the NPK unit. Therefore, it would appear that the yield from plot 28 could have been improved by the addition of lime.

E. Discussion

The problem of developing economical and simple methods for controlling the factors governing the growth and composition of crops probably is the most perplexing ever to confound the investigators of agricultural problems. One of the contributions of Lagatu and Maume and of Thomas and their collaborators is the division of the major problem into two distinct units; one concerning the study of the effects of fertilizer on the nutritional composition of the crop and consequently the determination of the nutritional status of the crop conducive to maximum yield, and one concerning a study of the soil itself in order to determine the amounts and kinds of fertilizer necessary for maximum yields. Whether this division is of any real value is doubtful, for surely it is less reasonable to confine the study of fertilizer requirements to the relationship between the plant composition and yield as is done in the foliar diagnosis method, than it is to study the soil in its relationship to plant composition, or even to the study of the soil alone. Further, it is well to recall that in Section II of this report, a great number of factors were described which affected the composition of plant tissues, but over which man had little or no control.

It seems very unlikely that a precisely defined chemical composition of a plant can be designated as optimum, except under the conditions under which the "optimum" composition was determined. But it is upon this rather weak foundation that Lagatu and Maume have attempted to apply the foliar diagnosis technique to the determination of the fertilizer requirements of a given crop. From data obtained over a number of years, Lagatu and Maume have calculated "standard" ratios of certain chemical components in the leaves of potatoes as N:P:K: = 38:4:58 (1934c, 1935a) and for grapes as 41:8:51 (1936, 1936c, 1938). Following the same techniques, Craig (1938, 1939) proposed 50:12:38 as the "standard" ratio for sugar cane. It is difficult to believe that these "standard" ratios represent inherently optimum chemical compositions which result in optimum yields regardless of other factors as claimed by Lagatu and Maume. Even if we assumed that such a biological constant could exist, it is much more likely to be in the form of Mitscherlich's c constant than in the form of a ratio between arbitrarily selected nutrients in plant tissue. It must be borne in mind that these ratios are confined to only three of the large number of equally significant nutrients found in plant tissue.

A further point detracting from the possibility of the existence of a physiologically important NPK unit as a biological constant is that the "standard" ratios proposed by Lagatu and Maume are affected by many factors, including, of course, all soil factors. Even if it were possible to accept these ratios as constants, which is difficult to do at the present time with the small amount of data available, they could not be used with the same ease of manipulation as is possible with the Law of Diminishing Returns.

The foliar diagnosis technique, weak as it is in its ability to forecast fertilizer requirements, does indicate changes in the nutritional status of a given crop. The technique may be used with considerable success to study the effects on the plant of such factors as cultural practices, irrigation, placement of fertilizers, pruning, climatic conditions, diseases, and abnormal soil conditions. This type of information may then be used as a supplement to the more appropriate techniques permitting the quantitative determination of the fertilizer requirements of a crop.

The foliar diagnosis method is nothing more than another form of tissue test. Currently there are two procedures used in interpreting the data on leaf composition. One interprets separately the effects of the individual nutrients and selects minimum values below which crop yield is affected (Hoagland, 1941; Ulrich, 1942, 1942a). This assumes that all other components will be present in amounts greater than their minima, and that only one dominant limiting factor is operating at a given moment. The second, based on the foliar diagnosis technique, converts the chemical composition of the crop into a form which indicates the equilibrium between three major nutrients, namely nitrogen, P_2O_5, and K_2O.

Unless it can be shown experimentally that the NPK unit is related more closely to the growth and yield of the plant than are the percentages of the individual nutrients from which it is derived, not only can the extra computional work not be justified, but even the nutritional relationships may be unnecessarily obscured. Such a comparison has never been made, and until it has been made, the foliar diagnosis technique can be considered no better than the critical concentration technique.

There is only one general conclusion that can be drawn concerning the foliar diagnosis. This procedure cannot be applied in a manner which will permit the estimation of the fertilizer required for optimum plant growth. Although such attempts have been made by Lagatu and Maume, their techniques are so weak in theory and so clumsy in practice that they are of no practical value.

IX. TRIPLE ANALYSIS

A. Introduction

The triple analysis technique for testing soil fertility and for predicting probable crop response to fertilization was developed by Lundegårdh (1938) in Sweden. The procedure is based on the assumption that the fertilizer requirements of the crop could be determined most readily by chemical analysis of the plant, and of the citric acid extract of the surface soil and of the sub-soil. Previous to the work of Lundegårdh, most investigators limited themselves to the use of soil analysis or of plant analysis in their quest for a method for determining the probable responses of crops to fertilizers. Joulie (1889) and Januszewski (1895) were probably the earliest workers to consider both plant analysis and soil analysis in the interpretation of crop response data. Januszewski (1895) analyzed two soils, set up fertilizer experiments with wheat on the basis of these soil analyses, and then analyzed the grain and straw at the time of harvesting. Januszewski altered the fertilizer application as a result of the soil analyses and attempted to correct the nutrient deficiencies in such a way as to obtain wheat with the desired chemical composition. He concluded that chemical analyses of crops were of value for the estimation of fertilizer requirements only if the soil is carefully evaluated as to its physical and chemical characteristics. Expressing the same conclusion, Lundegårdh (1938) stated that "a good practical test of the fertility of the soil and its fertilizer requirement must be built up from a scientific knowledge of the soil and its influence on the growth of the plant".

B. Technique

The triple analysis technique, as originally described by Lundegårdh (1938), varied according to the conditions of the experiment. In some instances, the ash analyses of the plant tissue and the analyses of the surface layer of soil were used, in other instances, analyses of the plant ash, surface soil, and sub-soil were used. While the term triple analysis was proposed to fit the latter case, it soon became a general name for the simultaneous use of chemical and biological data to determine fertilizer requirements. The analyses of the soil included the pH, phosphorus, potassium, calcium, magnesium, manganese, copper, and iron. Lundegårdh used quantitative spectroscopic analysis for determining the cations. For pH and phosphorus determinations, electrometric or colorimetric methods were used.

The analyses of the leaf sample was considered by Lundegårdh to indicate nutrient deficiencies in the soil, and to a certain extent whether an injurious lack of physiological balance exists between two or more nutrients. The analyses of the soil were considered to reveal the minimum availabilities of nutrient cations, and also inhibiting or antagonistic effects of certain ions such as the effect of lime on the uptake of manganese or the effect of potassium on the absorption of calcium. It is apparent that Lundegårdh regarded the plant as an ideal soil extracting agent and the analysis of the soil an aid in the interpretation of data obtained by analysis of the crop.

Unfortunately, this fruitful approach to the problem of the determination of crop fertilizer requirements was not developed on a practical level. Lundegårdh subsequently abandoned the very real possibilities of his triple analysis procedure, and limited it to the plant only, thereby following the example of Lagatu and Maume (1934, 1937, 1937a), Thomas (1945), and Thomas and Mack (1940, 1944). In other words "triple analysis" became "tissue diagnosis".

Lundegårdh's approach to leaf analysis was somewhat different than that of Lagatu, Thomas, and their collaborators. The Lundegårdh procedure was based on leaf samples collected at the flowering stage of the plant when the vegetative parts were fully grown but still vigorous, a practice in contrast to that followed in foliar diagnosis in which samples were taken throughout the whole season, and also in contrast to the techniques of the majority of tissue analysis methods which, if a single sample is taken, used samples taken as early as possible in the growing season.

Lundegårdh (1943) tested the practical value of his leaf analysis in 800 field experiments with cereal crops in different parts of Sweden in 1938 and 1939. By plotting the percentages of potassium in the leaves from unfertilized plots against the increased crop yields obtained with potassium fertilizers, Lundegårdh (1943) obtained a curve approximating a hyperbola. What he actually found was an approximate inverse relationship between the concentration of potassium in the leaves and the increase in yield obtained with fertilizer. Similar curves were obtained in respect to the nitrogen content and the effect of nitrogenous fertilizers, and in respect to the phosphorus content of the leaves and the effect of phosphatic fertilizers. The mathematical equation showing this inverse relationship for a single nutrient was expressed by Lundegårdh (1943) in the form

$$ya = \frac{b}{x^c}$$

where a, b, and c are constants, y the increment in the yield due to the addition of the fertilizer, and x the concentration of the nutrient in the plant at the time of flowering. The general form of the equation for more than one nutrient was given as

$$yA = \frac{b_1}{x_1^{c_1}} \times \frac{b_2}{x_2^{c_2}} \times \frac{b_3}{x_3^{c_3}} \times C_s$$

where y is the increase in yield, A is a constant representing the individual values a_1, a_2, and a_3; x_1, x_2, and x_3 are the percentages of potassium, phosphorus, and nitrogen respectively, and C_s is a correction constant which brings the values of b_1, b_2, and b_3 into numerical agreement with the scale of yield increments. Whether or not these equations correctly represent the relationships they attempt to express is a matter of conjecture, for the supporting evidence is far from conclusive.

Curves relating analyses of the crop and its response to added fertilizers were plotted by Lundegårdh from the mass of data he obtained in 1938 and 1939. These curves may be used to study the relationship between different amounts of two nutrients in the leaves and the increased yields resulting from fertilization. For example, figure 52 shows the effect of the interaction of potassium and phosphorus. If the soil suffers from a phosphorus deficiency, as is indicated by the low percentage of phosphorus in the leaves, fertilization with a potassium fertilizer alone will produce a smaller crop yield than if both potassium and superphosphate were added. Similarly leaf analyses may show whether superphosphate fertilization is profitable without simultaneous fertilization with nitrogen. The tabular method of presentation of these data used by Lundegårdh (1941) is shown in table 39.

Since the curves developed by Lundegårdh are based on data from a large number of observations over a wide area, they are of some value in suggesting recommendations of the fertilizer requirements. The technique is the only one that truly relates plant analysis and yield for a number of different factors, and if it will do nothing else, the technique demonstrates to the farmer how he can economize with mineral fertilizers.

C. Discussion

The triple analysis technique as described by Lundegårdh (1938) is the earliest significant approach to the study of the nutrient levels in the crop as they are related to the response of the crop to fertilizer. Any method for the diagnosing fertilizer requirements of crops is not directed primarily toward forecasting crop yields, but rather to forecasting the probable response of the crop to the addition of a fertilizer

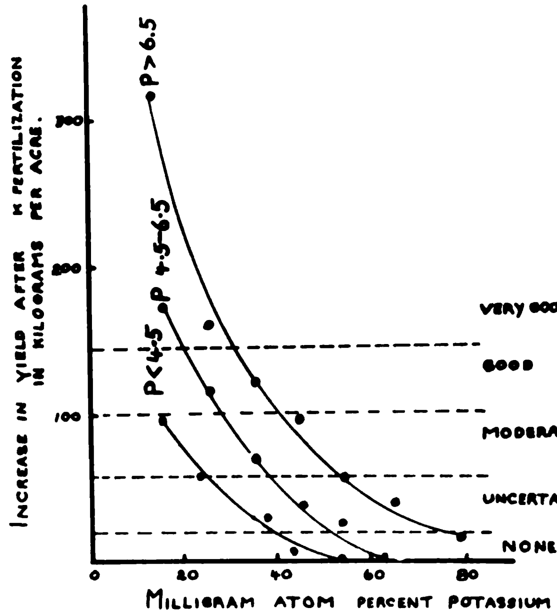


Figure 52. Probable effect on yield of cereals as a result of fertilization with 60 kilograms of 40 percent potassium salt per acre. As the potassium content in the tissue increases the effect decreases. Increasing supply of phosphorus raises the utilization of potassium. (Lundegårdh, 1943)

Table 39. Effect of fertilizers on the grain yield of oats. Analytical results expressed as percent milliequivalents in the dry matter of the leaves of oats at maturity. (Lundegardh, 1941)

Probable effect of fertilizer		Very good	Good	Average	Doubtful	None
Effect of 100-150 Kg. per hectar of muriate of potash (40%) in addition to 150-200 kg. per hectar of saltpetre.						
Potassium content	with P content <4.5	--	10-20	20-40	40-50	>50
	with P content 4.5-6.5	<20	20-30	30-50	50-60	>60
	with P content >6.5	<30	30-40	40-60	60-80	>80
Effect of 100-150-kg. per hectar of superphosphate (20%) in addition to 150-200 kg. per hectar of saltpetre.						
Phosphorus content	with N content <100	-	---	1-8	8-9	>9
	with N content <100	4	4-6	6-8	8-9	>9
Effect of 150-200 kg. per hectar of saltpetre.						
Nitrogen content	with P content <4.5	---	20-80	80-120	120-160	>160
	with P content 4.5-6.5	100	100-120	120-200	200-500	---
	with P content >6.5	120	120-200	200-250	----	---

so that yields may be improved. This may be accomplished either by applying the Law of Diminishing Returns as was done by Bray and Willcox or by the complicated procedure using tissue analyses. The minimal requirements for the development of such a tissue analysis method would be large numbers of data from plants growing on a series of soils containing various amounts of one nutrient while others are present in excess. The data must include the internal concentrations of the varying nutrient at a definite stage in specific organs, and also the increases in crop yield resulting from the application of definite amounts of nutrients. The increments in yield resulting from the addition of nutrients could then be represented as a function of the nutrient concentration within the plant at the time of fertilizer application. This procedure is essentially that used by Lundegårdh (1938, 1943), but it cannot be compared either in general applicability or in ease of execution with the practical value of applying the Law of Diminishing Returns.

X. CRITICAL CONCENTRATION

A. Introduction

The value and possible application of critically defined concentrations of nutrients and of nutrient ratios in plants as a biological method for the determination of fertilizer requirements of plants has been discussed by Thomas (1945), Goodall and Gregory (1947), Ulrich (1948), and others.

The biological methods discussed in previous sections of this report have been based primarily on accurate laboratory chemical techniques by which plant tissues of various ages were analyzed, and their compositions expressed on a dry weight basis. On the other hand, the analytical techniques used most commonly for the determination of "critical" concentrations of nutrients in plants have been the so-called quick-test types. These techniques probably were given their greatest impetus as a result of Hoffer's (1926) scheme of quick-tests for nitrates and potassium in the corn plant carried out under field conditions. Later they were "improved" by Thornton, Conner, and Fraser (1934), and by others, and were extended to include other nutrients. From this point onwards, the development of modified "quick" techniques proceeded at a rapid rate and contributions were made by many workers. Among these, Emmert (1934), Carolus (1938), Hance (1936, 1937, 1941), and Scarseth (1941, 1942) merit especial consideration.

At the same time that these techniques were being developed, Wallace (1932), Lagatu and Maume (1934) and somewhat later, also Thomas (1937), Nightingale (1942), Macy (1936), Ulrich (1941, 1942, 1942a), Goodall (1945) and Boynton and Compton (1945), adopted the more accurate analytical procedures for ascertaining the nutrient content of plants.

The development of the abbreviated chemical tedhniques permitted analysis of great numbers of samples to show the effect of single nutritional factors. With the universal acceptance of the statistical techniques developed by Fisher (1934), it became relatively easy to show apparent relationships and correlations between the nutrient levels in crops and crop yields. Consequently, at the present time, the majority of research projects involving the diagnosis of the nutritional needs of a crop utilize critical concentrations and ratios of nutrients in the crop as they are related to yields. Goodall and Gregory (1942) in particular have made an exhaustive study of the various tenhniques used by investigators and have compiled tables showing the specific tissues recommended for sampling, the times of sampling, and the critical concentrations or the standard ratios recommended for the large number of crops. This compilation shows that the majority of workers using tissue analysis have attempted to standardize their methods to take into consideration as many as possible of the causes of variation of the nutrient content of the plant.

Regardless, however, of the type or quality of analytical procedures used, most of the work might be divided into one of two classes:

interpretation through the correlation of an optimum, critical, or threshold concentration of nutrients in the plant against yield, and interpretation through correlations of ratios of nutrients in the plant with yield. Both of these approaches will be considered in the following pages. It is proposed to consider the background upon which the two above approaches are based and the various ways in which attempts have been made to correlate them with plant yields.

B. Correlation of Critical Concentration with Yields

1. Introduction

The possibility of using minimum concentrations of inorganic nutrients in plants as the bases for determining nutritional deficiencies has been recognized for many decades by workers growing plants in artificial culture media and in field plots. A few classic publications are those of Hellriegel (1867), Heinrick (1882), Von Dikow (1891), Atterberg (1901), Pfeiffer, Simmermacher, and Rippel (1919), Emmert (1935), Macy (1936), Ulrich (1944), and Hill (1950). It was found that plant growth ceases when the concentration of any of the nutrients in the leaves falls below a certain minimum value. This minimum is different for different species, varieties, types of growth etc.

Macy (1936) presented a theory that there is a critical percentage of each nutrient in each kind of plant above which there is luxury consumption of the nutrient and below which there is a poverty adjustment which is almost proportional to the deficiency until a minimum percentage is reached. Macy tested his theory by studying the nitrogen requirement of barley during 1933 and 1934. The data based on the straw are given in table 40. In this table, the response to the nitrogen fertilizer is calculated as $\frac{\Delta Y}{\Delta N}$ for each increment of nitrogen and is compared with the corresponding average percentage of nitrogen in the straw. The yield curves for the straw reproduced in figure 53 show that the treatments exerted marked effects on the yield. However, the relationship between percentage nitrogen in the straw and the response of the yield to nitrogen shown in figure 53 is independent of these factors. This situation is in agreement with the theory advanced by Macy. In this case, the minimum percentage of nitrogen in the straw below which growth ceased was 0.4 percent, and the critical percentage above which luxury consumption began was about 0.8 percent. Macy concluded that the critical nutrient composition of a plant is an "ideal but inherent characteristic of the plant," and that the critical and minimum percentages varied only under special conditions. Further, he concluded that these percentages were intimately related to vegetative growth and yield.

Macy believed that the use of his theory to determine the fertilizer needs of particular crops on particular soils was as reliable as were field plot tests. This conclusion assumes that the sufficiency of a nutrient is a function of its percentage in the plant.

(Macy, 1936)

Milli-grams N applied	Yield	Percent N	Average percent N	$\frac{\Delta Y}{\Delta N}$	Yield	Percent N	Average percent N	$\frac{\Delta Y}{\Delta N}$	Yield	Percent N	Average percent N	$\frac{\Delta Y}{\Delta N}$
	D-O				**V-O**				**V-PK**			
0	1.97	0.37	0.49	120	1.28	0.45	0.64	74	1.96	0.44	0.45	218
79	3.98	0.60	0.72	23	2.24	0.82	1.18	- 1	4.14	0.45	0.52	108
158	4.23	0.83	1.31	3	2.23	1.54	1.95	- 2	5.97	0.59	0.73	19
237	4.34	1.78	2.02	- 14	2.20	2.35	2.59	4	6.35	0.87	1.08	- 3
316	4.12	2.25	2.49	4	2.25	2.82	3.05	-24	6.28	1.29	1.38	30
395	4.19	2.72			2.07	3.27			6.82	1.46		
	V-PKL-25				**V-PKL-20**				**V-PKL-15**			
0	3.50	0.39	0.42	179	2.68	0.39	0.46	134	2.13	0.49	0.60	89
79	5.51	0.45	0.57	54	4.51	0.52	0.67	25	3.22	0.70	0.77	32
158	6.72	0.70	0.91	14	4.94	0.81	1.11	2	3.41	0.83	1.01	16
237	7.18	1.11	1.38	3	4.97	1.40	1.53	25	3.75	1.19	1.31	10
316	7.29	1.65	1.82	14	5.52	1.66	1.78	-12	3.80	1.42	1.54	10
395	7.70	1.98			5.40	1.89			3.91	1.65		
	D-PK				**FD-PK**				**FV-PKL**			
0	1.97	0.37			2.75	0.45	0.45	309	2.30	0.39		
10	----	----			3.02	0.44	0.44	226	----	----	0.39	280
20	----	----	0.45	156	3.84	0.44	0.49	118	----	----		
40	----	----			4.99	0.53	0.57	113	3.28	0.38	0.42	192
79	4.55	0.52	0.58	116	6.34	0.61			5.88	0.45	0.66	26
158	6.84	0.64			----	----	0.64	120	6.71	0.87	1.12	12
198	----	----	0.77	50	8.82	0.67			----	----		
237	8.23	0.89			----	----			----	----		
277	----	----	1.15	15	----	----	1.06	8	7.38	1.37		
316	9.22	1.40	1.54	4	----	----			----	----	1.68	9
395	9.33	1.68			9.50	1.44			8.98	1.98		

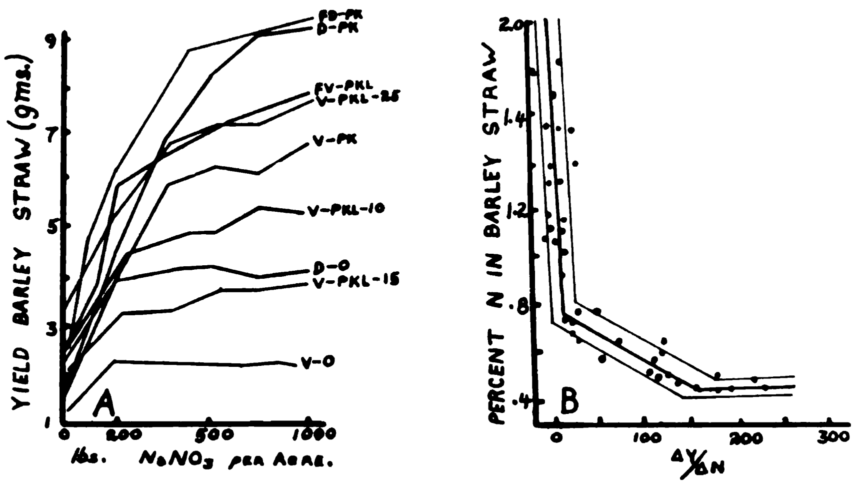


Figure 53. A. Yield cirves for barley straw under nine fertilizer treatments. B. Relationship between percentage nitrogen content and the yield response (barley straw) due to nitrogen fertilization (Macy, 1936)

While much work had been done previous to that of Macy which was based on definite minimum concentrations, the theory by Macy and its possible application resulted in furthering interest in this type of nutritional research. The general concept can be criticized because only one nutrient in the plant is considered and no consideration is given to the effects of other nutrients on the absorption and utilization of the one being studied.

Even with this limitation apparent, Ulrich (1942a, 1943, 1946, 1948) developed Macy's ideas further with a slightly different interpretation. Ulrich defined the critical nutrient level as "that range of concentrations at which the growth of the plant is restricted in comparison to that of plants at a higher nutrient level." He considered (1948) that the evidence indicated that the critical values varied only slightly in comparison with the range of concentrations above the critical level. Thus the potassium levels of the middle leaves of low potassium tomato plants grown in culture media varied from 0.91 percent in the dry matter in the early part of the season to 0.51 percent in the latter part of the season, while in high potassium plants the percentages varied from 4.66 to 2.29 during the same period. Ulrich (1946, 1946a, 1948) presented data supporting his statement that "there is no profound effect upon yield when large fluctuations in nutrient concentration well above the critical level occur within the plant." For example, his data show that in one sample, when the percentage of potassium in the middle blade of the tomato leaf changed from 0.47 to

0.51 percent, the yield changed from 146 to 214 grams of dry tops. On the other hand, when the concentration of potassium changed from 2.29 to 2.78 percent, only a slight effect was evident on the yield.

It would seem that Ulrich's data could be interpreted more easily on the basis of the Law of Diminishing Returns. The data, however, show the difficulty of working with critical levels of single nutrients for it was found that when low levels of potassium were in the plant added sodium exerted only a small effect upon the level of potassium in the plant but exerted a profound effect upon the yield. No better example could be desired to show that the addition of a second ion may effect the utilization of the first even though it is present in its "critical" concentration. It is necessary to determine the effects of other factors than the one being studied on nutrient concentrations within the plant and upon the final yield. This means, of course, falling back to the techniques of Lundegårdh (1941).

In any case, Ulrich (1943) believed that the critical concentrations of the various elements in the crop did not vary enough as a result of season, of the amount of sodium, or of the potassium concentration to prevent their use as points of reference to ascertain the potassium status of the crop, but the data he presented does not appear to justify this conclusion.

As a result of the work described above and of similar approaches, many workers have reported critical concentrations of an element in plants below which deficiency would be expected (see Goodall and

Gregory 1947). Among these workers are Carolus (1933, 1933a, 1935, 1937, 1938), Gilbert and Hardin (1927), Hester (1935), Scarseth (1943, 1943a), Emmert (1935, 1935a, 1937, 1941, 1942, 1949), Boynton (1942), Hill (1943), Hill and Cannon (1948), and many others.

A considerable amount of work has been carried out by English workers, particularly at Long Ashton, on the relationships between threshold, or minimum, values, visual symptoms of deficiency, the laboratory chemical analyses, and data obtained by tissue tests. For example, Nicholas (1944, 1948), Nicholas and Jones (1944), Jones and Nicholas (1945), Nicholas and Catlaw (1947, 1947a, 1947b) have shown that the appearance of visual symptoms of deficiencies, especially of potassium, magnesium, phosphorus, manganese and boron and of visible symptoms of excess chlorine, zinc, and manganese are correlated with the threshold content of these substances in the plants. The value of these data is trivial because it should be apparent that when nutrient deficiencies are acute enough to produce visual symptoms, or nearly so, there is no need for chemical analyses to show that the concentrations of the nutrients in the plants are low.

2. Determination of Critical Concentrations

The methods of determining critical concentrations of nutrients in plant tissue are as variable as the number of workers conducting investigations of this nature. There are, however, two distinct approaches to the general problem. Ulrich (1948) suggested that a preliminary estimate may be obtained by growing the crop in soil or in solution cultures. The averages of one such set of data based on Ladino clover supplied with various amounts of phosphate are presented in table 41. The yields and the phosphorus contents of the plants are presented graphically in figure 54. Similar data for a series of potassium fertilized series of field plots appear in figure 55. These data caused Ulrich to conclude that the critical concentration for phosphorus is approximately 600 parts per million in the dry material, although the data themselves indicate that this was only a rough estimate.

Under field conditions, Ulrich's (1948) calculation of the critical concentrations is based on comparing the concentrations of the nutrients in plants known to be deficient with those in plants amply supplied with the given nutrient. When this procedure is repeated over a period of seasons, correlation between yield and the concentration of the nutrient usually will be obtained, and the nutrient levels in the plants signifying deficient yields might be established, as was done by Ulrich (1946a) for Ladino clover.

Ulrich's work (1946a) showed that he could not always depend upon the results of field plot experiments, as is shown by figure 56. In this case, there appeared to be no luxury consumption, as was found in the pot tests, and he arbitrarily considered that the critical level again was 600 parts per million although the results of the field tests do not show that this was true.

Table 41. Fertilizer treatments, average yields, and average P_2O_5 as parts per million in the leaf stalks of Ladino clover. (Ulrich, 1946a)

Treatment	Fertilizer application gms. per pot	P_2O_5 (ppm)	Yield (gms)
Untreated	----	378	11.2
P_0	K_2O 3.02 gms. Lime 19.2 gms.	389	14.9
P_1	K_2O 3.02 gms. Lime 19.2 gms. P_2O_5 0.42 gms.	449	32.3
P_2	K_2O 3.02 gms. Lime 19.2 gms. P_2O_5 0.84 gms.	762	46.3
P_3	K_2O 3.02 gms. Lime 19.2 gms. P_2O_5 1.27 gms.	797	52.3
P_4	K_2O 3.02 gms. Lime 19.2 gms. P_2O_5 1.69 gms.	946	52.0

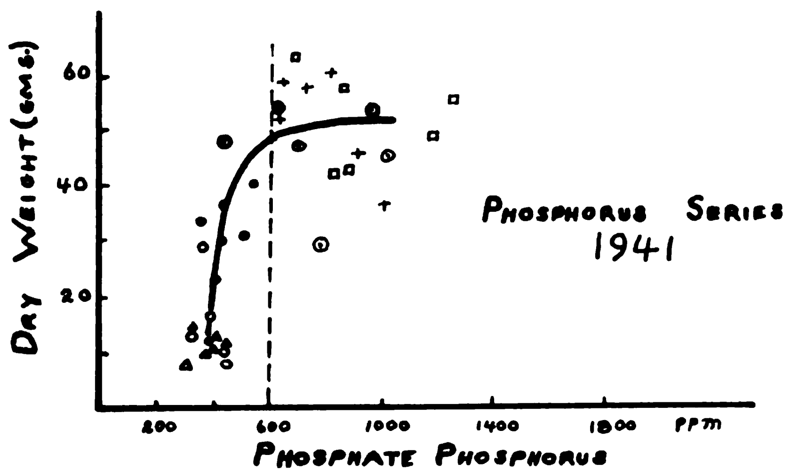


Figure 54. Relationship between the yield of Ladino clover and the phosphate concentration in the petioles when phosphorus is limiting in some of the treatments (Phosphorus series of plots). Solid line is a freehand line drawn through the average points. (Ulrich, 1946a)

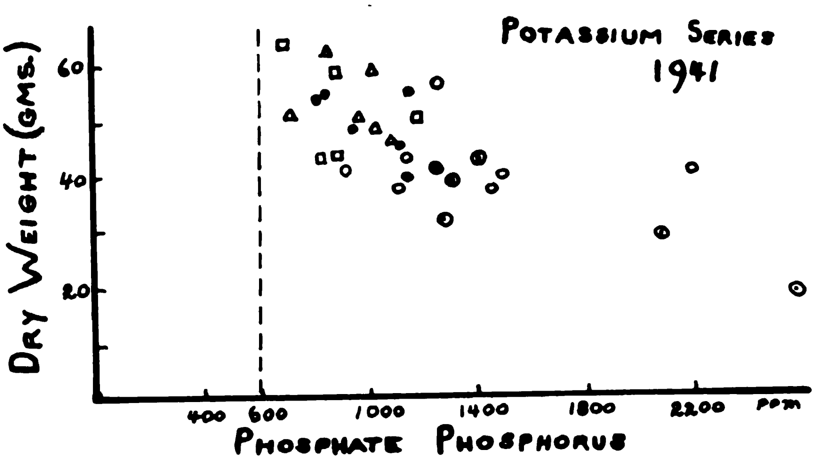


Figure 55. Relationship between the yield of Ladino clover and the phosphate phosphorus concentration of the leaf petioles when the phosphorus is not limiting growth (potassium series of plots). (Ulrich, 1946a)

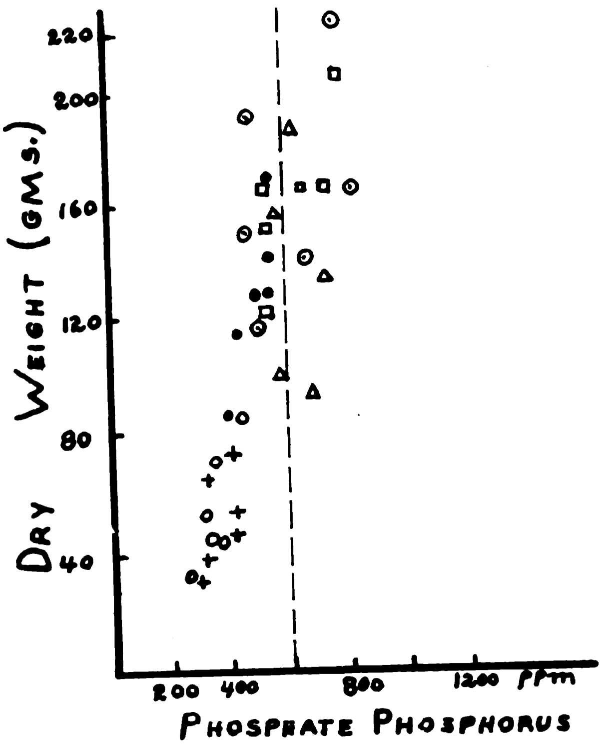


Figure 56. Relatioship between the yield of Ladino clover and the phosphate phosphorus concentration of the leaf petioles of clover grown under field conditions under various fertilizer treatments.(Ulrich, 1946)

Emmert (1935a, 1937) used such statistical procedures as correlations, partial correlations and partial linear regressions to determine the limiting values of nutrient concentrations in crops. The details of his procedure are discussed in his paper describing the correlation of yield with the amount of soluble nitrogen and phosphate in the conducting tissues of potatoes at various stages of growth. His data are given in table 42 and they show the effects of nitrogenous fertilizer on the nutrients in stem tissue and on the yields. His approach was purely statistical in that he used a linear correlation, but which, of course, was not strictly accurate for the population as a whole. Emmert, however, maintained that if the data were limited to values below the point where the curve flattens out, the correlation was almost linear, and while not representing the population as a whole, this segment of the linear curve showed the degree of correlation with this limited group of data. If the higher values of nitrogen in the tissue are associated with insignificant changes in yields, it indicates that a point has been reached where the curve begins to flatten and that the optimum for the set of conditions being studied is near this point. If, when the values above this point are removed, the partial correlation drops or remains constant, then there is an indication that the optimum value probably lies above this point. But if the partial correlation rises, it means that the high values are associated with lower yields and that the optimum was below the value selected.

It was by the above procedure, based on the data in table 42, that

Table 42. Effects of nitrogenous fertilizer on the concentration of nitrate nitrogen and of phosphate phosphorus in the stem tissue and on the yields of a spring crop of potatoes, together with various correlations, partial correlations, and optimal levels of nitrogen and phosphorus. (Emmert, 1935a)

Treatments in pounds per acre	Yields, average of 4 plots (pounds)	Date of sample: June 15 Sol. N ppm	June 15 Sol. P ppm	June 21 Sol. N ppm	June 21 Sol. P ppm	July 15 Sol. N ppm	July 15 Sol. P ppm	Average Sol. N ppm	Average Sol. P ppm
Check	127	321	228	160	198	218	116	233	181
100 lbs. $NaNO_3$	187	896	226	265	160	324	105	495	164
500 lbs. $NaNO_3$	200	747	210	780	180	627	270	718	220
Check	152	353	218	210	183	262	140	275	180
100 lbs. $(NH_4)_2SO_4$	207	545	207	453	132	547	118	515	153
500 lbs. $(NH_4)_2SO_4$	211	712	233	913	124	1030	100	855	152
200 lbs. $NaNO_3$ 300 lbs. $(NH_4)_2SO_4$	279	980	192	1267	128	1130	153	1126	158
8 tons of straw	155	342	262	568	155	613	205	508	207
check	170	284	245	260	160	218	127	254	177
10 tons of manure	199	290	268	233	167	169	196	231	210
40 tons of manure	202	239	218	467	146	327	155	344	173
check	185	288	208	217	117	242	187	249	171

Simple correlations (significance 0.273)	June 15	June 21	July 15
NY	0.395	0.556	0.424
PY	0.002	0.053	0.074
NP	0.272	0.689	0.144

Partial correlations (significance 0.281)	June 15	June 21	July 15
N Y . P	0.416	0.819	0.441
P Y . N	0.123	0.724	0.149
N P . Y	0.287	0.866	0.128

Partial correlations with plots with nitrogen above 1000 ppm removed and phosphorus above 200 ppm removed.	June 15	June 21	July 15
N Y . P	0.376	0.515	0.170
P Y . N	0.446	0.174	0.050

Indicated direction of optimum	June 15	June 21	July 15
Nitrogen	above 1000 ppm	above 1000 ppm	above 1000 ppm
Phosphorus	below 200 ppm	above 200 ppm	above 200 ppm

Emmert (1935a) was led to the conclusion that the optimum concentration of soluble nitrogen in the stem should be at least 1000 parts per million throughout the season and that the soluble phosphorus concentrations should be below 200 parts per million. When, however, his data for nitrogen are plotted as in figure 57, there does not appear to be any justification for the selection of the value of 1000 parts per million of nitrogen as the optimum concentration. Indeed, the data show a very sharp break at 400 parts per million and there is greater justification in taking this value than the higher value that Emmert selected. Similarly, the phosphorus data shown in figure 58 do not indicate any justification for selecting 200 parts per million as the maximum value other than the fact that the value associated with the highest yield is below 200. It is obvious that if this value is omitted, there is no correlation between yield and phosphate concentration.

The above examples have been cited to show that in a purely statistical study, there are serious pitfalls into which the investigator can fall and that there are occasions when unjustified conclusions are drawn from such data. Statistics are of value in such a study only if used as a supplementary aid and not as a necessarily authoritative indication.

Emmert, however, realized the errors of his earlier technique and in a later study he used different statistical methods. In 1937 he described his use of partial linear regression to analyze the curvilinear relationship between crop yields and the percentages of nutrients

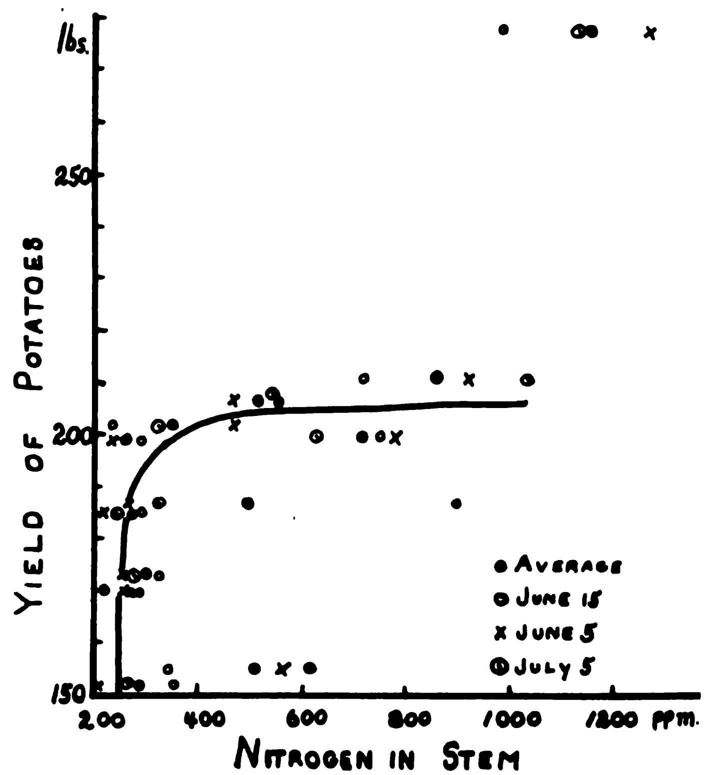


Figure 57. The relationship between the soluble nitrogen, expressed as ppm., in the stem tissue and the yield of potatoes at three different dates. (Plotted from data of Emmert, 1935a)

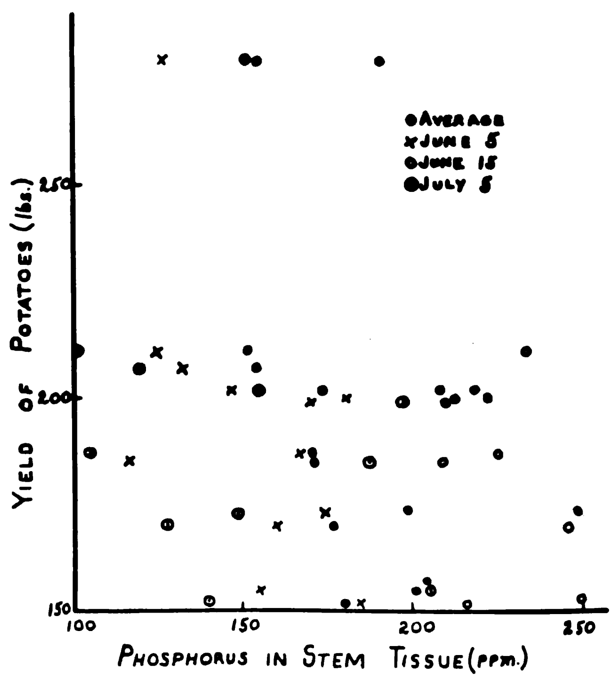


Figure 58. The relationship between the soluble phosphorus content expressed as ppm. in the stem tissue and the yield of potatoes at three different dates. (Plotted from the data of Emmert, 1935a)

in the plants.

The data given in table 43 illustrate Emmert's revised technique, and are based on the response of potatoes, grown in 1935 at Lexington, Kentucky, and fertilized with nitrogen and phosphate. The method involves grouping the data in groups consisting of approximately the same number of observations as indicated in table 43. It is obvious that the mean yields of these groups should be tested for statistical significance before proceeding further. In this case, there is no significant difference between the ranges of compositions, and further interpretation of the data normally would not be continued, but the procedure will be described to illustrate its possibilities. The next step is the calculation of the partial and single correlations shown in table 43. Then the partial regression lines can be obtained from the formula

$$y = y_M + r_{ny} \frac{\sigma_y}{\sigma_n} \left(N - \frac{N}{M} \right)$$

where, y is yield,

n is ppm. of soluble nitrogen in stem tissue of the plant,

p is ppm. of phosphorus in the stem tissue of the plant,

M is mean of group,

N is number in the group.

r is correlation coefficient

σ is standard deviation.

In this instance, the three partial regression lines are as shown in figure 59 and are noted as follows:

Table. 43. Yields of potatoes and the concentrations of nitrogen and phosphorus in the stem tissue, expressed as parts per million at Lexington, Kentucky, 1935. (Emmert, 1937)

	Range 1 N 139 - 233			Range 2 N 257 - 555			Range 3 N 555 - 1625		
	N (ppm)	Yield (lbs.)	P (ppm)	N (ppm)	Yield (lbs.)	P (ppm)	N (ppm)	Yield (lbs.)	P (ppm)
	139	33	195	253	38	160	556	42	188
	143	43	146	264	48	146	626	45	125
	147	40	120	264	39	146	700	63	146
	154	48	178	269	40	160	750	52	178
	162	43	266	264	38	250	834	46	188
	182	27	200	265	52	175	834	56	178
	186	55	260	286	49	114	408	65	105
	200	51	240	312	51	155	1125	46	160
	208	45	170	330	46	260	1200	58	122
	213	41	145	380	60	134	1250	80	140
	233	47	146	436	57	130	1625	70	150
	---	--	---	436	64	200	---	--	---
	--	--	---	470	55	134	---	--	---
	4	--	---	476	54	160	---	--	---
	--	--	---	555	37	155	---	--	---
Sum	1967	484	2116	5259	728	2499	10408	623	1680
Number		11			15			11	
Mean	178.8	44	192.4	350.6	48.5	166.6	946.2	56.6	152.7
σ	30.5	8.5	45.2	96.4	8.4	40.2	30.6	11.5	37.1
r_{ny}		+0.453			+0.381			+0.638	
r_{py}		+0.112			-0.254			-0.448	
r_{np}		+0.093			-0.095			-0.275	
$r_{ny.p}$		+0.447			+0.371			+0.599	

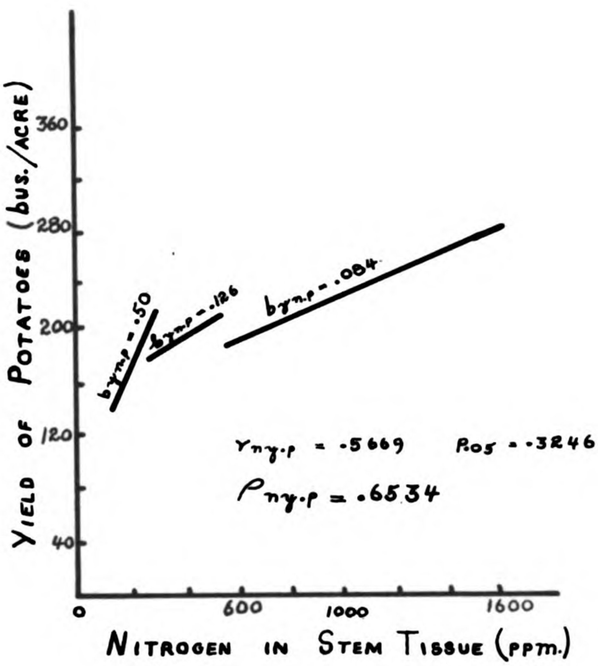


Figure 59. The relationship between the yield of potatoes and the nitrogen concentration in potato stems shown as regression lines for the three ranges indicated in table 43. (Emmert, 1937)

Range 1 $Y = 21.7 + 0.125\ n$

2 $Y = 37.5 + 0.0315\ n$

3 $Y = 36.8 + 0.021\ n$

These data exhibit several interesting aspects when plotted in the manner described. Since the data suggest that the optimum concentration of soluble nitrogen is 400 parts per million, a value suggested by the previous test, it first of all removes the danger of selecting the wrong breaking point in the data as was done in the previous work. Secondly, it shows that between 139 and 250 parts per million of soluble nitrogen in the tissue, the yield increases 0.5 bushels per acre for each part per million. Between 250 and 556 parts per million, the increase in yield is 0.126 bushel per acre, and above these values, the increase is 0.084 bushel per acre. These observations approximate the Mitscherlich type of yield curve. Finally, if this type of relationship were expanded to include a larger number of regression lines, it could be used to determine if fertilizer applications were profitable, but this could be done only if a relationship between the soluble nitrogen in the stem tissues and the rate of fertilizer application could be shown conclusively.

Another approach to the estimation of critical concentrations was proposed by Carolus (1937) and was based on experiments with potatoes at the Virginia Truck Garden Station. The crop was sampled every seven days during the season and a series of "optimum" weekly levels of nutrients was obtained as recorded in table 44. The analyses were obtained

Table 44. Optimum weekly nutrient concentrations in potatoes growing in Virginia conditions in 1936. (Carolus, 1937)

Age of plant (days)	Weight per plant (gms.)	Soluble Nitrogen (ppm.)	Soluble Phosphorus (ppm.)	K_2O (ppm.)	MgO (ppm.)	CaO (ppm.)
42	26	1228	216	3500	250	800
49	60	988	148	5125	650	1100
56	160	1025	215	3750	675	1000
63	340	891	150	3125	1075	1100
70	470	1295	167	8250	1050	1100
77	630	1104	140	6350	950	1250
84	743	927	90	6000	1075	1800
92	673	1204	84	5900	1225	1900
Average for season		1085	152	5250	882	1315

by "quick-tests" but Carolus states that these tests were "shorter and give a more definite indication of the nutrient uptake than complete methods, and are more reliable in diagnosing nutrient deficiencies than soil tests made after the fertilizer materials have been applied."

Carolus believed that under similar growing conditions plants that did not contain at least 75 percent of the amounts shown in table 44 should be considered to be deficient in that nutrient. The difficulty with this type of information is that, since similar conditions are difficult to define and may never be met, the optima are of little practical value.

3. Discussion

Whether the critical, or minimum, concentrations of nutrients in plant tissuee are of any real use to the research worker for the determination of the nutrient requirements of crops is a question surrounded by much controversy. Before considering this point, however, it should be pointed out that such critical concentrations are of undeniable value for certain uses.

One usable aspect of critical concentrations is their application to plant nutritional surveys. Surveys of this nature extending over wide areas disclose local deficient soils, suggesting areas which could be used to the best advantage for fertilizer investigations to be carried out later by more reliable methods.

Tissue analysis is of some value as a technique supplementary to field fertilizer experiments. There can be no doubt that it also is a valuable supplement to soil analysis for the interpretation of the effects of added nutrients on the yields of crops. But since we are considering only the critical or minimum concentrations, care should be taken in their use.

The use of critical concentrations and minimum levels for the confirmation of the diagnosis of nutritional deficiencies as expressed by visual symptoms is also important. Finally, such data are of value in following the seasonal trends of the nutritional status of the plant.

In spite of the possible uses of "critical" concentrations of nutrients in plants, such data cannot supply the information needed to determine fertilizer requirements of crops. The data are interpreted almost entirely by statistical methods, but no matter how these methods are applied, there is no way that the results can be put on a practical basis. Not until the investigator has data showing the yield responses of crops with varying amounts of fertilizer is he in a position to consider establishing standard values for the concentrations of nutrients in crops.

The basing of conclusions on data from a single element is in general unsatisfactory. It has already been pointed out that the increase in yield to be expected from increase in the supply of a nutrient is related not only to its internal concentration, but also to that of other nutrients. A method for interpreting the results of tissue diagnosis must take into account the concentration of single nutrients and also variations in the concentrations of other nutrients. The significance of this latter aspect of the problem will now be discussed.

C. Nutrient Ratios

The limitations of the use of critical nutrient concentrations in plants have been recognized by investigators for some time. In many instances, the approach has been to compute and use nutrient ratios in the plant for diagnostic purposes. According to Goodall and Gregory (1947), this procedure involves certain hidden assumptions concerning the curves relating crop yields to nutrient content, and they explain the problem by stating, "Let us suppose a three-dimensional figure in which the vertical axis represented whatever feature of development was being measured (say, the response to a particular fertilizer application) while the two horizontal axes represented the content of the two nutrients in question in the plant material, all other factors being held constant; if development of the plant depends on the ratio of two nutrients within it, and not on their individual values, then any vertical plane through the origin must cut the figure in a horizontal line. This has never been demonstrated." Gregory (1937), in fact, showed with barley grown in pot cultures that as the supply of nitrogen and phosphorus increased in constant proportion to each other, the ratio of these two elements in the plant remained constant although their concentrations individually increased and the crop yield increased as shown in figure 60 and table 45. Moreover, when luxury consumption occurred, an increase in the supply of the nutrient in

Table 45. Yield of barley plants fertilized with varying amounts of nitrogen and phosphorus, expressed in grams per pot. (Gregory, 1937)

	P_1	P_2	P_3	P_4	P_5
N_1	33.0	18.9	2.70	1.32	0.70
N_2	10.54	10.86	4.94	3.59	2.45
N_3	4.64	4.43	4.20	3.50	3.47
N_4	1.98	1.98	2.20	1.97	1.27
N_5	1.20	1.22	1.04	1.20	1.00

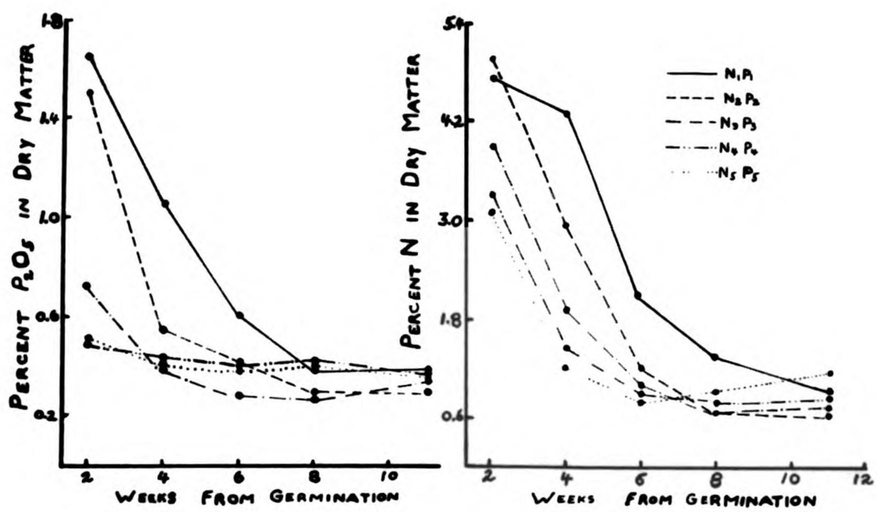


Figure 60. Drift of phosphorus (A) and nitrogen (B) expressed as percent of the dry matter of whole barley plants sampled 2, 4, 6, 8, and 11 weeks after germination. Nitrogen and phosphorus varying together. Balance of $N:P_2O_5$ constant at 3:1. (Goodall and Gregory, 1947)

excess may increase the ratio of its internal concentration to that of the "limiting" nutrient without affecting either the internal concentration of the limiting nutrient or the yield.

It appears that simple ratios, as distinct from the ratios devised by the foliar diagnosis school, can only serve as supplementary to those indications given by the actual concentrations. Richards (1944) believed that the use of such ratios, without consideration of the concentration of the individual components, is unjustified in most cases. He cites as an example that the results of Hunter, Toth and Bear (1943) concerning the calcium and potassium nutrition of lucerne are as readily explained on the basis of the individual percentages of these two cations in the plant as by their ratios. Munter (1919) stated that wheat was deficient in nitrogen if the P_2O_5:N ratio in the straw was greater than 0.6, but Goodall and Gregory (1947) pointed out that his figures showed that the correlation of yield increment due to nitrogen-containing fertilizer with his ratio was only -0.2969, while that with the percentage of nitrogen in the straw was -0.6375.

The fact that ratios give no information that can not be obtained from simple concentrations does not, of course, detract from the fact that the diagnosis of the nutritional status in respect of one element on a basis of its concentration in the plant may not have to be modified according to the levels of the other elements. This has been shown conclusively by Lundegårdh (1941) whose data have been discussed in the section on Triple Analysis.

Since the time of Atterberg (1888a, 1889) most workers have at one time or other expressed the nutritional status of a crop on the basis of the ratios of components in the tissues. This information, although considered to be of little value, has been compiled by Goodall and Gregory (1947) and arranged in tabular form for the different crops considered.

D. Presentation of Data

The majority of workers have merely reported or suggested certain values, or ranges, of the nutrient content of crops as representing a theoretical portion of curves of the type developed by Macy (1936). An example is the suggestion of Boynton and Compton (1945) that apple trees would produce an optimum yield if the leaves sampled in July contained between 1.85 and 2 percent nitrogen. Hill (1943) stated that a reduction in the yield of carrots would result if the petiole sampled in the active growth period contained less than 0.0125 percent P_2O_5 in the fresh material. Beauchamp, Lazo and Bonazzi (1934) reported that good growth of sugar cane resulted when the leaves sampled when the plants were nine months old contained K_2O and P_2O_5 in the ratio of 3:1.

There are a few cases in which the workers have reported successive ranges rather than isolated concentrations or ratios. Heinrich (1882), for instance, presented data showing the probable yield of oats in relation to the nutrient content of the roots at maturity. His data covered successive ranges through low, average, and high yields as indicated in table 46. Borden (1936) reported four levels of the potassium and phosphorus contents of the juice of sugar cane as indicative of the various levels of deficiency tabulated in table 47. He does, however, state that the results probably are only reliable for the particular variety of sugar cane that he used and then only under similar growing conditions. This method of presenting data has not had wide usage. This probably is due to the fact that investigators either seek to determine the critical levels and to bypass the intermediate gradations, or because they fail to visualize plant relationships as continuous, rather than discontinuous, functions.

Probably the best approach to the use of tissue analyses for the determination of the effects of several nutrients on yield is that suggested by Nicholas (1948). This worker shows interrelationships of the mineral nutrients by plotting their concentrations along the radiating spokes of polygonal figures. Theoretically, there can be as many "spokes" as there are influential elements, but Nicholas has as yet considered only N, P, K, Ca, Mg and Mn. Using a large amount of data obtained by tissue analysis, he estimated the concentration of these elements corresponding to normal growth and high yields, and estimated the "threshold" values, or values below which deficiency symptoms occur. Such a diagram is presented in figure 61, in which the healthy plant is represented by isometric "spokes" for each element, which results, of course, in a symmetrical figure. Intensity factors are then represented by the length of the spokes and

Table 46. Probable yield of oats in relation to the nutrient content of the roots at maturity, expressed as percentages of the dry matter. (Heinrich, 1882)

Nutrient	Probable yield of oats		
N	0.5-0.6	0.7-0.9	0.9 or more
P_2O_5	0.1-0.2	0.2-0.3	0.3 or more
K_2O	0.1-0.2	0.2-0.4	0.4 or more
CaO	0.2-0.3	0.3-0.4	0.4 or more

Table 47. Percentages of nutrients in the crusher juice of sugar cane at different levels of nutritional deficiency. (Borden 1936)

Level of deficiency	Percent P_2O_5	Percent K_2O
Low	0.025	0.15
Doubtful	0.030	0.20
Medium	0.035	0.25
High	0.045	0.35

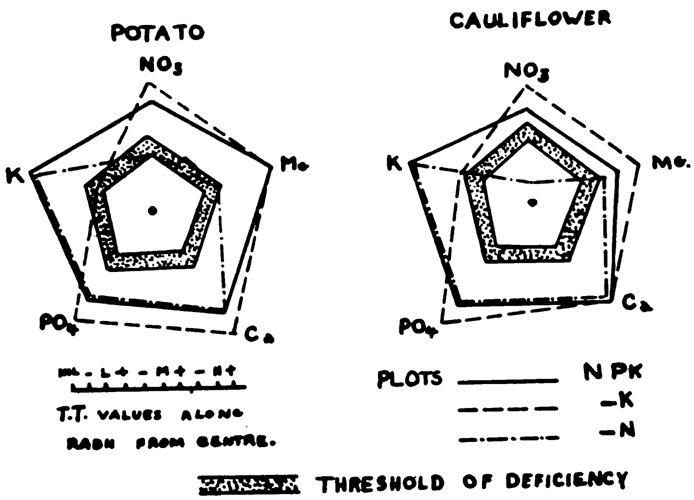


Figure 61. Diagrams showing the relationship between soluble potassium, magnesium, calcium, phosphorus, and nitrate nitrogen in the potato and in the cauliflower growing under various fertilizer treatments. The mean of six tissue test values from NPK, NP, and NK treatments are plotted along five radii of a circle. (Nicholas, 1948)

quality factors by the outline of the figure. At some place in the periphery lies the deficiency threshold region. The unsatisfactory nutritional condition is seen by the distortion of the outline due to the unequal length of the spokes. Thus, low nitrogen is often accompanied by low magnesium.

This approach of Nicholas possesses advantages over foliar diagnosis techniques because it is not restricted to the three major elements, and the relationships are not shown by a single point in a triangular system. It possesses the disadvantage that it is not able to show the seasonal trends as readily as does the foliar diagnosis system.

E. Discussion

Of all various methods for determining the fertilizer requirements of crops, the use of critical concentrations probably rests on the weakest experimental foundation. The current literature is replete with "critical" and "optimum" concentrations as evidenced by the monograph of Goodall and Gregory (1947), but they are of little real value except to the individual investigator in his own area because they differ under different conditions. This resulting confusion can be of little assistance in solving the perplexing problem of determining the fertilizer requirements of crops.

The greatest weakness of the critical concentration concept is its failure to permit the estimation of the amount and kind of fertilizer required to give optimum crop yields. Since the determination of critical concentrations is so rapid it can be used effectively in survey studies and it is in this category that these data must be placed in relation to the other and superior techniques.

Critical concentrations may also serve a purpose in the study of seasonal trends of the nutritional needs of crops and they may suggest the advisability of applying side dressings, nutrient sprays, etc., but in this capacity they will be only of supplementary value to other techniques which can more authoritatively estimate the major fertilizer requirements.

The use of critical concentrations should be completely re-examined experimentally to determine whether the use of this technique has been a detriment or an asset to agricultural research. Research workers in the field of plant nutrition and fertilizer use should divorce themselves from techniques designed for rapidity rather than for accuracy and they should attempt to develop new methods, or to re-examine and improve older methods in order to find more accurate procedures, for it cannot be denied that, even with the development of more rapid and less tiresome techniques, our agricultural soils in many areas are deteriorating more rapidly than these quick methods are restoring them.

XI. LEAF PAINTING AND INJECTION

A. Introduction

The advocates of leaf painting and injection techniques have implied that they can, in a limited way, be used to determine the fertilizer requirements of crops and consequently they merit consideration. The diagnostic use of paint injection has been reviewed at length by Roach (1939), and by Roach and Roberts (1945). These workers have proposed these techniques as routine diagnostic methods, particularly as supplements to the use of visual symptoms. In this connection, Roach (1943) states that for "the diagnosis of mineral deficiencies several methods are necessary to suit different circumstances; the method of leaf injection is to be used in conjunction with visual symptoms." This seems like a weak justification.

B. Technique

Roach and Roberts (1945) proposed a series of solutions to be used for diagnostic purposes. They state that these solutions in the concentrations recommended are applicable over a wide range of crops but that it may be necessary to modify them in rare cases. The solutions recommended for leaf injection techniques by Roach are as follows, but in some instances the addition of sulphuric acid may be necessary to prevent precipitation.

Element	Concentration (%)	Source
N	1	Urea
P	0.5	NaH_2PO_4
K	1	KCl
Ca	1	$CaCl_2$
Mg	0.5	$MgSO_4.7H_2O$
Fe	0.025	$FeSO_4$ + 0.025% (by volume) H_2SO_4
Mn	0.025	$MnSO_4$ + " " "
Zn	0.025	$ZnSO_4$ + " " "
Cu	0.025	$CuSO_4$ + " " "
Ni	0.025	$NiSO_4$ + " " "
B	0.1	Boric acid

The injection procedures discussed briefly below are described in detail by Roach (1939, 1943, 1943a) and by Roach and Roberts (1945).

In the interveinal method, the solution containing the element to be tested is held in a thimble-shaped vessel made from a cellophane drinking straw containing a small strip of filter paper glued inside. A slit is made in the interveinal area of the leaf and the end of the filter paper pushed through it acting as a wick. If a deficiency is present, there will be a localized improvement in the color of the nearby mesophyll cells.

The leaf stalk technique is the most highly recommended by Roach and Roberts (1943). It involves cutting off the blade and inserting the petiole into a glass vial containing the testing solution. Depending upon the vascular system of the plant, varying effects on the color of the leaves above and below the treated petiole will occur. In apple, pear, plum, and peach, the two leaves above and below the treated petiole are infiltrated with the solution on the side nearer the cut petiole, but not on the further side. Thus in four leaves, comparisons can be made between opposite sides of the same leaf. If on the other hand, only the tip of a leaf is removed part of the blade or all of it will be affected by the solution depending upon how much of the tip has been removed.

In the branch method, the branches of a tree may be injected each with a different nutrient solution. The solution is fed to the tree through a piece of rubber tubing leading from the base of the container to a hole bored in the branch. This technique permits the same tree to be used for studies of several possible deficiencies.

There are two methods by which the test solutions may be applied to the entire tree. One is the liquid method which involves supplying the solution through tubing leading into holes, one-fourth inch in diameter, bored in the tree. In the other method, the dry nutrient salt is forced into small holes at three inch intervals around the circumference of the tree and the hole plugged with a cork.

The leaf painting method was studied by Roberts (1944) in his attempt to overcome the slow uptake of nutrients by fruit trees when treated by the injection method. He simply painted the leaf with the nutrient solution. Roberts claimed that by using a series of concentrations, he could ascertain not only the deficient nutrient but also its concentration required for eliminating the deficiency.

C. Application

The injection and painting techniques may be used as diagnostic procedures or as curative procedures. As far as diagnosis is concerned, the techniques have had their greatest use in the study of leaf chlorosis of one form or another. The nutrient that corrects the chlorosis is the nutrient that is deficient, but in some instances, the response may be a local increase in size or a general increase in growth or yield.

Corky-core of apples was diagnosed as boron deficiency by the use of injection techniques, by Atkinson (1935), McLarty (1936), and Young and Bailey (1936), and the wither-tip of apples in Western Australia was diagnosed as copper deficiency by Dunne (1938).

The injection of test solutions into the plant or spraying them on the foliage is the quickest way of getting temporary recovery although permanent recovery is not likely. Roach (1934) reported that commercial apple trees in average health and vigor made twice as much growth after injection with K_2HPO_4 and urea, each at the rate of about 28 pounds per acre, as did untreated trees and they produced leaves thicker and healthier in appearance. In a further test, Roach (1939a) injected peach trees with manganese valued at four cents per tree, and was able to correct the deficiency. The trees previously had failed to produce a crop but produced a good average crop after the treatment.

D. Discussion

Probably the worst that could be said of leaf painting and injection techniques as far as their application to the fertilization of crops is concerned is that they are aimed at saving the cost of the fertilizer required by the immediate crop. Such a practice will do more damage to agriculture than good, for it eliminates, temporarily at least, the necessity for improving the soil. On the long time basis, it is more important to understand the soil and to improve it than to produce a crop.

Like many other types of tissue techniques, these methods permit only very vague conclusions as to fertilizer requirements, and cannot determine quantitatively the actual amounts of fertilizer required by crops. These methods cannot be considered more valuable than visual symptoms for they are not even applied until visual symptoms have appeared.

The only significant use of these procedures is for the identification of specific deficiencies, especially of the minor nutrients.

XII. VISUAL SYMPTOMS

A. Introduction

Visual deficiency symptoms have been used for the determination of the fertilizer requirements of a crop. The nutritional deficiencies are recognized by specific symptoms, mainly of the foliage, exhibited if the supply of one or more nutrients is insufficient to promote healthy growth. Before this method may be applied, the specific symptoms of each nutrient deficiency for each kind of plant must be recognizable. These specific symptoms have been determined by growing plants in artificial media under controlled conditions.

Many color plates illustrating these deficiencies have been published by the Chilean Nitrate Educational Bureau (1941), Wallace (1943, 1944), Cook and Millar (1949), Hambidge (1949), and others.

B. Application

Visual symptoms are intended for use in connection with other confirmatory methods for determining the nutrient status of the crop and are of no value when used alone. The various color atlases cited previously can be used for identifying the simple deficiencies, but not even the most experienced worker can interpret the symptoms of multiple deficiencies accurately in the majority of cases without resorting to confirmatory methods. Contrary to general opinion, all plants do not exhibit recognizable specific deficiency symptoms and this seriously limits their use. Only certain plants may serve as indicators of particular deficiencies. For example, the growth of sunflower plants grown under controlled conditions has been suggested by Stephenson (1940) and Colwell (1943) as a means of assessing the boron status of the soils.

Schemes in which special indicator plants and fertilizer treatments are combined have been proposed by Piper (1940) and Wallace (1943) for use in the fields where special difficulties are encountered or where an area of unknown cropping potentialities is being tested. These schemes aiming at solving during a single season the main deficiency problems likely to exist may have some value but they cannot give more information than adequate soil analyses.

Wallace (1943) suggested the use of certain indicator plants for diagnosing specific deficiencies. He arranges these species in groups as follows:

Group	Indicator plants	Mineral deficiencies indicated
1	Cauliflower, broccoli	N, Ca, Mg, Fe
2	Potato	K, Mg
3	Sugar beet, mangold, globe beet	B, Mn
4	Pape, Swede	P
5	Oats	Mn

The following fertilizer treatments are applied to the plot:

NPK	NK	NPKB
NP	NPKMg	NPK Mn

If the effects of liming are to be studied, the experimental plan will be as indicated in figure 62 which shows the arrangement of plots. The crops are sown in strips running the length of the area to facilitate cultural operations and the fertilizers are applied as indicated by the cross-hatched areas. If the effect of lime is not studied, only two blocks would be planted. Wallace believed that one might obtain a fair estimation of the deficiencies in the soil by observing the visual symptoms evident from the experimental plots.

C. Discussion

Frequently the symptoms produced by a deficiency of a specific nutrient on a particular species are specific, but in many cases this is not the case. The symptoms may vary among different species and sometimes even among varieties as described by Wallace (1943) and by Hill and Johnston (1940).

There are circumstances described by Wallace (1943) in which deficiency symptoms may be masked entirely by pests and disease, such as eelworms or virus diseases in potatoes, and Haas (1937), Spencer and Lavin (1939), and Wallace (1943) have described symptoms produced by pests, diseases, mechanical injury, or weather conditions that may be indistinguishable from certain mineral deficiency symptoms. For example, leaf symptoms on young cereals and Brassica plants may be identical for wire worm, cold weather, root injury, or phosphorus deficiency. Symptoms of chloride injury may be, and often are, almost

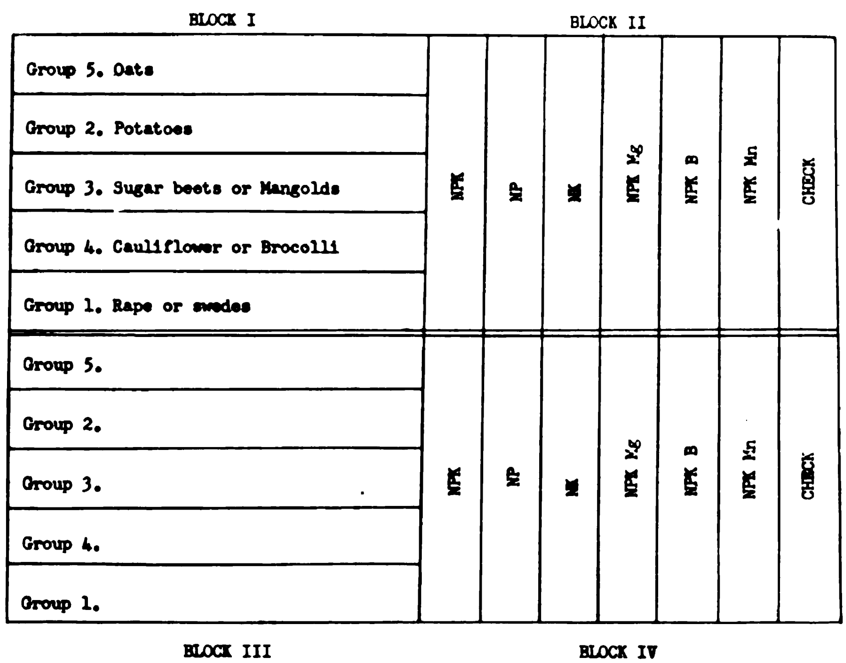


Figure 62. Field plan for determining soil deficiencies using indicator plants and visual symptoms. (Wallace, 1943)

identical with those of phosphorus deficiency as shown by Wallace (1943) on red currant foliage.

Symptoms of certain deficiencies are very similar and difficult to distinguish. Thus, chlorosis may be the symptom produced on apples, plums, and raspberries by deficiencies of iron, manganese, or nitrogen.

The value of visual symptoms also are limited by the fact that in the majority of cases only the most severe deficiency is apparent, and the effects of inadequate supplies of other nutrients are masked as described by McMurtrey (1949).

Finally, in most instances, nutrient deficiencies may cause decreased yields without inducing visible symptoms as has been pointed out by Hill (1943), Davis (1950), Batjer and Digman (1940), and Berger and Truog (1940).

There seems to be no possibility of using visible nutrient deficiencies for indicating a quantitative requirement of fertilizer. There is some value to the technique, however, for if a nutritional disorder can be diagnosed in the field, there may yet be time to correct the situation in the same season.

The conclusion that one must draw conerning the use of visual symptoms is that they may be of some value in the immediate correction of an apparent deficiency but no reliance can be placed on them without proper confirming techniques. They will point to acute nutritional deficiencies until such time as a more thorough study of the problem can be carried out by more accurate methods, but surely the farmer has failed to care for his soil if he waits until his crops exhibit visible symptoms of deficiency before he uses fertilizer.

XIII. WEEDS

The use of weeds as indicators of the fertilizer requirements of crops was suggested as early as 1882 by Heinrich who considered that the fertility of the soil might be measured by growing plants adapted to that particular soil and analyzing them to obtain indications of the nutrient content of the soil. In concept, this proposal essentially is a type of tissue testing. Weeds as indicators of soil fertility were first investigated by Hall (1905) whose work involved a comparison of the composition of the plants with that of the citric acid extracts of soil. He concluded that a universal test plant, such as a weed, was needed and that until such a plant was found, the interpretation of soil conditions from plant ash analysis was not practical as a substitute for chemical analysis of the soil.

Ecologists from time to time have suggested that the botanical composition of the natural flora or the weed flora could serve as an index of the nutritional state of the soil. Goodall (1949) analyzed weeds and compared their potassium and manganese contents with the requirements of barley and wheat for these substances. For example, figure 63 shows the correlations observed in the case of *Polygonum convolvulus* L. This figure represents the best correlation he obtained and even in this case there was no significant difference between site means, and with the exception of one station, the means of plots at a single station also were insignificantly different. There was, however, a highly significant correlation between the composition of the weeds and crop response to fertilizer when the data from all plots were grouped together as shown by figure 63. From these results, Goodall concluded that no response to potassium fertilizers could be expected if the percentage of potassium in the dry matter of the leaves of *Polygonum convolvulus* L. was greater than 1.83, but Goodall believed that his results were so inconclusive that no value could be gained from this approach.

A further example may be found in the work of Christensen and Larsen (1910) who considered that the presence of such species as *Scleranthus annuus* L. and *Rumex acetosella* L. indicated the necessity of lime for optimum crop production.

Although the distribution of a species may be generally dependent upon the nutritional status of the soil, such data are of little practical value for determining the magnitude of fertilizer requirements of crops.

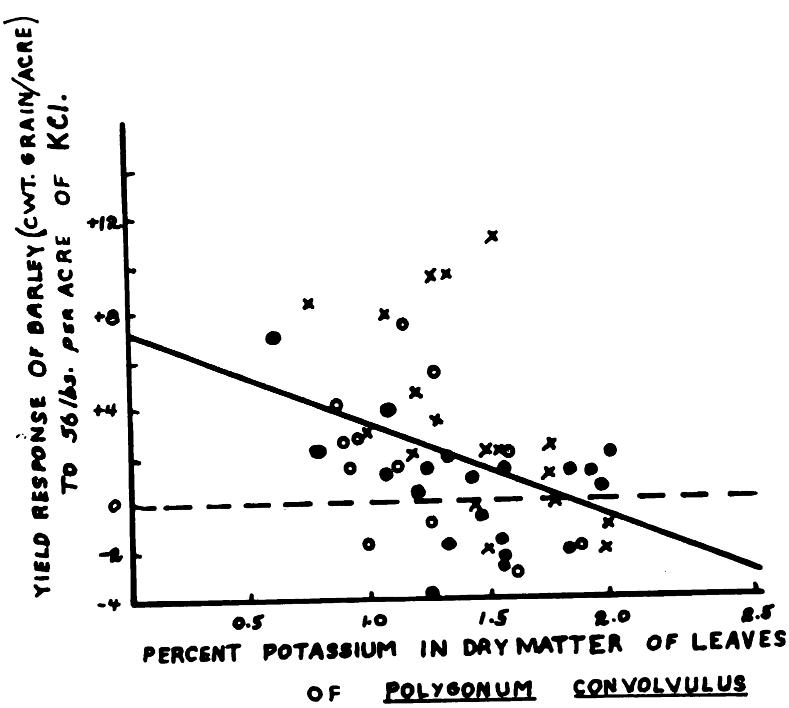


Figure 63. The relationship between the increase in grain yield of barley fertilized with potassium fertilizer and the potassium concentration in the leaves of *Polygonum convolvulus* L. at three different locations. (Goodall, 1949)

XIV. CHLOROPHYLL

Foliage of plants deficient in nitrogen has a lower intensity of green color than does the foliage of normal plants. This observation has suggested to a number of workers that the chlorophyll content might be used for assessing the nitrogen requirements of crops. The earliest attempt to do this was that of Gassner and Goeze (1936). Rye seedlings were grown in pots of soil and sand to which different amounts of nitrogen were added. After a growth period of 25 days, the chlorophyll was determined and the nitrogen requirements of the soil were estimated.

More recently workers in the United States have adopted this procedure and have attempted to apply it in practice. For example, the following workers have contributed to this work: Boynton, Compton and Fisher (1948), Boynton and Compton (1945), Compton and Boynton (1945), Compton, Granville, Boynton, and Phillips (1946), Boynton and Cain (1942), Judkins and Wander (1950), and Shear and Horsfall (1948).

The technique used in these studies for the determination of the chlorophyll content was devised by Comar and Zscheile (1941, 1942) who applied a colorimetric procedure using wave lengths 6600 Å and 6425 Å and an adaptation of Beer's Law. Compton and Boynton (1945) used a series of dilutions of pure chlorophyll for standardizing the method while Heeney (1950) set up standards with fresh material.

Compton, Granville, Boynton, and Phillips (1946) using McIntosh apples in two different orchards in the State of New York showed that there was a straight line correlation between the percentage of total nitrogen in the dry tissue and the amount of chlorophyll in the fresh tissue. Heeney (1950) carried out a survey of the Standard Orchard at Ottawa which involved three soil types, four kinds of rootstocks and four fertilizer treatments, and found a significant positive correlation ($r = +0.6630$) between the chlorophyll content of the leaves and the total nitrogen. In a later investigation, Heeney found a correlation of $r = +0.9974$ in a more uniform fertility block at Trenton. These data are presented in figure 64. Good correlations also were shown using tomato leaves as shown in figure 65.

Compton, Granville, Boynton, and Phillips (1946) set up color standards for use by the grower with which he could compare the leaves of McIntosh trees to determine the adequacy of the nitrogen supply. These standards were shown by Shear and Horsfall (1948) to be inadequate for other varieties or for different locations.

The results described appear to justify the use of the chlorophyll content of plants as indications of the adequacy of nitrogen supplies as in all cases good correlations were shown, but it should be borne in mind that the work was done on fertility trials in which nitrogen was generally the limiting factor. If the method is applied to general field surveys, interpretation becomes a more complicated problem. When applied in this manner, the results would doubtlessly be affected by deficiencies of other elements because nitrogen is not the only nutrient which affects the green coloration of the leaves. Deficiencies of

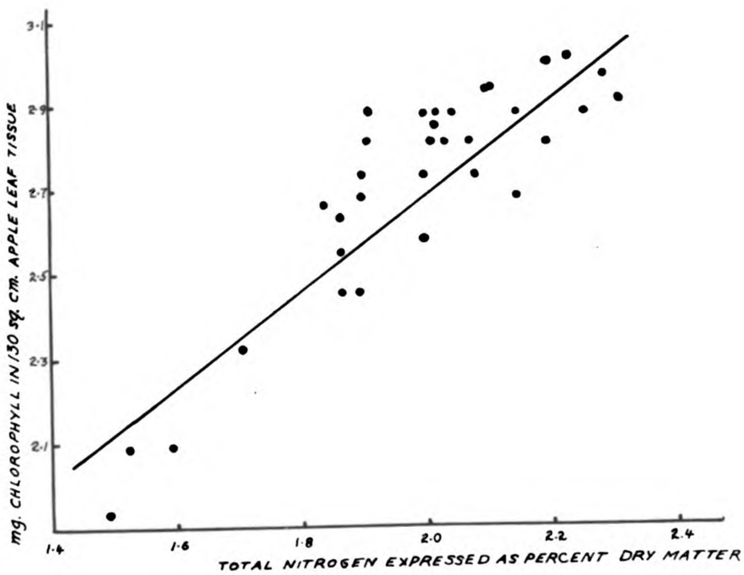


Figure 64. The relationship between total nitrogen and chlorophyll content in McIntosh apple leaves in Hendrick's Orchard, 1949. (Heeney, 1950)

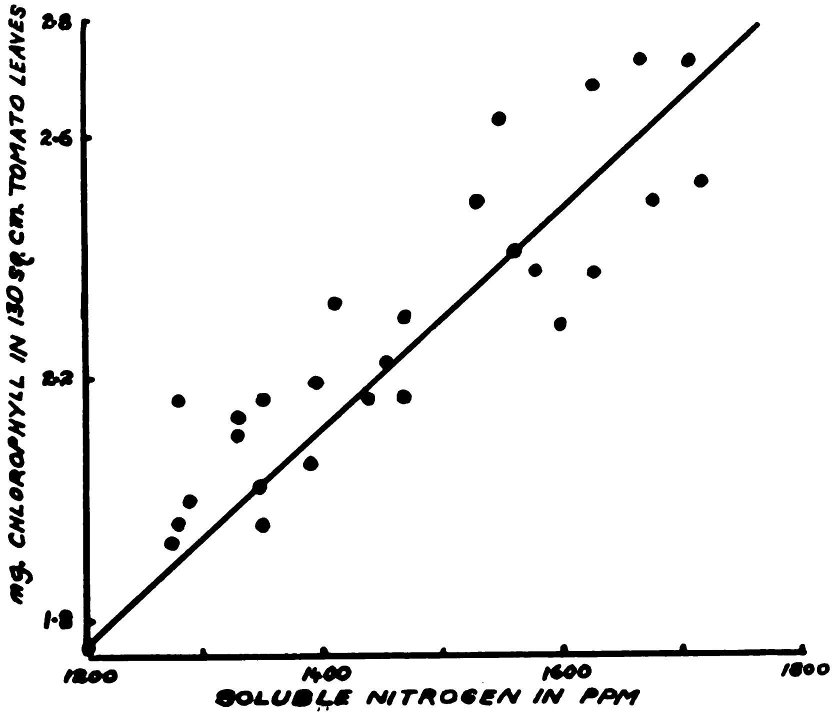


Figure 65. The relationship between soluble nitrogen and chlorophyll content in tomato petioles grown at Smithfield 1950. (Heaney, 1950)

iron, magnesium, sulphur and various other nutrients also induce chlorosis. The interaction of these other deficiencies will affect the practical value which might be attributed to the chlorophyll techniques.

A further obvious objection to the use of subtle color comparisons is that of their subjective nature, and judgments of color differences are as variable as the number of observers. This is especially important as the color variations correlated with adequate, deficient, or excess nitrogen are very slight. Judkins (1949) and Judkins and Wander (1950) attempted to overcome some of this error by using a photoelectric reflection meter. This, of course, removes the error due to variable interpretation of the color charts but does not in any other way improve the value of the technique.

There is no real value in this method for it is the estimation of a factor (chlorophyll) which is used to estimate a second factor (nitrogen) which is then used to estimate the third factor of the nutrient status of the crop, so that finally an estimation can be made of the fertilizer requirements of the crop. Any method involving so many successive estimations will be so subject to error as to be of little practical value.

XV. SUMMARY

Confusion in the theory of the responses of crops to fertilizer is a major factor obstructing agricultural progress. An understanding of the reasons for this confusion is necessary for the future improvement of the productivity of agricultural soils. Consequently, a detailed study of the original literature upon which present theories of fertilizer use are based is essential.

The overthrow of the humus theory by Liebig, and his recognition of the importance of mineral nutrients in crop production resulted in the initiation of our current concepts and increased interest in the nature of soil fertility and of cultural practices producing maximum crop yields. Although the problem of relating the numerous factors affecting nutrient concentrations in the soil and in plant tissues is very complex, a number of mathematical expressions relating these factors have been proposed. A study of the experimental data on which these expressions were based is essential to a correct understanding of fertilizer use.

The most important of these expressions, the Law of Diminishing Returns, in its implications and applications presents the most direct and practical method of studying efficient quantitative fertilizer use. This Law, however, has been severely criticized because it implies a mathematically expressed biological constant. This criticism does not seem justified because any method in which results of field trials, tissue tests, or other techniques are interpreted so as to permit

general field recommendations must imply such a constant whether or not it is mathematically expressed.

The applications of the Law have been replaced largely by tissue analysis at the present time, but the Law is based on a much sounder foundation theoretically than currently accepted procedures for the determination of the fertilizer requirements of crops. The techniques of Willcox, based on the Mitscherlich constant expressed in Baule units is a direct application of the Law. Bray, on the other hand, has introduced modifications which permit the application of the mathematical expression of the Law but does not imply the general acceptance of the constancy of a biological factor over widely different conditions. Techniques of this type are the only methods yet devised for the quantitative estimation of the fertilizer requirements of crops. In order to preserve our agricultural soils they should be given prime consideration in investigations of the use of fertilizers.

Various techniques have been suggested including biological methods of analyzing small samples of soil. These techniques cannot serve any useful purpose, except in special cases, until such time as it can be demonstrated that they are superior to chemical methods of soil analysis. Further, they cannot be used for the accurate quantitative determination of the fertilizer requirements of crops.

The techniques involving the use of the plant, leaf painting and injection, deficiency symptoms, and chlorophyll determinations can be used qualitatively only, even by the most experienced investigator, because of the difficulty of interpreting differences observed under normal and deficient conditions. It is also apparent that the most commonly used techniques based on the analysis of the plants, such as critical concentrations and ratios, foliar diagnosis, and triple analysis, cannot quantitatively estimate fertilizer requirements of present or future crops. Many of these methods are, however, of considerable value in studying the seasonal trends of crop nutrition and as rapid methods of conducting preliminary surveys of soil fertility over wide areas.

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