THE EFFECT OF CERTAIN MICRONUTRIENT ELEMENTS ON THE GROWTH AND YIELD OF PINEAPPLE PLANTS

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I—INTRODUCTION *

The pineapple was, almost certainly, native of Brazil (5), and very probably the Caribs or Arawaks took some to Puerto Rico before the sixteenth century.

The pineapple (66) is related to the bromeliads and air plants, and it can absorb nutrient constituents through its leaf axils and long-barbed and barbless bayonetlike leaves that protrude from numerous whorls on the main stalk. The so-called pineapple fruit is an aggregate of many individual fruits with their fibrous juice pulp surrounding the core. Pineapple plants are propagated under field conditions from three asexually produced vegetative organs, known respectively as suckers, slips, and crowns. (See figure 1). In some varieties each individual fruit has a number of seeds which can be used for propagation but the most common practice is to plant slips or suckers. The slip resembles a miniature plant and is produced near the base of the fruit. The suckers resemble the slips, but are larger. The crown slip is produced at the top of the fruit; it is rarely used for propagation.

After one crop of pineapples is harvested a second crop, or ratoon, is produced from a new plant resembling a sucker which is formed at the base of the main stalk in contact with the soil.

Pineapple plants respond to good soil that must be well managed, not too alkaline or wet, and well aerated. The acreage in pineapples in Puerto Rico is relatively low compared to that in other crops. The total value of the export crop is high, although very low if compared with sugar cane. Practically all fresh and canned pineapples exported go to the United States where they are considered of good quality and get the highest prices.

The pineapple growers of Puerto Rico claim that the yields from native slips show a yearly lowering in production, as if they were degenerating or as a result of a "rundown" of the stock. Slips imported from Cuba when first planted in Puerto Rican soil, show considerably greater vigor than native slips. They give higher yields than the native stock brought originally from Cuba. It has been observed also that Cuban stock will show signs of decreased vigor and yield after a few years of propagation. Since pineapples are,

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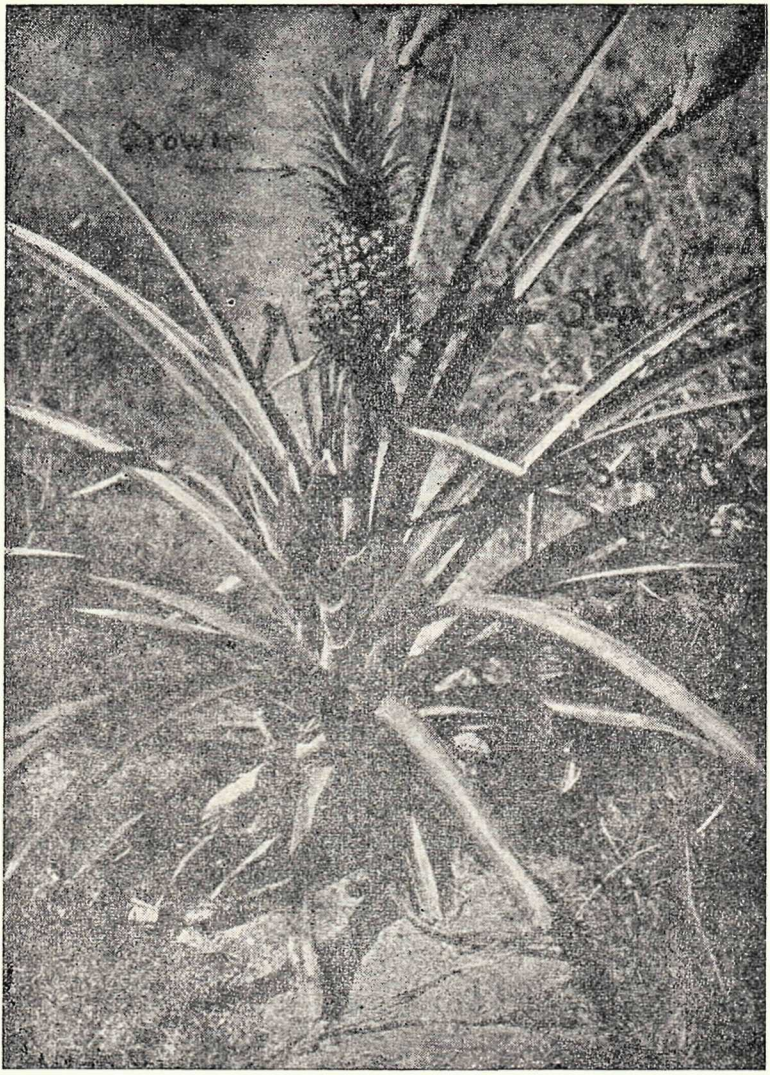


FIGURE 1.—A pineapple plant showing the position of slips, suckers and crown.

as explained above, propagated under field conditions from asexually produced vegetative organs, the degeneration is probably not on a genetic basis. On the other hand, no degenerative disease such as mosaic has ever been found. Hence, there is evidence of the possibility of a nutritional disturbance, perhaps in the micronutrient elements, that may produce vegetative organs with low reserve nutritional elements. These, on being planted in a soil causing such disturbance, may bring about the above-mentioned apparent degeneration of the stock.

This nutritional derangement may have been produced by changes in the soil as a result of faulty cultivation methods, fertilizer practice, constituents of the parent material of the soil, poor crop rotation and conservation. It is claimed, however, that slips planted on virgin soil or on soils not used before for pineapple planting, do not show this degeneration taking place so rapidly as on soils previously used for several years under continuous pineapple production.

Pineapple chlorosis has also been known in Puerto Rico from early times, as shown in works done by Gile (25) since 1911, and by Henricksen (31, 32) in 1925. Similarly, pineapple chlorosis was also known in Hawaii as shown in the experiments of Kelly (50, 51, 52, 53) from 1909 to 1914, also of Wilcox and Kelly (85) in 1912, and of Johnson (45, 46, 47, 48, 49) in 1916–1924.

This yellowing is a source of considerable loss. Spraying of the chlorotic pineapple plants with iron sulfate solution has been practiced in Puerto Rico and in Hawaii, this being an adequate remedy for this malady.

These problems were brought to the attention of Schapelle (67, 68, 69, 70, 71) in 1939, at the Agricultural Experiment Station of the University of Puerto Rico. He set up experiments in order to examine the effect of different nutrient elements under different treatments in the field and under different concentrations of macro and micronutrient elements in solution cultures. His investigations on the nutritional aspect of these pineapple problems in Puerto Rico were followed by the experiments of Hopkins, Pagán, and Ramírez-Silva (40). The experiments presented in this dissertation are part of this series of greenhouse and field investigations on the above-mentioned pineapple problems. The effect of the micronutrient elements: iron, manganese, aluminum, boron, copper and zinc, on pineapple growth and production was examined by means of different treatments in solution cultures.

In accordance with Hoagland (34) who considers the terms "minor elements," "rare elements," and "trace elements" as inappropriate, we use the term he suggested, that is, "micronutrient" elements. Among these are iron, manganese, aluminum, boron, zinc, copper, etc., called micronutrient elements because of the minute concentrations in which they are found in plants. Very minute quantities of them are required to perform their essential functions in plant nutrition. Also, small amounts of them are enough for plants to restore themselves from the specific abnormalities and impaired physiological functions caused by their deficiency in the nutrient medium.

In contrast with these "micronutrient" elements there is a group of elements once called "essential" elements and now usually called "major" elements in plant nutrition. Among them are: nitrogen, potassium, phosphorus, calcium and magnesium. As they are found in greater quantities, they will be referred to as "macronutrient elements." In this sense, the idea of either major or minor, or of essentiality, will not be erroneously conveyed. It may be the case that the so-called "minor elements" be of "major" importance in some cases of plant nutrition. No element is "major" or "minor"; they may be either macronutrient or micronutrient, according to their concentration in plants.

The artificial culture method has been found to be a valuable tool in plant nutrition research. This method has been termed "hydroponic," "water culture," "sand-culture," "gravel-culture," "solution-culture," etc. The term "water-culture" is widely used, but it is not as accurate as the term "solution-culture," since it is a solution of nutrient substances that is used in this artificial method. Of course, when sand or gravel is used in the culture media, the corresponding term of sand or gravel culture is appropriate.

II—REVIEW OF LITERATURE

A. *The growth of plants in artificial media in relation to the study of plant nutrition*

In order to study the role played by nutrient elements in the plant, and in an attempt to separate this from the problem of the availability of the nutrient elements in the soil, that is, from the soil-solution problem, the artificial culture method has come to be a valuable tool in this field of research.

The method of growing plants in nutrient solutions has been used as the best known means of controlling the concentrations, pH,

and proportions of nutrients fed to plants in experimental treatments. The convenience of this method, as well as the objections to it, depend upon the specific problem under experimentation.

Since the earliest recorded experiments with solution cultures by Woodward (61) in 1699, and later on by procedures developed by Sachs, Knopp, and Nobbe, from 1859 to 1865, the modifications introduced by means of different formulas and techniques have made this medium of growth for plants very useful for the purposes of fundamental experimentation and preliminary trials for field work and research.

Hoagland (34), on his discussion of the topic of growth of plants in artificial media in relation to plant nutrition, taking in consideration the dominating phases of this subject, appraises the far-reaching scientific significance of this method and its great service in understanding the nature of the soil-plant system.

According to Miller (61), nutrient solution formulas have been proposed by Tollens in 1882, Schimper in 1890, Pfeffer in 1900, Crone in 1902, Tottingham in 1911, Shive in 1915 (73), Hoagland in 1920, and many others. The osmotic pressure of the solutions has been considered, also, the relationship between proportion of salts, the growth effect of light and temperature, the relative absorption of ions, and the functions of the elements essential to plant life. Miller (61) shows a list of useful formulas for nutrient solutions.

Shive's (73) three-salt solution of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$ has been the basis of much experimentation by him and others. They have considered the osmotic pressure best fitted to certain crops, the supply of essential elements, and the relation of the elements as found in soil extracts. The formulas derived from Shive's three-salt solution have been classified in series according to osmotic pressures and the molecular proportions of the dissolved components. They are designated by serial numbers when reference is made to them in experimental work.

For experimental purposes it has been recommended that renewal of solutions be made at least every month, and if possible continual removal, aeration by bubbling air, adequate temperatures, uniformity in light supply, and the use of pure chemicals and distilled water.

The works of Lowhwing (54) in 1932, of Clark and Shive (19) in 1932, and of Bryant (14) in 1934, show that nonaerated nutrient solutions tend toward a greater ratio of roots to tops. This suggests the idea of growing plants for a certain period of time, in the beginning without aeration, later on aeration is applied as required for normal crop production.

The preparation of separate solutions to prevent precipitation of concentrated salts upon being mixed was recommended by Tottingham (81) in 1914.

B. *Researches on nutrition of pineapple plants as related to micronutrient elements*

During the last two decades mineral deficiencies in plants have been the subject of much experimentation in the field of plant nutrition. Nevertheless, the question of toxicity due to excess of certain nutrient elements has not been emphasized to a great extent from the standpoint of practical plant culture. The balancing, additive, and antidoting effects between certain nutrient elements have been the subject of still less investigation. Examples of this toxicity are shown by manganese in pineapple plants, as reported from Hawaii by Kelly (50, 51, 52, 53) in 1908–1912, by Johnson (45, 46, 47, 48, 49) in 1916–1924, by Wilcox & Kelly (85) in 1912, and by McGeorge (58) in 1923.

In 1911–1916, Gile (25, 26) and Gile and Ageton (27) found a type of chlorosis of pineapples in Puerto Rican soils that contained large quantities of calcium carbonate and no excessive manganese. They called such chlorosis, lime-induced chlorosis. They noticed that spraying with solutions of iron salts resulted in the restoration of the green color. Johnson (45) in 1916, however, traced the pineapple chlorosis as due to manganese toxicity with a condition of low or no iron absorption, and found that spraying with iron sulphate solution counteracted the effect of high manganese present in the soil. On the other hand, Gile (25) found that this spraying of plants had to be repeated so often that it was not economically practicable.

As we are dealing with the general term "chlorosis", it must be explained here that it stands for a diseased or unhealthy condition of the leaves shown by the loss of the green coloring matter. This yellowing may be due to several factors such as lack of the nutriments producing or intervening, directly or indirectly, with the process of chlorophyll formation, to poor drainage or bacterial effects. After the leaves become strongly chlorotic necrosis ensues, showing extensive black areas.

Schapelle (67, 70, 71) in 1940, attempting to elucidate the problem of the apparent degeneration of pineapple stock and the increasing chlorosis of pineapple plants grown in Puerto Rican soils,

made studies on the effects of macro and micronutrient elements on the growth, yield, chlorosis, and quality of fruits of pineapple plants. He found that potassium was an important factor in the quality of the fruits, that plants responded better to ammonia than to nitrate nitrogen. He noticed that chlorosis was a cause for lower yields. He found profitable the use of frequent spraying with ferrous sulfate solutions. In experiments with pineapples grown in nutrient solutions he used with success a modified form of the solution used by Sideris, Krauss, and Young (74). He had demonstrations of best growth under the conditions of the experiment at a pH around 4.5, that manganese and zinc tended to produce chlorosis, that aluminum and boron tended to counteract the effects of manganese and zinc, and that copper, added at the concentration of 2 p.p.m. controlled a root fungus that caused stunting of the pineapple plants.

After this work of Schapelle (67, 70, 71), Hopkins and co-workers (40) in 1944 started their work along this line of research not only using the pineapple plants but also beans and tomatoes. They found: (*a*) high amounts of water-soluble manganese in soils from pineapple growing areas, (*b*) observed the chlorosis of pineapples grown in those soils, and that beans would not grow on them on account of severe chlorosis, (*c*) that in spite of the correction of chlorosis in pineapples by spraying with ferrous sulphate, certain abnormalities occurred, such as reduction in size of the plant and development of more red pigments than normally, (*d*) slips imported from Cuba, of the variety "Red Spanish," produced larger and greener plants than slips from the same variety from Puerto Rican plants; but after being planted for two generations in Puerto Rico, plants originally from Cuban slips reverted to the inferior type, (*e*) many fruits produced in these soils were affected with "short top," that is, a very short crown in the fruit which appears to be brought about by high soluble manganese under high intensity of light. The pineapple soils have pH as low as 3.8 which brings manganese into solution. Tomato plants used for preliminary experiments showed high correlation of maximum growth with the wider ratio of Fe to Mn. Tomato plants show a great sensitivity to manganese, requiring a large ratio of Fe to Mn for normal growth, flowering and production. It was noticed that the reserve of iron in seeds or propagating organs supplied the plant during a certain period of the early growth with the iron necessary to antidote the manganese in the nutrient solution, in either soil or solution culture.

C. *Researches on plant nutrition as related to micronutrient elements used in these experiments*

The micronutrient elements used in this experiment: iron, manganese, copper, zinc, aluminum and boron, have been the subject of an immense amount of research. To review and make a thorough study of all this literature would be outside the scope of this dissertation, but a chronological review of investigations having a bearing on this experiment is necessary. In the following pages such a review is made, taking in consideration that some references have already been mentioned in previous sections.

1. *Iron*

Iron is essential for the growth of plants. In them it is almost universally present in small quantities. Molisch in 1892, as quoted by Miller (61), found that most iron in plants is in a combined, insoluble form. Iron apparently occurs in plants in two forms, namely, the "active and inactive", sometimes also called "available and non-available".

Although plants deprived of iron show marked chlorosis, it has been definitely proved by Willstatter and Stoll in 1913 that iron does not enter into the composition of chlorophyll. Gile and Carrero (28, 29, 30), studied in 1914–1916 the iron requirements of rice, pineapples, and other crops. They noticed that iron, after being transported to the leaves, is immobile, and that colloidal iron is not absorbed by plants. Warburg (83) proposed in 1925 the theory that iron is the oxygen-carrying component of the respiration ferment. Hopkins (36) 1930, believed in the important role played by iron in the cellular processes involving biological oxidation.

According to Miller (61), the researches made by Oddo and Pollaci in 1920, Deuber in 1926, and Pollaci in 1935 show a possible explanation of this role of iron as a catalytic agent in chlorophyll formation, catalyzing the formation of the pyrrole nucleus which is the center of the chlorophyll complex.

Gile and Carrero (30) in 1920, and Willis and Carrero (87) in 1923, agreed in considering that lime-induced chlorosis is due to a depression in the available iron. Rippeal (65) observed in 1923, that chlorosis produced by manganese in the form of soluble salts in solution cultures, was overcome by increasing the supply of iron. He concluded that manganese interfered with the iron within the plant and not with its absorption.

Gericke (24) noticed in 1923 that iron-deficiency symptoms were more acute in plants under lights of high intensity than in lights

of low intensity. This should be examined according to the results of experiments made by Hopkins, Pagán and Ramírez-Silva (40) in 1944. They noticed phototropic effects of manganese in the absence or low level of iron. It is possible that Gericke was taking for iron deficiency what may be called manganese toxicity.

Burk, et al (16), made a report on the experiments made on metallic humates up to the year 1931 and, with reference to iron humate, they pointed out important facts that show the advantages in using humic acid for furnishing iron in a permanently soluble form in soils and in artificial culture media. The humic acid itself does not act by increasing directly the availability of constituents added to or present in the culture medium; or by activating toxic metabolic products; or by affecting surface tension, viscosity, and potential differences between culture medium and organism, or the oxidation potential of the media; but provides iron for growth and nutrition in a more highly available form.

Horner, Burk and Hoover (41) explained in 1934 a method of preparing humate metals from the salts of the corresponding metal and humic acid made from sucrose. This method is simple and provides a soluble form of iron at a very wide range of pH values. It is of great utility in solution cultures where available iron is required for treatments at different pH values, and also when the culture solution is subjected to variations in pH. These humates are stable in alkaline, neutral and moderately acid media, and are not precipitated by phosphates.

The role of iron in plant metabolism should be related to enzymes called iron enzymes, namely, catalase, peroxide, citochromes, indophenol oxidase, and others.

2. *Manganese*

Manganese is widely distributed in nature. It is an essential element for plant growth and functions in the synthesis of chlorophyll and carbon assimilation. According to McHargue (60), previous to 1774 the compounds of this element were confused with those of iron. In that year Scheele discovered that the metal found in pyrolusite, manganese, is altogether different from iron. Gauhn isolated the metal shortly afterwards. Thus Scheele started the work to investigate the functions of manganese in plant economy and its occurrence in soils. Ninety years later Sachs was able to prove that plants assimilate manganese and that it cannot replace the functions of iron in plant growth. By 1894 Bertrand had already determined the chemical composition of the sap of the lac tree and found that

its ash contained 2.5 per cent of manganese. The importance of manganese, in spite of the small content of it in plants, was by that time already recognized.

The works of McHargue, in 1914, showed that manganese and iron play an important role in chlorophyll formation and that this element may be toxic to plants at certain levels of concentration in the nutrient solution.

Sachs in 1865 noticed yellowing and etiolation of leaves in an attempt to substitute manganese for iron. Field observations on pineapples grown in manganiferous soils show that the interior of the fruits have a whitish appearance and usually contain excess acidity. Lime application makes manganese toxicity worse.

In 1908 Fukutome (22), from Tokyo, in his experiments on flax, noticed beneficial action when iron sulfate was added to manganese treatments. This is sometimes termed antagonism, counteracting effect, or antidoting action. Masoni (55) also observed in 1911, in his experiments with corn and lupines, the beneficial counteracting effect of iron on the detrimental action of manganese.

Stocklasa, J., (79) noticed in 1911 that aluminum and manganese have an additive beneficial effect on the growth of several plants.

Wilcox and Kelly (85) in 1912, in their experiments with pineapples, grown in Hawaii, made a thorough study of the physiology and chemistry of pineapples as affected by manganese toxicity. They do not mention the antidoting action of iron. They observed the mechanism of chlorosis, and the bad effects on roots due to excessive soluble manganese in the nutrient solution.

Kelley (53) in 1914 indicated that manganese may have an effect upon soil so as to bring about the mobilization of calcium and magnesium, and that it may stimulate the oxidation going on within the plant and in the soil.

The antidoting action of other cations as Ca, K, Na, and Mg on Mn was observed by McCool (56) in 1913. It may be that the beneficial effect of manganese is only due to the association or counteraction of other cations. He noticed that the deleterious effect of manganese varied inversely with the intensity of light.

Funchess (23) noticed in 1918 that nitrates and nitric acid increased the toxic concentrations of manganese.

Hopkins (36, 37) in 1930 reported the increase of growth of chlorella six hundredfold by addition of manganese to the nutrient solution, and he suggested that manganese functions in an indirect

manner in plants by its action upon the oxidation of iron. Hopkins (39) also noticed slow growth of *Lemna Minor* with iron and no manganese.

Bortner (11)) in 1935 observed chlorosis produced in tobacco plants by manganese in concentration of 15 p.p.m. in the nutrient solution, and noticed the antidoting effect of phosphorus. On the other hand, Sherman and Harmer (72) in 1941 found symptoms of manganese deficiency in oats manifested by specks and chlorosis which were prevented with the application of manganese.

Hopkins, Pagán and Ramírez-Silva (40) in 1944 found increase in growth of beans and tomatoes when manganese in soil was immobolized in the soil, and still more growth if iron humate was added. Marked detrimental phototropic action was noticed with excess supply of manganese, and in this effect, the antidoting action of iron was very effective.

Arnon (3), in his report of a review of the research done in mineral nutrition in plants for the year 1943, presents the following hypothesis offered by Somers and Shive: The cells of plants can tolerate only a certain definite concentration of iron which is of ferrous valence. The function of manganese is to regulate the concentration of ferrous ion. Manganese ions oxidize ferrous to ferric ions which precipitate in the form of "ferric-phosphorus organic complex," rendering iron physiologically inactive.

3. *Copper*

Copper is widely distributed in plants in considerable quantity; but according to research done it has stimulating action only at very low concentration, and it is generally toxic to green plants. The content of copper in pineapple fruits, according to an analysis shown by Beeson (8), is about 8 milligrams per kilogram. McHargue (59) reported in 1925 the copper content in various plants and plant parts as ranging from traces to 46 parts per million.

Plants respond to adequate copper treatments, as explained by Miller (61), showing increase in vigor, yield, quality, and control of chlorosis. He quotes the work of Maquene and Demossy in 1920 where they show that copper is found in greatest abundance in cells that are active in growth, and that its translocation is controlled by metabolic processes.

In the raw peat soils of the Everglades of Florida, Allison, Bryan and Hunter (2) in 1927 found copper to be a specific limiting factor, giving response in growth and production in a remarkable way when 30 and 50 pounds of copper sulphate per acre were applied to the soil.

Miller (61) gives a review of outstanding research done up to 1945 with copper and he points out the beneficial effect in some crops like onions, and in some fruit trees, when used as a fertilizer or as spray.

Hopkins (38) found in 1933 no increase in growth attributable to additions of copper to culture of *Lemna* and *Chlorella*.

Skoog (76) claims that copper may be related to the respiratory process.

The essentiality of copper for many species has been recently demonstrated by Hoagland and others, as reviewed by Petrie (64).

Felix (21) experimented in 1927 with onions and lettuce on the reclaimed muck lands of Central New York and found copper to be a limiting factor. Lack of copper produced a specific anatomical abnormality known as "rabbit ear". Onions fertilized with Cu SO_4 produced better colored and thicker scales.

It should be noticed that according to the findings of Waddell and Steenbock (82) in 1929, copper is regarded as a necessary adjunct to iron in the regenerated of anemia in animals. This may be considered as parallel to its effects in chlorophyll regeneration. Schappelle (71) claims that copper at a rate of 2 p.p.m. in the nutrient solution controlled a root fungus that caused stunting of the pineapple plant.

The so-called copper enzymes and copper containing proteins should, in part, show the relation of copper to respiration and oxidation processes in plants.

4. *Zinc*

The effect of zinc on plant metabolism is one of the most interesting phases of the field of plant nutrition.

The essentiality of zinc in corn plants was recognized, according to Miller (61), by Maze in 1915, and by Somer (77) in 1928, who noticed abnormalities in the growth of buckwheat and sunflower, and in the flowering of *Vicia faba*.

Bertrand and Andreitcheva (9) in 1933 considered the zinc content correlated with a high chlorophyll content.

According to Miller (61) zinc deficiency caused the plant disease called "little leaf," as demonstrated by works of Chandler, Hoagland and Hilbard in 1933, on peaches, apricots, tobacco, squash, corn, mustard, tomatoes and other plants.

Mowry and Camp (62) in 1934 found that spraying with zinc sulphate, or its addition to soil, made tung trees recover from bronzing.

Chapman, Vanselow and Liebig (18) produced mottle leaf by omitting zinc from culture solutions. The "mottle leaf" disease in citrus orchards is caused undoubtedly by zinc deficiency. Hoagland (34) describes the fight against this disease in California during twenty years, and says that not a good clue to its cause has been found yet.

Spraying with appropriate zinc compounds, he says, is effective and commercially practical. In Florida, the disease on pecans called "rossette," in Australia a disease of pine trees, and in Hawaii pineapples showing distorted blades, are conditions remedied by zinc sprays. It has been observed that for various reasons zinc in the soil is sometimes not made available to plants. It might be fixed to soil colloids. Certain soil organisms are a recognized factor in the non-availability of zinc by competing with the plant. Some plants have more capability than others for absorbing zinc when it is in a low supply from nutrients. Certain plants like alfalfa show a high ability to absorb zinc from the nutrient media.

Hoagland (34) considers that the quantitative requirement of zinc, as well as the deficiency symptoms, are in part governed by climatic or seasonal factors. High intensity of light aggravates the zinc-deficiency symptoms. (This is an important factor in tropical agriculture.) According to Hoagland (34), and the work of Skroog (76) about this phototropic action of zinc, auxin formation in plants is connected with zinc nutrition. Auxin breakdown is promoted by short-wave light.

The translocation of zinc is effected through the breakdown of the zinc protein compounds under the action of reduced light, thus releasing the zinc which is transplanted to regions of active growth. So, zinc is directly or indirectly connected with protein synthesis in plants. As it does not undergo reversible valence changes, its action in oxydation-reduction systems, if any, must be an indirect one, or due to its influence upon oxidizing enzymes and its interrelation with iron. Hence, zinc is related to respiratory processes and the maintenance of normal concentration of auxin in tissues as claimed by Skoog (76).

Thatcher (80) believed that copper and zinc are mutually counterbalancing catalyzers for hydrogen exchange, as shown by their strikingly opposite effect upon reversible oxidation-reduction reactions of both glutathione and ascorbic acid.

Leaf chlorosis in grape fruit trees in Puerto Rico, resembling the "mottle-leaf" in California and the "frenching" in Florida, was successfully controlled by Jensen (44), at the Federal Agricultural

Experiment Station of Puerto Rico, by spraying with zinc sulfate solution. Pineapple, under certain conditions of zinc deficiency, according to Nightingale (63) in 1942, show characteristic spots or blisters from which they recover by spraying with zinc sulfate solution.

As quoted by Beeson (8), a pineapple fruit analysis showed 20 milligrams of zinc per kilogram. Willis (86) showed that during the last fifteen years experimentation on this micronutrient element has demonstrated the essentiality of it for the normal growth of green plants, and its deficiency as causing characteristic chlorosis, mottle leaf or frenching, and "rossete" or "little leaf" in fruit trees.

5. *Aluminum*

Aluminum is very abundant in soils. It has been found in all plants that have been analyzed but, as a rule, the percentage of aluminum in plants is very low. It may thus be considered a micronutrient element. Grains and vegetables analyzed by Meyers and Voegtlin, as quoted by Miller (61), contained from 0.045 per cent dry basis in wheat flowers, up to 0.996 per cent in cotton seed. As to the role of this element in the growth and production of plants, Miller (61) reviewed the works of Yamano in 1905, who found injurious effects caused by 0.2 per cent ammonium alum on wheat and rye grown in nutrient solutions, and 0.8 per cent to be a fatal dose. Prianishnikov, in 1911, grew wheat, oats, barley, peas and buckwheat in sand cultures fertilized with aluminum phosphate and calcium carbonate alone. Baguley found in 1912 the iron and aluminum phosphate combination to be better. Kratzman observed in 1914 toxic effects of 0.005 per cent concentration of aluminum salts. Others, in experiments done after these, have found toxicity of aluminum salts at certain low levels of concentration in the nutrient media.

The soil-plant aspect of aluminum has been studied more than others. In fact, considering soil work, aluminum shows a great complexity in relation to other elements, it being one of the principal components of soils and soil colloids. Its availability is greater at lower pH.

Schapelle (71) showed beneficial action and recovery from injuries brought to pineapple plants grown in nutrient solutions lacking in aluminum. But Abbot (1) found in 1913 aluminum to be a toxic agent in the marsh regions of peaty sand, and also in culture solutions.

Barnette (6) in 1923, using solution cultures and upon observing the toxic effects of aluminum ions, determined that such toxicity was not due to acidity per se, but to the hydrolysis of aluminum salts.

The function of aluminum within the cell of the plant is ignored yet. Miller (61), reviewing the work of Fluri in 1907, mentioned the consideration that aluminum has an indirect effect in starch disappearance from the cell by increasing protoplasm permeability, diastatic action, and slowing photosynthesis.

Stocklassa (79) found in 1911 that aluminum and manganese together stimulated growth of several species of plants.

Blair (10) observed in 1923 the detrimental effect of soluble aluminum in soils upon roots. McGeorge (57) reported in 1925 that he noticed toxidity of aluminum on roots, in culture solutions, at the pH of acid soils. Haas (35), nevertheless, observed beneficial effect of aluminum in solution cultures of lemon, leafy-twig cutting, when a good supply of phosphorus is present.

Burgess (15) determined, 1923, the availability of aluminum in some soils. At pH 4 to 5 he found 388 parts per million while at pH 5 to 5.8 it was lowered to 36 parts per million.

Arnon (3), in a review on plant nutrition for the year 1945, says that Liebig, Vanselow and Chapman claim that they found that aluminum at low concentrations counteracts copper toxicity in citrus grown in culture solutions. It seems, according to them, that the case of the beneficial action of aluminum depends on its action against the toxicity of copper.

At high concentrations (2.5 to 5 p.p.m.) aluminum gave a curious stimulation of root growth accompanied by depression of top growth. In the absence of aluminum, excessive copper caused a brownish appearance in citrus roots and short swollen laterals which gave the roots a dwarfed, knotty, and unhealthy appearance. Top growth often exhibited iron chlorosis.

6. *Boron*

Boron is the micronutrient element that has received the greatest attention; and still the mechanism of the function of this element in plant growth is hidden to us. As claimed by Chapman (17), up to now we have not passed beyond the knowledge of the effects of its deficiency upon the meristematic tissue, and its interrelation with calcium. There is a marked similarity between the symptoms of calcium and boron deficiencies. Boron is widespread in the plant kingdom, it probably occurs in all green plants. Since 1857 its

presence was detected in plants by Whittstein and Apogier (88), followed by Baumert (7) in 1888, Hotter (42) in 1890, and Jay (43) in 1895, who analyzed various plants and believed in the universality of boron in the plant kingdom.

The study of the influence of boron upon plant growth called the attention of many investigators. Sand, solution, and soil-culture experiments were made by Augulhon (4) in 1910 and by Brenchley (12) in 1914. Their findings point toward the beneficial effect of boron when supplied to the plant in the right amount. The work of Warington (84) in 1923 on boron compounds on beans, in solution cultures and field experiments, was the beginning of the consideration of the mechanism of boron nutrition in plants. She pointed to the catalytic action and its effect on meristematic tissues.

The effect of boron nutrition on nodule formation in leguminous plants was studied by Brenchley and Thornton (13) in 1925. They found beneficial action on the production of nodules as due to the anatomical conditions of the plants with good boron nutrition. From there on, the boron requirements for nutrition of many crops have been studied, as well as the symptoms of deficiency and toxicity for different plants.

Miller (61) reviews the studies made on boron deficiency, and from the works of Warington in 1926, points out that disintegration of phloem and ground parenchyma, poor development of the xylem, and hypertrophy, discoloration, and disintegration of cambial cells occur when boron is omitted from the nutrient solution. Growth is arrested in the meristematic tissues of root tips, as found by experiments of Sommer and Sorokin in 1928. The effects of boron deficiency in tomato plants, "the guinea pig of the plant nutritionist," are very noticeable: death of terminal growing points of stem, characteristic brittleness of the stem and petiole, and poor brownish roots.

That the function of boron cannot be performed by other elements was found by Warington in 1927. She tried fifty-two other elements. Sugar beet and alfalfa are plants very much affected by lack of boron, and show specific deficiency symptoms. Boron, indeed, will show harm on plants when supplied in excess, bringing about chlorosis. This is probably due to its action against the solubility of iron, as claimed by Rodríguez in 1935. (66[a]) An effect of boron toxicity is stimulation of undifferential cell division causing abnormal growth in the regions of its maximun effect.

The essentiality of boron for higher plants is no longer open to dispute. Brenchley (12) and Warington (84) proved that boron

is absolutely indispensable for satisfactory growth of many crops. Both report on the retardation of the development of meristem tissues and discoloration of the stem in plants as specific symptoms of boron deficiency.

Eaton (20) made in 1944 careful observations on the nutritional effects of boron, and noticed that in most plants it accumulates in soluble but largely immobile form. He suggests that boron becomes attached to some large molecule which, though soluble, is unable to pass through the plasma membrane of the mesophyll cells. Owing to this immobility of boron in leaf tissue, plants may show symptoms of boron excess in old leaves, and yet not be supplied with excess. Thus, there may be an overlapping of beneficial and toxic effects in the same plant. High-light intensity may be responsible for its immobility in the leaves. Boron deficiency is aggravated by increase of calcium in the nutrient solution, but its toxicity is lowered. Variations in potassium concentration affect indirectly boron deficiency and toxicity due to the effect of potassium on calcium absorption.

III — OBJECT OF THE WORK

It is the object of these experiments to study:

1. The effect of iron, manganese, zinc, copper, aluminum and boron on the growth and production of pineapples.
2. The antidoting effect of iron on manganese toxicity.
3. The mutual action of these micronutrient elements, and their deficiency or toxicity as affecting root growth, leaves, flowering, fruiting, yield, and quality of the crops.
4. The causative agents of pineapple chlorosis.
5. To verify the data already obtained in other experimentation in this field.
6. To guide future experimentation with pineapple plants along this line.
7. To suggest possible methods to remedy injuries on the pineapple plants as caused by malnutrition of the plant.

IV — EXPERIMENTAL

A. General Plan

Solution-culture methods were used in this experiment with a formula of macronutrient elements already found to be good for growing pineapple plants. The facilities of the greenhouse and hydroponic equipment of the Agriculture Experiment Station of the University of Puerto Rico (Fig. 2 and 3) as designed by Schapelle (71) were used. These experiments are a part of a research project of this Station.

The experiments consisted of two series of treatments. One series had nine treatments of micronutrient elements, to study the individual effect of the micronutrient elements when added to the culture solution. Treatment number one had no micronutrient elements added. This treatment showed the combined effect of all the reserve micronutrient elements in the planted slips. It served as a check on the other treatments. Treatments 2, 3, 4, 5, 6, and 7 corresponded, respectively, to additions of iron, manganese, boron, copper, zinc, and aluminum as the only micronutrient elements added to the nutrient solutions. These showed the beneficial effect of the presence of these elements, or their toxic effect, when acting independently at the concentrations added, as compared with treatment 1, to which no micronutrient elements were added. The eighth treatment contained all the micronutrient elements in the concentrations used in the previous treatments. This treatment was another check on the other six treatments. Treatment 9 was the same as 8 except that copper was not added. This showed the effect of lack of copper and was intended to check the results on root injury shown by lack of copper in the experiments of Schapelle (71). This treatment may be used to examine the effect of copper added in treatment 5 and, from this, infer its effect shown in treatment 8 where all micronutrient elements were added, and those of the following series.

Treatments 1 to 9 were designated as follows:

Treatment 1 as –ME (no micronutrient elements added)
Treatment 2 as Fe (iron added)
Treatment 3 as Mn (manganese added)
Treatment 4 as B (boron added)
Treatment 5 as Cu (copper added)

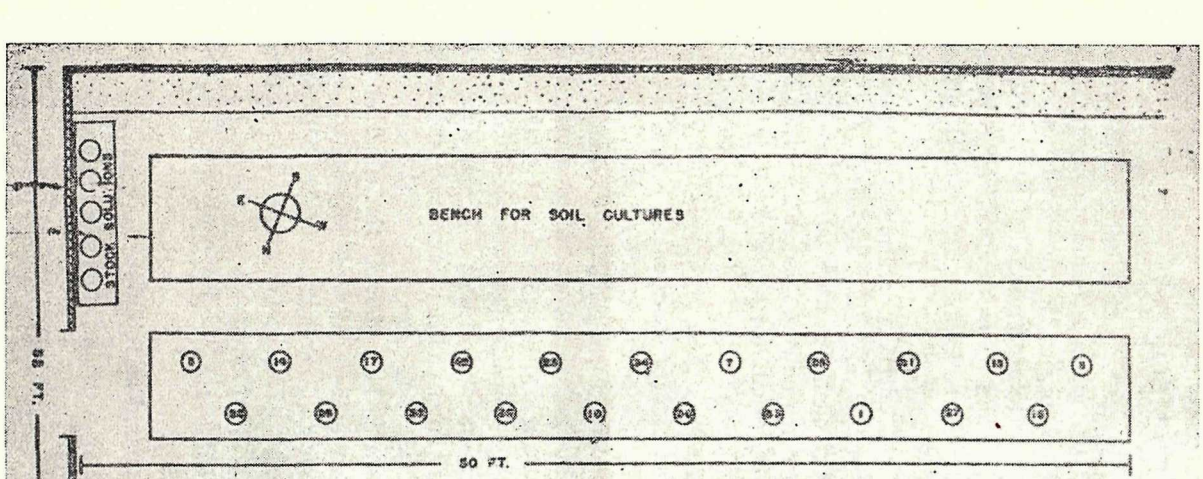


FIGURE 2.—Floor plan of the greenhouse showing the arrangement of the solution cultures. The numbers and position of the pineaple plants are shown within the circles.

Treatment 6 as Zn (zinc added)
Treatment 7 as Al (aluminum added)
Treatment 8 as ME (all micronutrient elements added)
Treatment 9 as –Cu (all micronutrient elements minus copper)

The series of treatments 10 to 14 was intended to study the antidoting action of iron against the chlorosis-producing effect of manganese. For this purpose 5 p.p.m. of manganese were added to each one of the five treatments, and a different level of concentration of available iron was added in each. So, treatment 10 had no iron added, number 11 had one p.p.m. added, number 12 had three p.p.m., number 13 had five p.p.m., and number 14 ten p.p.m. All these treatments were supplied with 2 p.p.m. of copper to prevent root injury as reported by Schapelle (71). The micronutrient elements: boron, zinc, and aluminum, were added also, in a concentration of one half p.p.m. each, in order to prevent deficiency of these elements. Treatment 2 with 5 p.p.m. Fe added as the only micronutrient element may also be considered as a member of this series for the purpose of the study of the antidoting effect of iron. The study of the results of the treatments 1, 3, 4, 5, 6, 7, 8, and 9 will throw light on the study of the series of treatments 10 to 14 and viceversa.

The effect of aeration of the culture solution was studied. During the initial period of growth the solutions were not aerated.

For the purpose of this study, observations and data on roots, plant growth, chlorosis, flowering, fruiting, yield, and quality of fruit were taken.

B. Methods

1. *Nutrient solutions*

Pineapple slips of the variety "Smooth Cayenne," 12 inches long and selected for uniformity, were "planted" in culture solutions. All the treatments contained the following concentration of macronutrient elements:

SALT	*Grams per liter*
$K H_2 PO_4$	0.1316
$Mg SO_4 7H_2O$	0.4100
$Ca(NO_3)_2 4H_2O$	0.4720
$NH_4 NO_3$	0.1260
K_2SO_4	0.1657

which furnish:

112.1 parts per million of K
30.0 parts per million of P
40.5 parts per million of Mg
80.1 parts per million of Ca

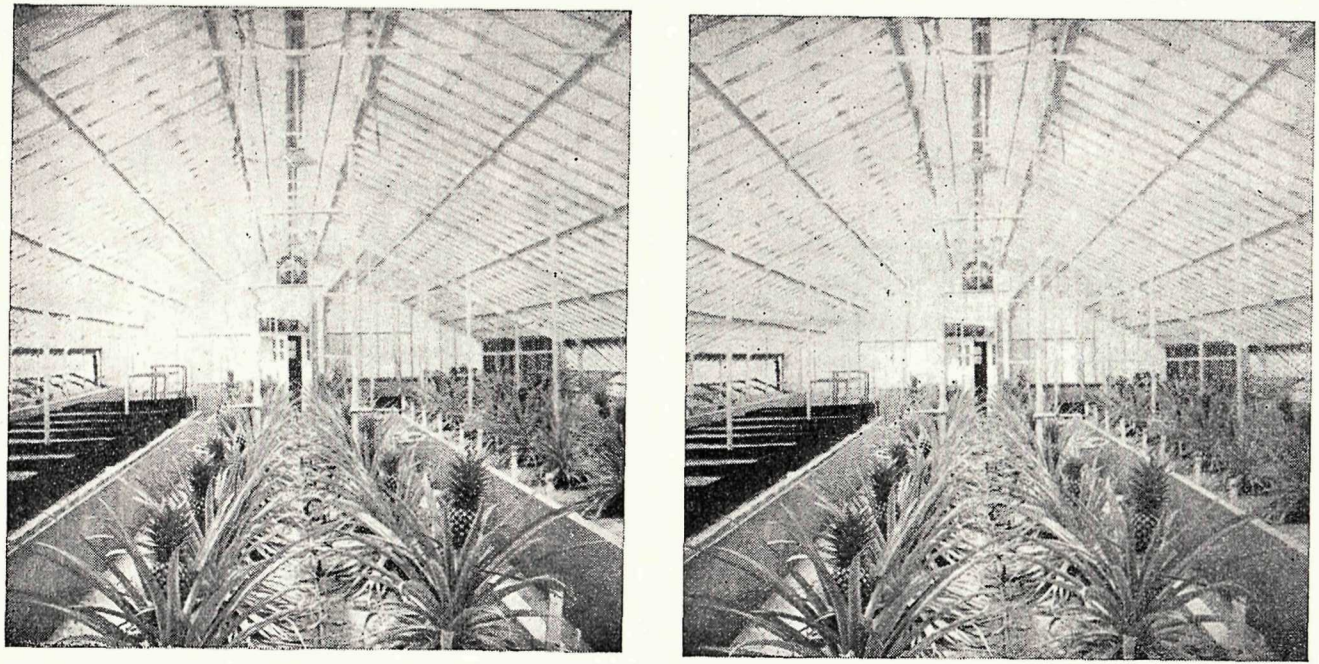


FIGURE 3.—Photographic views of the Greenhouse and the Solution Culture Experiment. Pineapple Plants, 493 days after planting. The air supply system for aerating the solution is shown.

83.6 parts per million of S
77.9 parts per million of Nitrate N
22.0 parts per million of Ammonia N

The following concentrations of micronutrient elements were added to the different treatments:

TABLE NO. I

TREATMENT			PARTS PER MILLION					
No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as $FeSO_4$
1	All M. E.	1- 2- 3						
2	Fe	4- 5- 6						5
3	Mn	7- 8- 9	2					
4	B	10-11-12				1		
5	Cu	13-14-15		2				
6	Zn	16-17-18					2	
7	Al	19-20-21			1			
8	All M. E.	22-23-24	2	2	1	1	2	5
9	Cu	25-26-27	2		1	1	2	5

THE IRON-MANGANESE SERIES

No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as humate
10	5 ppm Mn.	28-29-30	5	2	½	½	½	0
11	5 ppm Mn Fe 1 ppm.	31-32-33	5	2	½	½	½	1
12	5 ppm Mn Fe 3 ppm.	34-35-36	5	2	½	½	½	3
13	5 ppm Mn Fe 5 ppm.	37-38-39	5	2	½	½	½	5
14	5 ppm Mn. Fe 10 ppm.	40-41-42	5	2	½	½	½	10

The solutions of micronutrient elements were prepared as follows:

Element	Parts per Million of the Element	Salt used C. P.	Grams of Salt per Liter
Mn	2	$Mn\,SO_4\,H_2O$	0.00615
Cu	2	$Cu\,SO_4\,5H_2O$	0.00785
Al	1	$Al_2(SO_4)_3\,18H_2O$	0.012355
B	1	$K_2B_4O_7\,5H_2O$	0.007476
Zn	2	$Zn\,SO_4\,7H_2O$	0.008796
Fe	5	$Fe\,SO_4\,7H_2O$	0.02489

In every case stock solutions were prepared, so as to furnish the requisite amount of micronutrient elements, by using a definite adequate aliquot for the corresponding jar used in each treatment. The solution of ferrous sulphate was prepared in small quantities, at the required moment, to be used immediately. It was slightly acidified with sulfuric acid to prevent hydrolysis. Distilled water and C.P. chemicals were used always.

The iron humate added to treatments 10 to 14 was prepared according to the method of Horner, Burk and Hoover (41), and taking into consideration the recommendations of Burk, Lineweaver, Horner and Allison (16).

The macronutrient formula used in these experiments is a modified form of the solution used by Sideris, Krauss, and Young (74). It was used successfully by Schapelle (70, 71) in his work with pineapple plants.

The pH values of the nutrient solutions were determined periodically, by means of a Leeds and Northrup Universal glass electrode potentiometer. A pH value near to 4.5 was maintained by adding the required amounts of a normal $H_2 SO_4$ solution or a dilute solution of $Ca(OH)_2$, according to the change in pH of the nutrient solutions. According to Schapelle (71), pH 4.5 is the optimum value for growth of pineapple plants in culture solutions.

2. *Method of growing the pineapple plants.*

The pineapple slips selected for these experiments were planted in quart culture jars of good-grade white glass which were prepared with suitable wooden lids with a hole in the center big enough to hold the slips. Three cultures were planted for each of the fourteen treatments, so, there were forty-two culture jars. These were distributed at random in the greenhouse. The arrangement and number of the treatments are shown in figure 2. Two photographic views of the experiments, when the plants were already fruiting, are shown in figure 3. The jars were buried in sand to exclude light rays from the solutions.

Table I shows the concentrations of micronutrient elements used for each treatment and the jars numbers.

The macronutrient solution used was the same for all the treatments. Its concentration is shown on page 216. The solutions were changed every month. The concentration of the solution of macronutrient elements was reduced to one half from the 214 day on.

On the 84 day after the slips were planted, the plants were transferred from the quart jars to wide-mouth gallon jars because the root system had grown already too big for the quart jars. For the same reason they were again transferred to 17–liter pyrex jars on the 214 day. The lids of these jars were made of wood and the center holes were lined with cork rings to hold the plants in place.

The roots were carefully drained of the residual solution in them every time that the solutions were changed. To change the solution, the plant was removed together with the lid and the jar was emptied

with a siphon. It was then cleaned of any solid residue and of the old solution. Then the jar was filled to about one half its volume with water. The corresponding aliquots of the stock solution of nutrient elements were added independently one by one, dissolving each one after added. The volume was completed to the 17–liter mark, and the plant put back to its place. The solution was brought to the 17–liter level with distilled water every day.

While the plants were growing in the quart jars, that is, during the first 83 days, the solutions were not aerated. From the 84 day on air was bubbled continuously through the solution. The air bubbles were passed at a uniform rate through all the jars, as exactly as possible. An automatically-pressure-controlled electric air compressor furnished the supply of air. Glass tubing was used to pass the air from the main air pipe into the solutions (see Figure 3).

The greenhouse, where this experiment was set up, was well ventilated by means of an electric fan located near the roof at one end of the building. (See Figure 3).

3. *Observations and data to be obtained.*

The plants were observed every day to note: (*a*) any change in conditions of growth, (*b*) root volume and development, (*c*) date of appearance and magnitude of chlorosis, (*d*) necrosis in leaves, (*e*) intensity of greenness, (*f*) condition and pH of solutions, (*g*) date of the beginning of blooming and of attainment of mature fruit, (h) weight and size of fruit and crown when harvested, (*i*) analysis of the juice of the fruit ripened out of the plant, for reducing sugars, total sugars, total acidity and density.

4. *Chemical methods of analysis.*

(*a*) *Extraction of juice*

The ripe pineapple fruits were carefully peeled from the non-edible rind, and the whole fruit cut in pieces and subjected to 500 pounds pressure per square inch in a Carver laboratory press. The juice expressed from the whole pineapple fruit was collected for analysis.

(*b*) *Density determination*

The degree brix of the juice was determined using brix hydrometers calibrated at 20°C. The readings made were corrected to 20°C, using the scale for corrections attached to the thermometer within the body of the hydrometer.

(c) *Determination of reducing sugars*

The official method (75) for the determination of reducing sugars and total sugars was used, that is, Lane–Eynon general volumetric method, as described in section XXXIV 32 of the Official and Tentative Methods of Analysis (75). This method uses Soxhlet's modification of Fehling's solution, as reagent. Solution (A) consists of 34.639 grams copper sulfate ($CuSO_4$ $5H_2O$) dissolved in distilled water and diluted to 500 ml., filtered through prepared asbestos in a Cooch crucible. Solution (B) is the alkaline tartrate solution, and is made by dissolving 173 grams of Rochelle Salts and 50 grams of sodium hydroxide in distilled water and diluted to 500 ml., then allowed to stand for two days and filtered through prepared asbestos. This solution was standardized according to the procedure in Section XXXIV, 33 of the Official Methods of Analysis (75), taking 5 ml. of solution (A) and 5 ml. of solution (B).

The determinations were made on a portion of the freshly extracted juice diluted 1 to 10. A one-per-cent-aqueous solution of methylene blue was used as the internal indicator. A preliminary incremental titration was made on the first sample, and the determinations made in triplicate, according to Section XXXIV, 34 (75), following a similar procedure to the one used for standardization of the reagent.

The calculation of the reducing sugars was made using Table XV Section XLIII of the Official Methods (75), taking the factors in the column for solutions having one gram of sucrose in 100 ml. of the juice solution used for titration. Of course, the readings of the volume in ml. of solution required to react to the end-point with the amount of reagent used, was divided by the concentration factor of the reagent's solution, to obtain the ml. that would be required for a reagent with a concentration factor of 1.0.

As the values thus obtained give the grams of reducing sugar in 100 ml. of the solution of juice used in the titration, multiplying these weights by ten will give the grams of reducing sugar in 100 ml. of juice. To obtain the percentage of reducing sugars weight in the juice, the weight in grams of reducing sugars in 100 ml. of juice is divided by the specific gravity 20°/4°C of the juice as determined from the brix reading, from Spencer (78).

(d) *Determination of Total Sugars*

Total sugars were determined promoting total inversion by adding 2 ml. of concentrated HCl (specific gravity 1,1029 at 20°/4°C)

to 10 ml. of the freshly extracted juice in a 100 ml. volumetric flask, left overnight for total inversion. Then the solution was neutralized with NaOH and brought up to the 100 ml. volume. From this, 10 ml. were diluted to 50 ml. and analyzed for reducing sugar by the same method given above for the fresh uninverted juice. The weight in grams of invert sugar in 100 ml. of the titrated solution of juice as calculated from the factors of Table XV, in the Official Methods (75) on the first column for no sucrose, must be multiplied by 50 to account for the two dilutions made: 10 to 100 and then 10 to 50. This gives the weight in grams of total sugars as invert sugar in 100 ml. of fresh juice. This value divided by the specify gravity 20°/4°C gives the percentage by weight of total sugars, as invert sugar in the juice.

(*e*) *Total Acidity*

The total acidity was determined by titrating the freshly extracted juice against a standard NaOH solution, taking 10 ml. of the juice. Phenolphthalein was used as the indicator.

According to E. K. Nelson of the Bureau of Chemistry, U.S.D.A., quoted by Hericksen (33), citric acid constitutes about 87 per cent of the total acids of pineapple juice. The acidity of the juice is calculated on the basis of per cent of citric acid in juice. This is done by multiplying the normality of the acid in the juice by one tenth of the gram equivalent weight of citric acid, that is, 6.4, and dividing by the specific gravity 20°/4°C of the juice so as to reduce it to gravimetric basis.

C. *Results and Discussion of the Effect of Micronutrient Elements.*

1. *Chlorosis*

The observations on the chlorosis shown by the plants, are expressed numerically in table II, indicating the total severity of chlorosis per treatment of three plants, the day of appearance, and of any change in the severity.

The numerical evaluation has been made on the following scale of values per plant:

Slight chlorosis	1.0
Medium chlorosis	2.0
Strong chlorosis	3.0
Very strong chlorosis	4.0
Necrosis	5.0
Very strong necrosis	6.0
Death of each plant	8.0

TABLE No. II

OBSERVATIONS ON CHLOROSIS OF PLANTS EXPRESSED NUMERICALLY FOR THREE PLANTS

Number	Treatment	Days after planting													Weighted averages 414 days
		88	90	94	97	101	107	114	119	132	143	170	214	414	
1	M. E					1	2	2	3	4	5	6	6	6	4.2
2	Fe														0
3	Mn	1	1	2	2	3	5	6	6	6	10	10	10	10	7.1
4	B			1	1	2	2	3	3	4	5	8	8	8	5.3
5	Cu			1	1	2	3	3	6	9	14	14	14	14	9.2
6	Zn					1	1	2	2	4	4	5	5	5	3.5
7	Al			1	1	1	2	2	3	3	3	4	4	4	2.6
8	M. E.														0
9	Cu											1	1	1	0.6
	IRON-MANGANESE SERIES—5 PPM OF MANGANESE														
10	Fe 0 ppm	3	3	6	9	12	12	12	12	12	16	18	18	18	13.0
11	Fe 1 ppm														0
12	Fe 3 ppm														0
13	Fe 5 ppm														0
4	Fe 10 ppm														0

BASIS OF NUMERICAL EVALUATION PER PLANT

slight chlorosis	1.0	very strong chlorosis	4.0
medium chlorosis	2.0	necrosis	5.0
strong chlorosis	3.0	strong necrosis	6.0
dead plant	8.0		

See Table I for concentration of micronutrient elements in each treatment.

For the purpose of comparison on the chlorosis produced by each treatment, averages have been calculated as "weighted averages". The magnitude of the severity is multiplied by its duration in days, and each summation of these products is divided by 414, that is, the number of days from the time of planting to the time of blooming of the plants.

The following method was used, taking treatment three as an illustration.

According to table II, in this treatment chlorosis appeared on the 88 day.

The chlorosis value	1	lasted	6 days
The chlorosis value	2	lasted	7 days
The chlorosis value	3	lasted	6 days
The chlorosis value	5	lasted	7 days
The chlorosis value	6	lasted	29 days
The chlorosis value	10	lasted	271 days

Multiplying each value of intensity of chlorosis by the number of days it lasted, then this gives:

		Without chlorosis	88 days
1	multiplied by	6 equals	6
2	multiplied by	7 equals	14
3	multiplied by	6 equals	18
5	multiplied by	7 equals	35
6	multiplied by	29 equals	174
10	multiplied by	271 equals	2710
Total "chlorosis days"			2957
Total number of days			414
Average per day (three plants)			7.1

The average is calculated on the total growing period of 414 days. Some treatments showed chlorosis before others. Averaging for the total period of growth takes this into account.

Apart from the information given in table II, the following observations were made:

a. Treatments 2, 8, 11, 12, 13, 14, that is, all the treatments containing iron, showed plants with a good green color, especially treatment 2 that had iron as the only micronutrient element added.

b. The three plants of each treatment showing chlorosis were not affected equally in each case, some showed very slight chlorosis too low to be evaluated. The variations in reserve iron in the planted slip may be the chief reason

for this. The table gives information about the starting date, intensity in each case, and the rate of increase.

c. One plant in treatment 10 never bloomed and finally died of an extremely strong necrosis.

d. One of the plants of (copper) treatment number 5, showed also strong necrosis but was able to survive and bear fruit.

e. Attention should be given to the fact that treatment 3 had only two parts per million of manganese, while number 10 had five parts per million and the other micronutrient elements.

f. The degree of greenness in treatments 11 to 14 increased with the increase in iron in these treatments, but was not as high a green color as in number 2 that had iron as the only micronutrient element added. (Treatments 10 to 14 had other micronutrient elements added besides the manganese and iron. See Experimental Methods).

Discussion of the results on the chlorosis produced

All the treatments with iron added: 2, 8, 9, 11, 12, 13 and 14, were exempt from chlorosis.

Treatment 2, with iron as the only element added at 5 ppm, was the greenest of all treatments. Treatment 1, with no elements added, showed chlorosis; so, the iron added in treatment 2 was enough to prevent the chlorosis caused by chlorosis-producing elements in reserve in the planted slip. In other words, the reserve iron in the slip was not sufficient to counteract the chlorosis-producing tendency of the other elements through the whole period of growth. Extra nutrition of iron was required, as shown in treatment 2, to prevent chlorosis. Treatment 2 was greener than treatment 8 where the other elements were added and in which still no chlorosis was produced.

Treatments 10, 11, 12, 13, and 14 of the iron-manganese series, showed more definitely the counteracting action of iron against the chlorosis-producing action of manganese, and possibly of the other elements added in this treatment. While treatment 10 showed marked necrosis (one plant could not survive); treatment 11 (only one part per million of iron added) produced green chlorosis-free plants. Treatment 10 had five parts per million of manganese added and other micronutrients in smaller concentrations. (See Experimental Methods.) As will be shown later, these other elements have some tendency to produce chlorosis, especially copper.

Figure 4 shows plants under treatments 10 and 11, on the 102 day after planting. Notice that the plant of treatment 10 was strongly chlorotic, and that of treatment 11 was normal. Figure 5

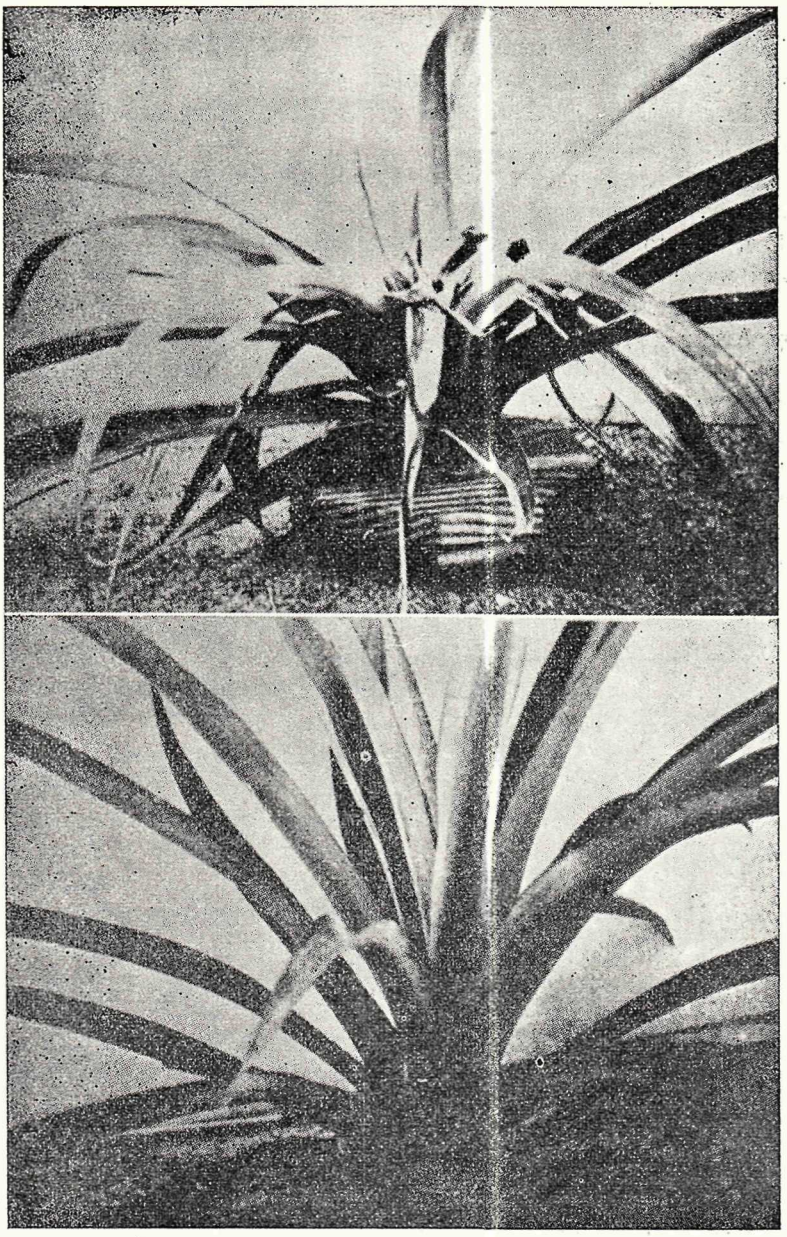


FIGURE 4.—Photographs of plants, 102 days after planting.
Treatment 10, above. Micronutrient elements added to the culture solution: 5 ppm of Mn, 2 ppm of Cu, ½ ppm of B, ½ ppm of Zn, and ½ ppm of Aluminum. The plant is strongly chlorotic and necrotic.
Treatment 11, below. The same micronutrient elements as above but with one ppm of iron added. The plant is growing normally.

shows plants of the same treatments 493 days after planting. The plant of treatment 10 was almost dead and that of number 11 produced a normal fruit with a beautiful crown, a good sign of vigor and good quality.

The appearance of treatments 11, 12, 13, and 14, showed an increase in greenness with the increase of iron. No detrimental effect due to iron was noticed in any one of the treatments where iron was added.

The chlorosis produced by toxic concentrations of iron mentioned by Arnon (3), and the necessity of certain concentration of manganese to bring about a balance of the ferrous and ferric ions for proper chlorophyll formation, are not shown in these treatments. It may be that in the case of treatment 2, where iron was the only element added in five parts per million, the concentration was low enough, or the reserve manganese in the slip was at the right level; but in any case, there were no signs of iron toxicity. There are good reasons to believe that the toxicity of iron, if any, would be at a very high level in pineapple plants.

Treatment 5, that had copper as the only minor element added, showed strong chlorosis and even necrosis. This points out the conclusion that copper produces chlorosis at certain levels of concentration in the nutrient solution, if in the absence of iron. These results show the action of copper stronger even than that of manganese, which in treatments 3 and 10 induced chlorosis very markedly and in proportion to its concentration in the nutrient solution. See figure 6.

Boron also produces chlorosis when added to the nutrient solution in the absence of iron, or in the presence of insufficient iron to antidote its action. Compare treatment 4 with 1.

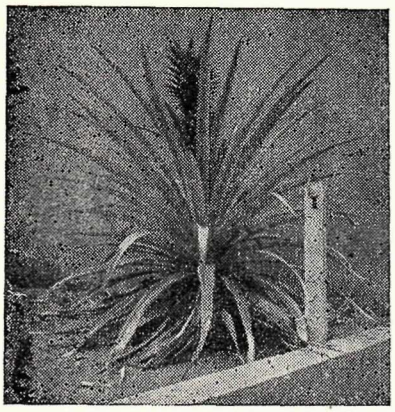
The above conclusions can be shown numerically as follows:

The average of the "weighted averages" of chlorosis of all treatments from 1 to 9 having iron, i.e., treatments 2,8,9, is 0.2; while the average for all treatments 1, 3, 4, 5, 6, 7 with no iron added, is 5.3. Also the chlorosis produced by treatments 1, without micronutrient elements added; 3 with manganese; 4 with boron; 5 with copper; and 6 with Zn; make a total of averages of 31.9. This was antidoted by 5 p.p.m. of iron added in the treatment 8, value 0, in which all the micronutrient elements were added.

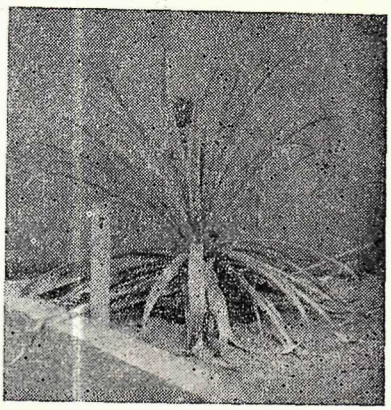
Evidence is given to definitely establish that, in the pineapple plant, iron antidotes the action of the chlorosis-producing elements copper, manganese and boron, when these are in a toxic concentration in the nutrient solution.

The treatments of zinc and aluminum showed beneficial effect against chlorosis. Treatments 6 and 7 gave lower values of chlorosis than treatment 1.

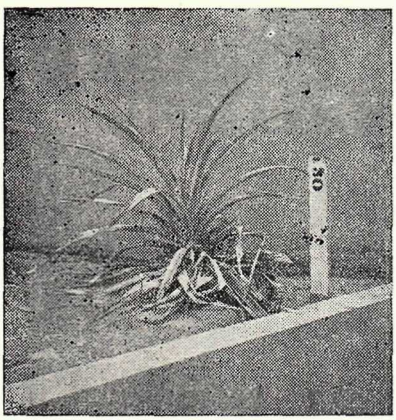
PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING



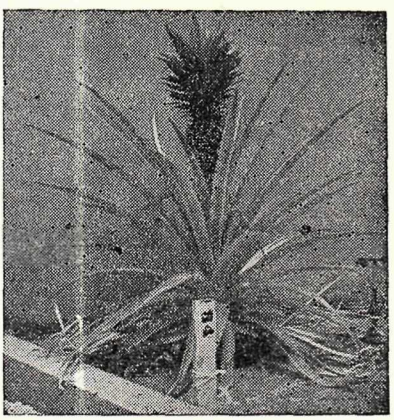
A. Treatment 1.
No micronutrient elements added.



B. Treatment 3.
2 parts per million manganese added.



C. Treatment 10.
No iron added.

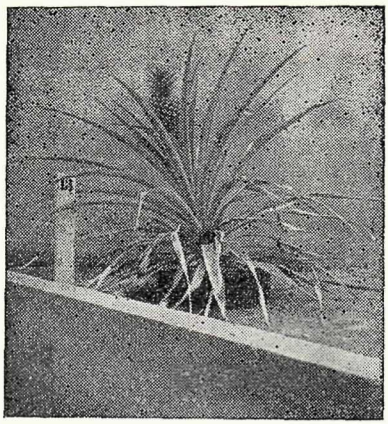


D. Treatment 11.
1 ppm of iron added.

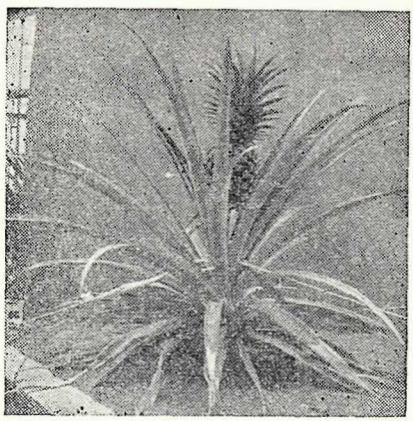
FIGURE 5.—Treatments 10 and 11, each contain: 5 ppm of manganese, 2 ppm of copper, ½ ppm of zinc, ½ ppm of boron, ½ ppm of aluminum.

Note that "C" is strongly chlorotic and necrotic, and has not flowered yet; the plant in "D" is heathly, and produced a fruit better than in "A" and "B". The plant, fruit and crown of "A" are better than those in "B". The preence of iron in "D" counteracted the detrimental action of other micronutrient elements affecting the plants in "A", "B" and "C".

PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING



E. Treatment 5.
2 ppm of copper added.
The plant is necrotic. The fruit and crown are very small.



F. Treatment 9.
No copper added.
The plant is healthy. Fruit and crown are normal.

FIGURE 6.—Compare the plant in "E" with the plant in "D", figure 5. The detrimental effect of copper on plant and fruit is noticed.

2. *Root Growth*

The roots started to come out on the eighth day. On the 79 day they were rather crowded in the quart-jar vessels and were to be changed to gallon jars.

Browning or burning of the root tips was noticed in some treatments. The severity of this injury is expressed numerically in table III. This root-rot injury in pineapple roots was noticed by Schapelle (71) in cultures deprived of copper.

As is shown in table III, up to the 79 day the solution had not been aerated, and on that day the root injury due to this burning or browning of the tips was noticed in the absence of iron and copper.

In nonaerated solutions, copper showed the greatest beneficial action. Treatment 5, in which copper was added, showed almost no injury; while treatment 9, with no copper added, showed a very high severity of injury.

Aluminum showed the highest intensity of injury to roots, in the nonaerated cultures. Zinc also showed injurious effects in nonaerated solutions. Compare the results of treatments 6 and 7 with treatment 1.

The aeration of the solution, as shown on the 108 day, tended to reduce the injury in all treatments. The aluminum treatment recovered rapidly and instead of the detrimental action a highly beneficial action was noticed. In the copper treatment it was the reverse, the beneficial action of copper turned into a detrimental effect. Zinc showed recovery also.

TABLE NO. III

OBSERVATIONS ON THE ROOTS

Treatment		Total Browning of Roots		Average volume in ml.	Appearance on the 149 day
Number	Elements	unaerated 79 day	aerated 108 day		
1	ME	6	6	125	Good
2	Fe	1	0	114	Very good
3	Mn	3	3	159	Good
4	B	4	4	137	Short
5	Cu	1	5	86	Hairless, stiff & brown
6	Zn	7	4	85	Good
7	Al	10	2	173	Very good
8	ME	3	3	72	Good
9	-Cu	8	1	107	Very good
IRON MANGANESE SERIES. Mn. 5 p. p. m.					
10	Fe 0 p p m	1	0	77	Good
11	Fe 1 p p m	0	0	73	Good
12	Fe 3 p p m	0	0	101	Good
13	Fe 5 p p m	0	0	86	Good
14	Fe 10 p. p. m.	0	0	70	Good

Table I gives the concentrations of nutrient elements in each treatment.

Numerical evaluation of browning based on: Very slight = 1, slight = 2, medium = 3.

Volume of roots obtained by displacement of water.

The iron treatments showed beneficial action with aerated as well as non-aerated solutions. Manganese and boron showed some beneficial action.

This interesting response of the micronutrient elements, as related to the aeration of the nutrient solution, points out that as far as root growth is concerned, an oxidation-reduction process plays an important role. It seems that iron and copper have a beneficial oxidizing action under anaerobic conditions; while aluminum and zinc do not show this property. On the other hand, the action of aluminum and zinc has a beneficial effect under the conditions of ample oxygen supply in aerated solutions.

The findings of Lochwing (54), Clark and Shive (19), and Bryant (14), as to the beneficial effect of nonaerated nutrient solution on root growth, should be conditioned by saying that copper or iron are required to be present.

The beneficial action of copper in raw peat soils, pointed out by Allison, Bryan and Hunter (2), may be attributed in part to the anaerobic conditions of these soils. The detrimental effect of aluminum on root growth, as noticed by Blair (10) Barnette (6), Abbot (1), and McGeorge (57), can be explained also as due to conditions of low oxygen supply in the nutrient solution. On the other hand, the beneficial action of aluminum as observed by Schappell (71) is due to the presence of a good supply of air.

The reports of Liebig, Vanselow and Chapman, as quoted by Arnon (3), on copper toxicity in citrus roots, and the stimulating action of aluminum, are confirmed by the results shown in these experiments as to the action of copper and aluimnum on root growth under aerobic conditions. That is, aluminum and copper counteract each other under either aerobic or anaerobic conditions.

The volumes of roots, as shown in table III, point out that copper and zinc tend to inhibit root growth; while aluminum, boron and manganese appear to stimulate it. Iron does not show any particular effect, for treatment 2 showed about the same results as treatment 1.

The combined effect of copper and zinc tended to produce low volume of roots in treatments 8 and 10 to 14. Treatment 9 without copper showed the best volume.

3. *Flowering and fruiting*

The first plant to flower and bear fruit was one of culture treatment 14, with 5 p.p.m. of manganese and 10 p.p.m. of iron. The fruiting stalk appeared at the 214 day, and the mature fruit was harvested on the 323 day. Table IV shows the average day when

the flowering started, and the average day when the fully mature fruit was picked from the plant in each treatment. The average weight of the fruit at maturity with and without crown and the weight of the crowns are given for each treatment. The figures on table IV show very interesting results.

TABLE NO. IV

FLOWERING AND FRUITING

MATURE FRUITS—AVERAGE FOR THREE PLANTS

Treatment		Days Taken		Weight in Grams		
Number	Elements	To Flower	To give mature fruit	Fruit	Crown	Fruit & Crown
1	-ME	424	536	1,033	389	1,422
2	Fe.	423	534	1,170	274	1,444
3	Mn	423	539	1,130	263	1,393
4	B.	494	611	1,165	275	1,440
5	Cu	519	624	863	258	1,121
6	Zn	450	555	853	246	1,099
7	Al	431	533	1,209	338	1,547
8	ME	405	533	1,067	199	1,266
9	-Cu	431	529	1,353	357	1,710
	IRON-MANGANESE SERIES—5 P. P. M. OF MANGANESE					
10	Fe 0 p. p. m.	570	675	521	160	681
11	Fe 1 p. p. m.	421	523	1,126	258	1,384
12	Fe 3 p. p. m.	412	519	1,133	319	1,452
13	Fe 5 p. p. m.	412	514	1,156	331	1,487
14	Fe 10 p. p. m.	409	509	1,230	376	1,606

See Table I for concentrations of elements in each treatment.

The treatments 11 and 12 showed flowering and fruiting earlier; and the more iron, the earlier.

Copper, boron, and zinc showed a retarding influence on flowering and fruiting, as shown in treatments 4, 5, and 6 as compared with 1. Copper exerted the most detrimental effect. See figures 5 and 6. It is remarkable that no retarding action was shown by manganese in treatment 3, thus showing that it is the retarding effect of copper, boron and zinc that have been antidoted by iron in treatments 8, 9, 11, 12, 13, and 14. The retarding action in treatment 10 may be attributed to the indirect effect of manganese-chlorosis and the retarding effect of other micronutrient elements added.

The treatments with iron showed the earlier flowering and fruiting, but it appears that iron and manganese have an additive action superior to their independent action. Compare treatments 2 and 3

where these elements are alone, one in each treatment, with 8 and 11 to 14, where they are together, and against the retarding influence of copper, boron and zinc.

The weights of fruits, either with or without crown, showed to be affected quite similarly to flowering and fruiting. The treatments of copper and zinc gave the lowest yield comparing treatments 5 and 6 with 1.

It should be pointed out that the minus-copper treatment 9 showed the highest yield. The aluminum treatment also gave a high yield. Iron and boron favored high yields also.

The production of fruits was not so badly affected by the injury on roots and by chlorosis when the injury did not reach the advanced stage of destroying the plant to a certain degree, as it did in the plus-copper treatment 5 and in the no-iron treatment 10. In the iron-manganese series the weight of crop increased with the increase in iron.

The desirable vigorous crown was at its best in the manganese and iron treatments, where increasing quantities of iron were added. The treatments without zinc did not show detrimental action on the crown. It was expected that treatments without zinc might give short crowns, Hopkins et al (40), connecting it with the "little leaf" disease in citrus, or any distortion in the leaves as discussed by Hoagland (34), due to lack of zinc in the nutrient solution.

It is noticed that the aluminum treatment produced very good fruits as to weight of the fruit and of the crown. This may be due to its beneficial effect on roots when aerated.

4. *Composition of the juice of the ripe fruits.*

Table V shows the degree brix of the juice, a figure approaching the per cent of total solids by weight in solution, called also "apparent gravity solids" in sugar technology. The total sugars in juice are expressed as invert sugar. The ratio of total sugar to brix has been calculated and multiplied by 100, so as to show the relation between sugars on the total (gravity) solids, and so as to give a figure independent of dilution. This may be called the "gravity purity of invert sugars". It is the figure that should give the best criteria of the quality of the juice as to its sugar content.

The percentage acidity is reported on the basis of citric acid as discussed in the Experimental Section. The percentage of reducing sugar in the juice is also given.

TABLE No. V

COMPOSITION OF THE JUICE OF THE RIPE FRUITS

Treatment		Juice Analysis—Average for three Plants				
Number	Elements	Reducing Sugar %	Total Sugar as Invert Sugar %	Degree Brix	o/o of solids which are Sugars	Acidity as Citric Acid %
1	-ME	2.75	10.70	15.7	68.2	0.73
2	Fe	2.61	9.80	13.1	74.8	0.67
3	Mn	3.02	9.34	14.7	63.5	0.76
4	B	3.29	10.84	14.6	74.0	0.68
5	Cu	2.46	9.06	12.7	71.3	0.66
6	Zn	1.95	7.60	11.5	66.0	0.88
7	Al	2.45	8.28	11.7	70.8	0.74
8	ME	3.73	11.48	14.8	77.5	0.61
9	-Cu	2.72	9.80	13.3	73.7	0.68
	IRON-MANGANESE SERIES—5 P. P. M. OF MANGANESE					
10	Fe 0 p. p. m.	2.47	9.23	13.9	66.4	0.72
11	Fe 1 p. p. m.	4.38	9.28	12.6	73.7	0.61
12	Fe 3 p. p. m.	3.05	11.22	15.6	76.8	0.63
13	Fe 5 p. p. m.	2.66	11.35	14.8	76.8	0.64
14	Fe 10 p. p. m.	3.22	11.50	14.3	80.4	0.53

See Table I for concentrations of elements in each treatment.

The best quality of the fruits is associated with the highest ratio of sugar content to total dissolved solids, and the lowest acidity in the juice.

The sugar content of the juice was lowest in the zinc, manganese and aluminum treatments. The treatment with no micronutrient elements added showed a low sugar content. The acidity was higher in these treatments. The copper treatment showed also some detrimental action, as shown by comparing treatment 5 with copper, and treatment 9 without copper. Iron and boron showed beneficial action for increasing the sugar content and lowering the acidity. Compare treatments 2 and 4 with 1.

The iron-manganese series showed low quality of juice in treatment 10, without iron. The other treatments, 11 to 14, showed increasing content of sugar and decreasing content of acid, with the increase in iron. Iron was the most important agent of high quality. All treatments with iron were superior to treatments without iron.

The reserve micronutrient elements in the slip were not sufficient to produce good quality, The values for treatment 1 showed rather low sugar content and a high acidity. Compare treatment 1 with treatment 8, which gave the best analysis next to number 14, that had 10 p.p.m. of iron. It seems that the chlorosis-producing effect of the elements is related to low sugar content, and high acidity.

V — SUMMARY AND CONCLUSIONS

A. *Work done*

Pineapple plants were grown in nutrient solutions from uniform and healthy slips used as the propagating organ. The solutions were prepared with a mixture of macronutrient elements containing ammonia and nitrate nitrogen, potassium, phosphorus, magnesium, calcium, and sulfur. The solutions proved to be good for the growth of pineapples.

Fourteen different treatments of the micronutrient elements: iron, manganese, boron, zinc, copper and aluminum, were used in triplicate. Combinations of these elements were made in order to trace their effect, either toxic or beneficial, on pineapple plant growth and production, on root growth, on flowering and fruiting, and on the quality of the fruit. The antidoting effect of iron against the chlorosis-producing action of manganese was also studied. Plants also were grown without adding micronutrient elements to the nutrient solution.

B. *Conclusions* (See footnote on page ~~287~~ 241)

1. Iron antidotes the chlorosis-producing action of manganese in pineapple plants. With 5 parts per million of soluble manganese in a nutrient solution containing one-half part per million of boron, of zinc, and of aluminum, and 2 parts per million of copper, severe chlorosis and necrosis appeared before the pineapple plant was able to attain full growth. But, with one part per million of soluble iron humate added to a similar treatment, a healthy, normal plant was produced.

2. Iron counteracts the chlorosis-producing effect manifested by copper and boron. It raises their chlorosis-producing level.

3. Copper and manganese, at a concentration of 2 parts per million in the nutrient solution, produce strong chlorosis if iron is not present.

4. Aluminum and zinc show beneficial effects against chlorosis.

5. Iron shows no toxic effects on pineapple plants when added as the only micronutrient element to the culture solution, up to 5 parts per million. If chlorosis-producing elements like manganese or copper are present, higher concentrations are beneficial.

6. The reserve iron content in slips of the variety "Smooth Cayenne" is quite enough to counterbalance the detrimental chlorosis-producing effect of the other reserve micronutrient elements in the slip. However, plants grown in nutrient solutions deprived of all micronutrient elements produced fruits of lower sugar content than those supplied with micronutrient elements.

7. Pineapple plants respond to increase in iron in the nutrient solution, giving increase in green color, in yield, in sugar content and in decreased acid content. Treatments with a constant supply of manganese and other micronutrient elements, as explained above in (A), and with iron added in concentrations ranging from 1 to 10 parts per million, showed that the above-mentioned beneficial effect varied directly with the increase in iron added to the nutrient solution.

8. Iron prevents browning of root tips, or root rot, of pineapple plants under either anaerobic or aerobic conditions. Manganese and boron show also a somewhat beneficial action.

9. Copper has a highly beneficial action in preventing root-rot injury, when under anaerobic conditions. However, when the nutrient solution is well aerated the effect of copper is somewhat detrimental to the roots. Nonaerated solutions must be well supplied with copper and iron.

10. Aluminum has a beneficial action on root health and volume under the aerobic conditions of well aerated nutrient solutions, but it is highly toxic to the roots under anaerobic conditions.

11. Zinc exerts a somewhat detrimental action on roots under anaerobic conditions of the nutrient solution.

12. It seems that as far as root growth is concerned aeration plays an important role; copper having a beneficial action under anaerobic conditions; and aluminum when the nutrient solution is well aerated. Iron, manganese and boron are beneficial under any of the two conditions of oxygen supply.

13. Aluminum and manganese promote increased volume of roots when the nutrient solution is well aerated. Copper and zinc tend to reduce the volume of roots, but iron and boron show no direct effect.

14. Iron has a beneficial effect on early flowering and early maturity of the pineapple fruit.

15. Copper, zinc and boron, and the chlorosis-producing effect of manganese, have a retarding effect on flowering and fruiting. In

this action they are counteracted by iron. Aluminum shows no specific effect upon flowering and fruiting.

16. Zinc and copper tend to produce low yields. Aluminum and boron increase the yield. Manganese does not show specific effect on yield when added alone to the nutrient solution.

17. Zinc, copper, manganese and aluminum show a tendency to produce fruits with low sugar content and high acidity.

18. Iron and boron show a beneficial effect by increasing the sugar content and lowering the acidity. Iron is an important agent of high quality of pineapple fruits.

19. Zinc deficiency in the nutrient solution shows no signs of anatomical abnormalities in the pineapple plant.

20. Chlorosis in pineapple plants causes lower sugar content and higher ratios of acid to sugar in the fruit.

21. Experimentation on the mutual effect of the micronutrient elements on pineapple should take into consideration the reserve of elements in the planted slips.

22. The antidoting action of iron against the detrimental effects of copper should be studied by using varying concentrations of iron and different concentrations of copper.

23. Field experiments with iron humate added to the pineapple soils should be made to study its effect under soil conditions.

24. More available iron in soils will greatly improve the Puerto Rican pineapple crops.

NOTE: The above-mentioned conclusions on the independent action of the micronutrient elements refer to treatments in which they were added separately as the only micronutrient elements to the nutrient solutions in which the pineapple plants were grown. The concentrations used for each element were: 5 ppm Fe, 2 ppm Mn, 1 ppm B, 2 ppm Cu, 1 ppm Zn, and 1 ppm Al.

C. SYNOPSIS OF THE EFFECT PRODUCED BY MICRONUTRIENT ELEMENTS ON PINEAPPLE PLANTS, WHEN THE ELEMENTS ARE ADDED INDEPENDENTLY TO THE NUTRIENT SOLUTIONS

Element	PPM added to the nutrient solution	Chlorosis	Root-rot injury		Volume of roots aerated solutions	Production		
			Solutions			Early flowering and fruiting	Yields	Quality
			non-aerated	aerated				
Iron	5	Prevented Chlorosis	Prevented injury		No effect	Beneficial effect	High	High
Manganese	2	Severe Chlorosis	Beneficial effect against injury		Good	Retarding due to chlorosis	Not Specific	Low
Boron	1	Produced Chlorosis	Beneficial effect against injury		Not effect	Retarding effect	High	Good
Copper	2	Severe Chlorosis	Beneficial effect against injury	Detrimental effect	Detrimental effect	Retarding effect	Low	Low
Zinc	2	Prevented Chlorosis	Detrimental effect	No detrimental effect	Detrimental effect	Retarding effect	Low	Low
Aluminium	1	Prevented Chlorosis	Detrimental effect	Beneficial against injury	Beneficial effect	No effect	High	Low

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