

RELATIONSHIPS OF IRON AND COPPER  
TO ASCORBIC ACID IN PLANTS

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## I. INTRODUCTION

Because of the importance of ascorbic acid in human diet, numerous studies have been carried out in order to obtain information concerning its natural occurrence. The data obtained by these studies show the great variability in the concentration of ascorbic acid in its biological sources.

The factors influencing the synthesis of ascorbic acid in plants have been investigated during the past two decades. Various opinions, many of which are contradictory, concerning the importance of species, varieties, light, location, soil type and fertilizers, on the concentration of this vitamin in plants are expressed in the literature.

The data dealing with the effect of inorganic nutrients on the ascorbic acid status in plants are especially confusing even though a very large number of investigators have studied this phase of the problem. Reviews on this subject have been made by Dutcher (1932), Hamner and Maynard (1942), and by Carrol (1943).

Studies have been carried out in the field, greenhouse and in controlled nutrient solution; and the effect of the "macro-elements" on the ascorbic acid status of plants have been described by many authors. However, the effect of the "minor-elements" on the

concentration of ascorbic acid in plants has not been as well studied although elements such as copper and iron are known in vitro to be powerful catalysts of the oxidation of ascorbic acid.

Most workers have assumed that any relationship between environmental factors and the amount of ascorbic acid present in the plants reflects the effect of these factors on its synthesis. It is possible, however, that the stability rather than the rate of formation of ascorbic acid in the plant tissues is influenced by these factors. There are thus two main possible reasons for the great variations in the concentrations of this vitamin in similar plant tissues.

It is not known whether the ascorbic acid is stored in the vacuoles or in the cytoplasm of the plant cells. Reedman and McHenry (1938) reported that in certain plant tissues such as potato, cauliflower, a certain amount of the ascorbic acid is combined with protein; but the fraction of ascorbic acid extracted by the methods commonly used to determine its amount in the plant material is water soluble and in a free state in the protoplasm or the vacuolar sap. The stability and subsequent accumulation of the water soluble fraction of ascorbic acid in the plant cell may thus be dependent on the factors affecting its stability in water solution.

Before describing the experiments carried out, a review of the literature on the effects of copper and iron on the concentration

of ascorbic acid in biological material and its stability in vitro will be presented.

### A. Review of Literature

#### 1. Relationship Between Copper and Iron and the Concentration of Ascorbic Acid in Biological Material

Hamdallah (1939) was the first worker to test the effect of depriving plants of iron on the ascorbic acid concentrations in the tissues. He found that beans, spinach and corn grown on a Knopp's iron free solution contained as much or more ascorbic acid than did plants grown on a complete nutrient solution.

Randouin and Le Gallic (1940), after analyzing many plant species, discovered that species with high protein content usually have a high concentration of iron and ascorbic acid. However these authors did not duplicate their experiments with single species in order to test if this relationship was consistent.

The addition of iron sulfate to the soil, depressed the amount of ascorbic acid in the orange fruits according to investigations carried out by Roy and Bahrt (1940) in Florida.

In a very meticulous nutrient solution, Lyon, Beeson and Ellis (1943) studied the effect of deficiencies of minor elements on the vitamin content of tomatoes. They found that fruits from the iron deficient plants contained 30 per cent more ascorbic acid



on a fresh weight basis than did the plant growing on a nutrient solution supplied with iron. On the other hand, the ascorbic acid content of the fruits was not affected by limiting the supply of manganese, zinc, copper and molybdenum, although the number of fruits of each plant was then significantly lessened by a lack of these nutrients.

As a continuation of their first study Lyon and Beeson (1948) published their results on the effect of toxic amounts of minor elements on tomatoes and turnips. Their plants were grown in sand supplied with nutrient solutions.

The concentration of ascorbic acid in tomato plants grown on nutrient solution supplied with 50 parts per million of copper was found 60 per cent greater than in the plants grown without iron. However, the ascorbic acid concentration of turnip greens was lessened with increased amounts of copper in the nutrient solution. On the other hand, no significant difference in the ascorbic acid concentration of tomato and turnip green was observed with increase of the amount of iron in the nutrient solution up to 150 parts per million.

Sideris and Young (1948) reported that pineapple leaves accumulated more ascorbic acid when the plants supplied with nitrate nitrogen were deprived of iron, than when they were supplied with iron. However, when the plants were supplied with

ammonium nitrogen, the concentration of ascorbic acid in the leaves was the same irrespective if iron was present or not in the nutrient solution.

In 1949, the same authors, studying the effect of different iron-manganese ratios on the ascorbic acid content of pineapple leaves, found a greater ascorbic acid concentration in the plants deprived of iron than in those supplied with 0.5 parts per million of iron in the nutrient solution and when a relatively high amount of manganese was present.

The effect of the absorption of iron on the concentration of ascorbic acid in oat plants grown on field plots in Texas, was investigated by Wynd and Noggle (1950a), who reported that

. . . the concentration of vitamin C was inversely related to the concentration of iron found in the plant. This relationship was true for all groups of control data and for amounts of ammonium sulfate, ammonium phosphate, urea or sulfur. The data supporting this generalization were obtained from plants which showed wide ranges in the amount of dry matter produced, percentage nitrogen and concentration of iron. The soils on which the plants were grown varied widely in the pH value.

However, the same workers published (1950b) that in rye plants grown on field plots in Canada, the concentrations of ascorbic acid were positively related to the percentage of nitrogen, calcium and iron. From a personal communication with F. L. Wynd it was learned that the positive relationship found in this case between

the concentrations of iron and ascorbic acid was the only instance in which such a relationship occurred to these workers. In their article, the authors emphasized that the "accumulation of ascorbic acid in plant tissue probably is not intrinsically positively correlated with iron, but rather with a subtle secondary relationship between iron and copper." This hypothesis is based on the thought that the concentration of ascorbic acid in plants may be the image of its stability in the plant tissue. From in vitro studies, the stability of aqueous ascorbic acid solution is related to copper and iron, as will be shown in the next paragraph.

## 2. Relationship Between Copper and Iron and the Stability of Ascorbic Acid in Vitro

As early as 1921, Hess and Unger found that milk treated at 60° C for 40 minutes in a copper vessel caused scurvy to guinea pig in four weeks.

In 1928, Szent Gyorgy, measured the oxygen uptake of solution of his preparation of ascorbic acid in a Barcroft apparatus. He found that in the presence of copper the solution showed an intense uptake of oxygen, but he did not find any noticeable effect of  $\text{Fe}^{+++}$  and  $\text{Mn}^{++}$ .

Increasing concentrations of copper at a pH value of about 6 in an acetate buffer were shown in 1933 by Euler, Myrback and

Larsson, to result in increasing consumptions of oxygen in a Warburg apparatus from an ascorbic acid solution. These authors obtained a small action from iron on the oxidation of the ascorbic acid in the solution.

The following table presented by Kellie and Zilva (1935) shows that copper is a more efficient catalyst than iron in the oxidation of ascorbic acid in water solution.

| Time<br>in<br>Hours | Milligrams of Ascorbic Acid per<br>100 Milliliters of Water |                                                     |                                                     |
|---------------------|-------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|
|                     | Quartz<br>Distilled<br>Water                                | Quartz Distilled<br>Water 0.01 mg.<br>Fe per 40 ml. | Quartz Distilled<br>Water 0.01 mg.<br>Cu per 40 ml. |
| 0                   | 37.0                                                        | 34.5                                                | 34.1                                                |
| 1                   | 35.2                                                        | 31.8                                                | 24.2                                                |
| 2                   | 33.0                                                        | 29.6                                                | 21.2                                                |
| 3                   | 30.9                                                        | 28.2                                                | 15.2                                                |
| 4                   | 29.4                                                        | 25.8                                                | 8.0                                                 |
| 20                  | 25.7                                                        | 10.3                                                | 0.0                                                 |

Mawson (1935) found that ferrous or ferric ions catalyze the oxidation of ascorbic acid in aqueous solution, but to a lesser extent than does copper. He also observed that a mixture of copper and iron has a catalytic effect on the aerobic oxidation of ascorbic

acid greater than have either metal alone. He pointed out that in such biological media as serum, the amount of free copper and iron is so large that some inhibitors must protect the ascorbic acid from being oxidized. He found that some protective action was exerted by cysteine, cystine and glutathione. He also showed that in lemon juice there is no such protective mechanism, but if traces of animal tissues are added to the juice, its ascorbic acid is protected from oxidation at a pH value of 7.4 and at a temperature of 37° C.

The amount of ascorbic acid in various animal tissues was observed by McFarlane (1936) to reduce a proportional amount of iron. He added sodium diethyldithiocarbamate to the list of inhibitors of the oxidation of ascorbic acid and he also found that if dipyridyl is added to sodium diethyldithiocarbamate the aerobic oxidation of ascorbic acid in orange juice is inhibited.

Barron, Barron and Klemperer (1936) studied the oxidation of ascorbic acid added to media such as blood serum, urine, milk and fruit juices, by measuring the amounts of oxygen taken up by these media in a Warburg apparatus. He found that most plant extracts do not contain inhibitory mechanisms; as exceptions were listed the extracts from plants containing high concentration of ascorbic acid such as orange juice, tomato juice and grapefruit juice. This experimental result suggests the importance of the

stability of ascorbic acid on its concentration found in the fresh tissue. These authors observed that the stability of ascorbic acid, added to plant extracts which do not possess an inhibitory mechanism, was not directly related to the amount of copper in the extract. They considered hemochromogens as possible catalysts for the oxidation of ascorbic acid in tissues which contain no oxidizing inhibition mechanism.

Barron, DeMeio and Klemperer (1936) studied the rate of oxidation of ascorbic acid in various buffer solutions and the catalysts which accelerate this oxidation. They found that if the aqueous solution was not contaminated with heavy metal and especially with copper, the oxidation of ascorbic acid at 25° C failed to take place up to a pH value of 7.6. They found that in a citrate buffer of pH 3.17, the increase in the rate of oxidation of the ascorbic acid was logarithmically related to increase in the amount of copper in the solution. These workers stated that iron was a powerful cell catalyst when it combines with porphyrin to form hemin. When hemin is joined with nitrogenous derivatives such as nicotine, pyridine, pilocarpine, its oxidation reduction potential increases and the authors suggest a possible role of hemochromogen in the oxidation of ascorbic acid in plant tissues.

Mack and Kertesz (1936) studied the oxidation of known amounts of ascorbic acid in the acetic extracts of pea plants.

Because the rate of oxidation of the ascorbic acid was less in the plant extract than when the same amount of ascorbic acid was added to the ash of the same plant material, they concluded that only a small fraction of the copper naturally occurring in peas was in a catalytically active form. They also stated that iron ion does not catalyze the oxidation of ascorbic acid but greatly increase the catalysis by copper.

Stotz, Harrer and King (1937) investigated the oxidation of ascorbic acid in the pressed juice of squash and cauliflower to which known amounts of various form of copper were added. They concluded that copper combined with protein can oxidize ascorbic acid at about the same rate as inorganic copper.

Another aspect of the importance of copper in regard to ascorbic acid in plants was emphasized by Powers, Lewis and Dawson (1943) who purified the enzyme ascorbic acid oxidase and showed that it contained at least 0.24 per cent of copper.

#### B. Object of the Present Study

The most pertinent studies on the effect of copper and iron on the concentration of ascorbic acid in plant tissues have been reviewed and found to be very limited in number. On the other hand, no study was found in the literature on the effect of copper and iron on the stability of ascorbic acid in plant tissues. Since

in vitro, the reactions of copper and iron are very well known and have an obvious effect on the stability of aqueous ascorbic acid solution, it seems to be of importance to find out if the same factors affect the stability of ascorbic acid in plant tissues.

This problem is of especial importance in grass because of its value as a forage crop and also because of the increasing use of immature cereal grasses as source of vitamins for human consumption.

It may be noted, that studying the effect of nutrients on the physiology of plants, most authors have indicated the changes which might occur under the conditions of the experiment, but have not correlated the effects with the amounts of the nutrient actually taken up by the plants. The fact is known, however, that the mere presence of one nutrient in the substrate may sometimes greatly affect the absorption of another. In the present study the effects of different amounts of copper and iron on the concentration and stability of ascorbic acid will be correlated with the amounts of these nutrients supplied to the plants and found in the plants.



## II. EXPERIMENTAL PROCEDURES

### A. Equipment

The plants studied in the following experiments were grown in a room, arranged especially for nutrient solution studies. A concrete floor, often washed, reduced contamination from dust particles. Two rows of twenty glazed earthenware containers of one gallon capacity were placed on wooden benches. The mechanical arrangement of the cultures has been described by Wynd (1951). The pots were filled with number eight crushed quartz sand previously washed with tap and distilled water. A hole, three-quarters of an inch in diameter was located at the bottom of the pot, which permitted the nutrient solution to flood the pot from below. Sixteen liters of nutrient solution were stored in five-gallon soft glass carboys, which had first been painted black to prevent growth of algae and then with aluminum paint in order to reflect as much heat and light as possible. Each carboy (C) was connected to two pots (A) and (B). The nutrient solution was automatically forced up from the carboy into the pots by means of an air pump blowing air into the closed system connected to all the carboys. The air pressure was maintained constant by a hydrostatic column (E) and the height to which the solution rose in the culture pots was regulated

by the amount of water in the tank. The mechanical arrangement of the cultures is illustrated in Figure 1.

The greenhouse bench supporting the culture pots is indicated by (F), the common air-pressure line by (D), and the height of the water in the container (E) is indicated by (G). Very accurate and dependable control of the rise of the nutrient solution in the culture pots was attained by this device. Every four hours an electric time switch turned on the air pump, and the nutrient solution was forced up into the pots where it remained for about ten minutes. When the air pressure was released the solution drained back into the carboys.

### B. Nutrient Solutions

The complete nutrient solution was that of Shive (1915) for best growth of wheat tops: Its final composition was:

| Composition of the Nutrient Solution                  |                 |
|-------------------------------------------------------|-----------------|
| Salts                                                 | Grams per Liter |
| $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$            | 3.697           |
| $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ | 1.228           |
| $\text{KH}_2\text{PO}_4$                              | 2.450           |

Concentrated solutions of each salts were made up as follows:

| Composition of the Stock Solutions                    |                    |
|-------------------------------------------------------|--------------------|
| Salts                                                 | Grams in 16 Liters |
| $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$            | 5502.0             |
| $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ | 1827.3             |
| $\text{KH}_2\text{PO}_4$                              | 3646.0             |

Fifteen liters of triple-distilled water were added to each carboy, and then 178 milliliters of each of the stock solution and 534 milliliters of water were added, which brought the final volume to 16 liters.

Micro elements were supplied by adding one milliliter of a stock solution per liter of nutrient solution. The stock solution of micro elements had the following composition:

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Stock Solution of Micro Elements

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| Salts                                      | Grams per 2 Liters |
|--------------------------------------------|--------------------|
| $\text{H}_3\text{BO}_3$                    | 5.720              |
| $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ | 0.440              |
| $\text{MnCl}_2$                            | 2.290              |
| $\text{MoO}_3$                             | 0.015              |

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The final concentrations of the micro elements in the nutrient solution were as follows:

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Concentrations of the Micro Elements in the Nutrient Solution

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| Element    | Parts per Million |
|------------|-------------------|
| Boron      | 0.50              |
| Zinc       | 0.05              |
| Manganese  | 0.50              |
| Molybdenum | 0.005             |

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Copper and iron were added separately at various concentrations as will be explained for the different experiments.

The initial pH of the solutions was about 5.2. This pH was checked at regular intervals during the growth period of the plants. Water was added periodically to compensate for that lost by transpiration.

Other factors which were varied for the different studies will be explained for each experiment.

### III. EXPERIMENT 1

#### A. Purpose

The following experiment was planned in order to discover whether or not iron and copper supplied to wheat plants might catalyze the oxidation of the ascorbic acid present in the plant tissue, in the same manner as it has been mentioned in the literature in the case of in vitro experiments.

The purpose of this first experiment was to investigate the relationships between increasing amounts of copper and constant iron concentration in the nutrient solution on the concentration of ascorbic acid in the fresh wheat tissue and on the stability of this vitamin in the dried tissue. In the same manner, it was proposed to investigate the same relationships with increasing amounts of iron and constant copper concentration in the nutrient solution.

Just before jointing stage, the ascorbic acid was determined in the fresh tissues. The remainder of the plant material was dried and ascorbic acid was determined at intervals to obtain its rate of oxidation. Analyses of iron and other components of the plant materials such as carbohydrate, nitrogen and chlorophyll, were carried out on the plant tissues. Any possible relationships between these

components and the ascorbic acid status of the wheat plants will be discussed in the presentation of the data.

### B. Experimental Conditions

#### 1. Iron and Copper Levels in the Nutrient Solution

After the complete nutrient solutions were made up, the following amounts of iron and copper were added:

| Concentration of Iron and Copper in the Nutrient Solutions of the First Experiment |                           |                           |                           |
|------------------------------------------------------------------------------------|---------------------------|---------------------------|---------------------------|
| Iron Series                                                                        |                           | Copper Series             |                           |
| ppm of Fe in the Solution                                                          | ppm of Cu in the Solution | ppm of Fe in the Solution | ppm of Cu in the Solution |
| 0.0                                                                                | 0.02                      | 1.0                       | 0.00                      |
| 1.0                                                                                | 0.02                      | 1.0                       | 0.01                      |
| 2.0                                                                                | 0.02                      | 1.0                       | 0.02                      |
| 3.0                                                                                | 0.02                      | 1.0                       | 0.03                      |
| 4.0                                                                                | 0.02                      | 1.0                       | 0.04                      |
| 5.0                                                                                | 0.02                      | 1.0                       | 0.05                      |
| 6.0                                                                                | 0.02                      | 1.0                       | 0.06                      |
| 7.0                                                                                | 0.02                      | 1.0                       | 0.07                      |
| 8.0                                                                                | 0.02                      | 1.0                       | 0.08                      |
| 9.0                                                                                | 0.02                      | 1.0                       | 0.09                      |

These two series of cultures will later be mentioned as the "iron series" and the "copper series."

The stock solution of iron was prepared by dissolving 1.6 grams of electrolytic iron in three milliliters of concentrated sulfuric acid. The volume of this solution was brought to 500 milliliters with distilled water. Five milliliters of this stock solution in 16 liters of nutrient solution correspond to one part per million of iron. No attempt was made to keep the iron level constant during the experimental period.

The stock solution of copper was prepared by dissolving 63 milligrams of copper sulfate ( $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ ) in distilled water. The volume of this solution was adjusted to 500 milliliters. Five milliliters of this stock solution in 16 liters of nutrient solution correspond to 0.01 part per million of copper.

During the growth of the plants, the nutrient solutions were renewed 13 days after seeding.

## 2. Plant Material

Thirty seeds of wheat, "Illinois No. 1-128," harvested in 1947, which was supplied by the Michigan State College Farm Crops Department, were sown in each pot on May 24, 1948. The seeds were evenly spaced and covered with about one-half inch of gravel. During germination, the pots were covered with cardboard to prevent evaporation and to secure even germination. The plants were harvested on June 28, 1948, 36 days after seeding. All the plants



at that time were starting to joint. The jointing stage was chosen as the time of harvest because, as shown by Kohler (1944), the composition of grass at that stage is especially high in ascorbic acid as well as many other organic constituents.

### C. Experimental Results

#### 1. Observations on the Plants

##### a. Description of the Plants at Time of Harvest

At harvest time all the plants had formed the first joint. The plants of the iron series had generally thicker stems and greener leaves than the ones of the copper series. However, the differences between the two series were not very striking.

Plants grown on the nutrient solution lacking iron started to show chlorosis about 20 days after seeding. An increase in the growth of the plants was observed to be about proportional to the increments of iron in the solution up to six parts per million. At that point the growth showed a tendency to lessen.

The plants on the copper series, though smaller in size than the ones of the iron series, were all normal and showed increased growth from no copper to 0.06 parts per million of copper in the nutrient solution. Then growth was lessened and the plant

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looked poorer in the following cultures containing increasing amounts of copper in the nutrient solution.

#### b. Fresh Weight of the Plants

##### (1) Effect of Iron

The data indicating the average fresh weight per ten plants expressed in grams are assembled in Table 1, and are graphed against the amount of iron in the solution in Figure 2. These data are the average values of two duplicate cultures. If the values obtained for the plants grown on three parts per million of iron are disregarded, an increase of the fresh weight up to six parts per million of iron is apparent. Then it falls off to reach approximately the same value as for the plants grown without iron.

##### (2) Effect of Copper

The data indicating the average fresh weight per ten plants expressed in grams are assembled in Table 3 and are graphed against the amounts of copper in the solution in Figure 45.

The effect of copper on growth as reflected from the fresh weight of the plants is pronounced. The growth was increasingly stimulated from the culture without copper to the one with 0.06 parts per million of copper. Then, a sharp decline brought back

the fresh weight per plant to a value lower than for the plants grown without copper.

### c. Dry Weight per Plant

#### (1) Effect of Iron

The data for the average dry weight per ten plants expressed in grams are indicated in Table 1 and are graphed against the amount of iron in the solution in Figure 3.

A general upward tendency was presented by the dry weight of the plants with increasing iron in the solution. This tendency did not decline as in the case of the fresh weight when the amount of iron in the solution was larger than six parts per million.

#### (2) Effect of Copper

The average dry weights per ten plants of the copper series are arranged in Table 3 and graphed against the amount of copper in the solution in Figure 46.

In this series of cultures, the dry weight of the plants paralleled the fresh weight values per plants, showing first an increase up to 0.06 parts per million of copper in the nutrient solution and then a decrease to a value lower than for the plants grown without copper.

## 2. Chemical Analyses of the Plants

In order to be able to compare more easily the data for iron absorption and the amounts of ascorbic acid found at the various determinations, these two components will first be discussed. The other plant components studied will be taken in consideration at the end of this paragraph on chemical analyses.

### a. Iron

#### (1) Amounts of Iron per Plant

(a) Effect of increasing the amounts of iron in the solution on the iron content of the plants. The milligrams of iron obtained per ten plants are presented in Table 1 and graphed against the amount of iron in the nutrient solution in Figure 4.

An increase in the iron content of the plants was observed following increase of iron in the nutrient solution but this increase was not a straight relationship. On the other hand, differences in the accumulation of iron corresponded to changes in dry weight of the different cultures. This might suggest that in the plants most of the iron was tied to the constituents forming the dry material. This consideration is strengthened by the fact that the nitrogen portion followed very closely the amount of iron accumulated. Another criterion of the importance of iron in the plant is the close

correlation between the amount of chlorophyll and the total amount of iron, as shown in Figure 13. The correlation between chlorophyll and iron has been observed by many authors but usually only a relationship between the so-called active iron and chlorophyll is noted. The present data show positively that straight relationship between total iron and chlorophyll may occur.

(b) Effect of increasing the amount of copper in the solution on the iron content of the plants. Table 3 indicates the milligrams of iron absorbed per ten plants in the copper series; these values are graphed against the amount of copper in the solution in Figure 47.

Inasmuch as the same amount of iron had been added to the complete nutrient solutions at the different levels of copper, it should follow that any difference in the amount of iron accumulated in the plant is intrinsically due to the influence of differences in the copper content of the nutrient solution. Up to 0.06 parts per million of copper in the nutrient solution corresponded to an increase in the amount of iron taken up which paralleled very closely the amount of dry matter (Figure 44) and carbohydrate (Figure 43). It should be noted that such a relation with the carbohydrate fraction had not been found in the iron series.

## (2) Amount of Iron per 100 Grams of Fresh Plant Material

(a) Effect of iron. The milligrams of iron per 100 grams of fresh plant material appear in Table 5 and Figure 16.

If the results obtained for the amount of iron in each sample are calculated on a fresh matter basis it is possible to get an idea of the actual concentration of that element in the living plant tissue. A slight upward tendency in the amount of iron in the fresh matter corresponding to increased amount of iron added to the nutrient solution can be noticed but this increase is not a linear function of the amount in the solution. The positive relationship with nitrogen which has been found in the data per plant is again found when these data are calculated on a fresh weight basis but it does not hold as closely. This relationship is presented in Figure 25. In the same manner the relationship with chlorophyll is not as clear as before, Figure 38.

(b) Effect of copper. The data indicating the milligrams of iron per 100 grams of fresh plant material of the copper series are shown in Table 7 and Figure 51.

The concentration of iron in the fresh tissue showed a slight decreasing trend with increasing copper in the solution. This trend is well correlated with the data for dry weight as shown graphically in Figure 60 and loosely with the total carbohydrate, Figure 61.



Both of these relationships are very striking when the data for the amount of iron per plant are computed.

(3) Amount of Iron per 100 Grams of Dry Material

(a) Effect of iron. The amount of iron in milligrams per 100 grams of dry plant material are presented in Table 9 and Figure 31.

The amounts obtained for the iron series do not show any precise tendency. After examining all the data obtained, no relation could be found and the ones which existed in the case of the amount of iron per plant or in the fresh tissue do not hold true when calculated for the iron in the dry plant material.

(b) Effect of copper. The data for the amount of iron in milligrams per 100 grams dry material in the copper series are assembled in Table 11 and Figure 63.

With increasing amount of copper in the solution less iron was obtained in the dry plant material. The relationship which had been found between iron and carbohydrate does not hold true when calculated on a dry matter basis and no other relationship was found.



## b. Ascorbic Acid

### (1) Description

For the purpose of studying the possible effects of iron and copper on the total amounts of ascorbic acid in the wheat plants and on its stability after drying, four different determinations were made at various intervals. The first determination on both series was done at the time of harvest, the second after the remainder of the plant material was dried in a forced air oven at 60° C for three days, the third 31 days later and the last 105 days after drying, the samples being kept at room temperature. The values obtained at each determination were calculated on a basis per 10 plants, per 100 grams fresh material and per 100 grams dry material; each result will be discussed separately,

The percentage of the original amount of ascorbic acid left after each determination was also calculated in order to get an idea of the relative rate of oxidation of the ascorbic acid in the plants from the different cultures. When these values are subtracted from hundred the percentages of loss is obtained.

### (2) Amount of Ascorbic Acid per Plant

(a) Effect of iron. The data expressing the amounts of ascorbic acid, at harvest, after drying, 31 days and 105 days

after drying per ten plants grown at various levels of iron are assembled in Table 2 and are graphed against the amount of iron in the solution in Figures 5, 6, 7, and 8.

The total amounts of ascorbic acid at harvest when calculated per plant did not show any relationship neither with the amount of iron in the solution nor with the amount of iron taken up by the plants. We might say that there was a slight tendency to decrease with increase iron in the solution but this trend was not at all definite.

(b) Effect of copper. The data for the amounts of ascorbic acid at harvest after drying, 31 days and 105 days after drying per ten plants of the copper series are presented in Table 4 and Figures 48, 49, 50, and 51.

A definite trend in the amount of ascorbic acid in the plants grown on the copper series would be difficult to indicate although it can be seen that as the fresh weight and as the dry weight, the amounts of ascorbic acid increased in the plants from the no copper level to the one grown on 0.06 parts per million of copper and then decreased in the subsequent cultures. The same trend persisted but less accentuated for the values of ascorbic acid obtained just after drying and 31 days after drying. Eighty-five to 95 per

cent of the initial amount of ascorbic acid had been lost after 105 days and no trend was shown anymore.

### (3) Amount of Ascorbic Acid per 100 Grams Fresh Matter

(a) Effect of iron. The data in milligrams per 100 grams fresh matter of ascorbic acid at harvest, after drying, 31 days and 105 days after drying, of the iron series appear in Table 6 and in Figures 17, 18, 19, and 20.

The amounts of ascorbic acid at harvest did not bear any apparent relationship with the other constituents of the plant. After drying the values for ascorbic acid had a tendency to be higher in the plant grown on higher iron content in the solutions and this relation was kept till 105 days after drying.

(b) Effect of copper. The milligrams of ascorbic acid per 100 grams of fresh plant material grown in the copper series and determined at harvest after drying, 31 days and 105 days after drying are recorded in Table 8 and Figures 52, 53, 54, and 55.

The amount of copper in the solution did not have any apparent effect on the ascorbic acid at harvest in the fresh material, however after drying the plants which had been grown on the highest amount of copper kept the most amount of ascorbic acid.

#### (4) Amount of Ascorbic Acid per 100 Grams Dry Plant Material

(a) Effect of iron. The data expressing in milligrams per 100 grams of dry plant material the amount of ascorbic acid at harvest, after drying, 31 days and 105 days after drying of the plant grown on the iron series are presented in Table 10 and Figures 32, 33, 34, and 35.

When calculated on a dry matter basis the amount of ascorbic acid at harvest decreased with the amount of iron in the solution. After the material had been dried the amount of ascorbic acid left was almost the same in all the cultures, pointing out the fact that in the plants grown without iron the ascorbic acid was lost faster. This conclusion should not be generalized in regard to the effect of iron in the plant on the ascorbic acid present, because the amount of iron found in the dry matter was not proportional to the amount of iron in the solution and no relationship could be drawn between ascorbic acid and the amount of iron in the plant.

(b) Effect of copper. The amount in milligrams of ascorbic acid per 100 grams of dry plant material grown on the copper series determined at harvest, just after drying, 31 days and 105 days after drying are assembled in Table 12 and graphed against the amount of copper in Figure 64, 65, 66, and 67.

In the dry matter of the plants grown at different levels of copper, the amount of ascorbic acid at harvest did not follow any clear relationship. After drying the amount of ascorbic acid became almost inversely proportional to the amount of iron in the dry matter as can be seen in Figure 72, but this relationship did not hold at the next determinations.

(5) Percentages of the Original Amount of Ascorbic Acid Found at Each Determination

(a) Effect of iron. The values of ascorbic acid calculated as percentages of the original amounts at each determination are presented for the iron series in Table 13 and Figure 73.

The percentage values obtained are independent of the basis of calculation of the ascorbic acid and accordingly can be related to the other chemical data obtained on a per plant, per 100 grams fresh matter and per 100 grams dry matter basis.

Upon drying, ascorbic acid was lost at a slower rate in the plant materials grown on the cultures supplied with high amounts of iron. Indeed, only 14 per cent of the original amount of ascorbic acid were lost after drying from the plant material grown on nine parts per million of iron, whereas 38 per cent were lost from the plant material grown on the culture where no iron was added. A positive correlation between the percentage of the original amount

of ascorbic acid found after drying and 31 days later and the concentration of iron in the solution can be seen from Figure 73; after 105 days this correlation still holds but not as strong as for the other values.

When the percentages are graphed against the amount of iron in the fresh material or in the dry matter (Figure 74) no apparent correlation is brought about, but a positive correlation, as seen in Figure 27, was observed between the percentage of the original amount of ascorbic acid found just after drying and the percentage of dry matter in the fresh tissue.

(b) Effect of copper. The data for the percentages of the original amount of ascorbic acid found at each determination in the plants grown on the copper cultures are reported in Table 14 and Figure 74.

An upward trend in the percentage of ascorbic acid left after drying can be detected with increase of copper in the solution but this tendency is not kept for the percentage left 31 days after drying. After 105 days almost all the ascorbic acid has disappeared, only 5.4 to 14 per cent were left, when these values are graphed against the amounts of copper in the solutions, a slight decrease is shown from no copper to 0.02 parts per million of copper and then an increase to 0.08 parts per million is apparent.

From the observation of Figure 76, it can be seen that the rate of loss did not show any proportionality with the amount of iron in the dry matter. However, to a small extent it showed a negative relation after 105 days after drying as shown in Figure 76.

No other correlations were found with any of the components analyzed and calculated on a per plant, per 100 grams fresh matter and per 100 grams dry matter.

#### c. Other Chemical Constituents

The nitrogen, chlorophyll and carbohydrate contents of the plants have been calculated in the same manner as for iron and ascorbic acid. No further discussion will be undertaken about these constituents since any relationship between them and iron and ascorbic acid have already been discussed.

The following table will mention the places where the data can be found:

| Constituents      | Amounts per<br>Ten<br>Plants |         | Amounts per<br>100 Fresh<br>Material |         | Amounts per<br>100 g. Dry<br>Material |         |
|-------------------|------------------------------|---------|--------------------------------------|---------|---------------------------------------|---------|
|                   | Tables                       | Figures | Tables                               | Figures | Tables                                | Figures |
| Nitrogen          | 1 & 3                        | 10 & 41 | 5 & 7                                | 22 & 57 | 9 & 11                                | 28 & 69 |
| Chlorophyll       | 1 & 3                        | 11 & 42 | 5 & 7                                | 23 & 58 | 9 & 11                                | 29 & 70 |
| Carbo-<br>hydrate | 1 & 3                        | 9 & 40  | 5 & 7                                | 21 & 56 | 9 & 11                                | 36 & 68 |

#### D. Discussion of the First Experiment

If we only had the data on the total amount of ascorbic acid found at harvest, no significant effect of the amounts of iron and copper added to the solution could be reported on the ascorbic acid in wheat plants. Indeed from one culture to another, the values of ascorbic acid varied widely with no consistent relationship neither with the amount of copper and iron in the nutrient solution nor with the amounts of other components (iron, nitrogen, carbohydrate, chlorophyll) analyzed in the plant material.

However, a new outlook on the problem emerges when the data on the amount of ascorbic acid left after drying are studied. It can then be concluded that the over-all effect of a greater amount



of iron or copper in the nutrient solution corresponded to a greater stability of the ascorbic acid in the wheat plants during drying.

These relationships become apparent when the percentage of the original amounts of ascorbic acid left at the successive determinations made on the material left after drying are calculated.

The plants from the iron series showed the most significant increase in the stability of their ascorbic acid with increase of iron in the nutrient solutions and this relationship held true at the various determinations after drying. On the other hand, a positive relationship between increase of copper in the nutrient solution and stability of ascorbic acid in the plants existed just after drying but disappeared 31 days after drying.

It would seem likely that since a greater stability of ascorbic acid occurred in the plants grown on higher amounts of iron and copper, this stability would be correlated to the amount of iron and copper in the tissue. Of these two elements only iron was determined and in the conditions of the experiment there was no proportional increase of iron in the tissue with increase of the amounts in the nutrient solutions and subsequently no relation could be found between the plant-iron and the ascorbic acid stability.

It was noticed that in the copper series, where the amounts of iron added in the nutrient solutions were similar for the whole series, the final concentration of this element in the plant varied widely

but no clear relationship could be drawn between the amount present and the ascorbic acid stability.

From these conclusions, two suggestions will be advanced to explain the correlation between the increase of stability of ascorbic acid in the plant material after drying and the increase of iron in the nutrient solution. It is conceivable that only a fraction of the total iron present in the plant is responsible for the increase in the stability; this fraction would then be proportional to the amount of iron present in the nutrient solution. Indeed it is known that for other processes such as chlorophyll formation only a portion of iron is active as has been reported by Jacobson (1945). The other suggestion is that by increasing the amount of iron in the nutrient solution, another factor is proportionally affected which has a direct bearing on the ascorbic acid system of the plant; a more complete fractionation of the plant components should then be conducted in order to find this possible factor.

As can be seen from the results obtained in this experiment and if we assume that no ascorbic acid is synthesized after the harvesting of the plant, it can be concluded that the apparent behaviour of ascorbic acid in drying plant material is quite different from its behaviour in vitro.

It should be noted that the actual differences in amounts of iron absorbed by the plants grown on various levels of iron in the

nutrient solution were not great and not proportional to the amounts added in the nutrient solution. Lyon and Beeson (1948), on the other hand, were able to obtain proportional increase of iron in the plants grown on increasing amounts of iron. It is suggested that the gravel itself on which the plants were grown might contain impurities which should be taken out before starting a new experiment. A possible reason for the difference in the amount of iron taken up by the plants on the copper series might also lie in the impurities of the gravel.

It is suggested that a new experiment using the same plant material grown over a wider range of iron and copper concentrations in the substrate should be undertaken to find out if the same tendencies would be duplicated and hold true at the higher concentrations of these elements.

#### E. Summary of the First Experiment

1. Wheat plants were grown in gravel on two series of cultures; the basic medium of each culture was a complete three salts nutrient solution. In the first series, each culture was differentiated from the other by an increment of iron of one part per million, the whole series ranging from no iron to nine parts per million. In the second series, each culture differentiated from the

other by an increment of copper of 0.01 parts per million, the whole series ranging from no copper to 0.09 parts per million.

2. The plants were harvested at jointing stage. Ascorbic acid and chlorophyll were then determined on the fresh tissues. Iron, nitrogen and carbohydrate were determined on oven dry samples of the tissues. Ascorbic acid was again determined just after drying, 31 days and 105 days after drying. The data were calculated on a basis per plant, per 100 grams fresh matter and per 100 grams dry matter. Fresh and dry weight of the cultures were recorded.

3. In the iron series, the fresh weight of the plants increased with higher iron concentration up to six parts per million of iron in the solution. The dry weight showed an upward tendency with increase iron up to the last culture. In the copper series, the fresh and dry weight paralleled each other, increasing with higher copper concentration in the solution up to 0.06 part per million of copper and then decreasing until the last culture of the series.

4. At harvest, no correlation between the amount of ascorbic acid could be found with the amount of iron in the nutrient solution or in the plant calculated on a basis per plant, per 100 grams dry matter or per 100 grams of fresh tissue.

5. The rate at which ascorbic acid was lost during and after drying in the plants grown on the iron series was inversely correlated with the amount of iron in the solution on which the plants were grown. No relationship, however, could be found between the amount of iron in the fresh tissue and the rate at which ascorbic acid was lost.

6. The amount of iron found in the plants grown on the copper series was positively correlated with the dry weight of the plants. The relationship between iron and dry matter held true when the data were calculated on a fresh tissue basis but disappeared on a dry weight basis.

7. At harvest, no correlation between the amount of ascorbic acid could be drawn with the amount of copper in the nutrient solution or the amount of iron in the fresh tissue of the copper series..

8. The rate at which ascorbic acid was lost during and after drying was inversely correlated with the amount of copper in the solution on which the plants grew.

## IV. EXPERIMENT 2

### A. Purpose

It was suggested in the discussion of the first experiment that the positive relationship found between the stability of ascorbic acid and the concentration of iron and copper in the solution be subjected to a new trial.

In order to check the results obtained during the first experiment and at the same time to explore the effects of higher concentrations of copper and iron in the solution, it was thought to start the series of culture at the same level as for the first experiment, i.e., no iron or no copper but to add in the following cultures larger increments of these elements. In this manner by overlapping with the previous experiment and using the same plant material and the same experimental methods, the results obtained would be a complement to the first observations.

### B. Experimental Conditions

#### 1. Iron and Copper Levels in the Nutrient Solution

To complete three salts nutrient solutions, as used in the first experiment, were added the following amounts of iron and copper:

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Concentration of Iron and Copper in the Nutrient Solutions  
of the Second Experiment

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| Iron Series                  |                              | Copper Series                |                              |
|------------------------------|------------------------------|------------------------------|------------------------------|
| ppm of Fe in<br>the Solution | ppm of Cu in<br>the Solution | ppm of Fe in<br>the Solution | ppm of Cu in<br>the Solution |
| 0.0                          | 0.02                         | 1.0                          | 0.00                         |
| 2.0                          | 0.02                         | 1.0                          | 0.02                         |
| 4.0                          | 0.02                         | 1.0                          | 0.04                         |
| 6.0                          | 0.02                         | 1.0                          | 0.06                         |
| 8.0                          | 0.02                         | 1.0                          | 0.08                         |
| 10.0                         | 0.02                         | 1.0                          | 0.10                         |
| 12.0                         | 0.02                         | 1.0                          | 0.12                         |
| 14.0                         | 0.02                         | 1.0                          | 0.14                         |
| 16.0                         | 0.02                         | 1.0                          | 0.16                         |
| 18.0                         | 0.02                         | 1.0                          | 0.18                         |

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Iron and copper were added in the same form as in the first experiment from a stock solution of ferric sulfate and cupric sulfate.

## 2. Plant Material

Thirty seeds of wheat Illinois No. 1-128 were seeded in the quartz gravel in each pot on October 25, 1948, and were harvested 19 days later just before jointing stage on November 13, 1948.

## C. Experimental Results

### 1. Observations on the Plants

#### a. Description of the Plants at Time of Harvest

When the plants were harvested, they had not yet started to joint.

As was observed at the end of the first experiment, the plants on the iron series were somewhat better than those grown on the copper series, showing thicker stem and greener leaves.

On the iron series, the healthiest looking plants were grown on the culture containing six parts per million of iron.

The plants growing on the various levels of copper were all healthy and the best growth was obtained on the highest level of copper, i.e., 0.18 parts per million, contrary to what was observed during the first experiment where best plants were obtained at 0.06 parts per million of copper.

#### b. Fresh Weight of the Plants

##### (1) Effect of Iron

The data for the average fresh weight per ten plants expressed in grams are given in Table 15 and are graphed against the amount of iron in the nutrient solution in Figure 82.



According to these values, the highest weight per plant was obtained on the highest concentration of iron in the nutrient solution. However this value is somewhat out of the general line of the other results and if it is taken out, the curve then shows a maximum at six parts per million which corresponds to the observation made on the appearance of the plants at harvest.

## (2) Effect of Copper

The data indicating the average of the fresh weight per ten plants expressed in grams are assembled in Table 16 and are graphed against the amount of copper in the nutrient solution in Figure 119.

A general increase in the weight of the plants was shown from the culture without copper to the one with 0.18 parts per million of copper. This tendency was different from the one obtained during the first experiment, where the best growth of the plants occurred at 0.06 parts per million of copper and then decreased.

## c. Dry Weight per Plants

### (1) Effect of Iron

The data for the average dry weight per ten plants expressed

in grams are shown in Table 15 and are graphed against the amount of iron in the solution in Figure 83.

The variations in the dry weight of the plants showed the same tendency as the fresh weight and this tendency was even more pronounced. If the value for the plants grown on the solution with 18 parts per million is not considered, then a maximum of dry weight is obtained at a concentration of six parts per million of iron in the nutrient solution.

## (2) Effect of Copper

The average dry weight per ten plants of this series are arranged in Table 16 and graphed against the amount of copper in the solution in Figure 120.

The dry weight of the plants of the copper series did not parallel the fresh weight and showed two maxima, one at 0.06 parts per million and the other at 0.14 parts per million of copper. The reason for the irregularities in the dry weight of the plants is rather obscure but as we will see later it was followed by the amount of iron in the plants.

## 2. Chemical Composition of the Plants

### a. Iron

#### (1) Amount of Iron per Plant

(a) Effect of increasing the amounts of iron in the solution on the absorption of iron by the plant. The milligrams of iron absorbed per ten plants are presented in Table 15 and graphed against the amount of iron in solution in Figure 77.

To an increase of iron in the solution corresponded an increase of amount of iron absorbed per plant. This response was similar to the one obtained during the first experiment. A positive correlation between iron and dry weight of the plant was also obtained as during the first experiment. The other relations dealing with ascorbic acid will be considered later.

(b) Effect of increasing the amounts of copper in the solutions on the absorption of iron. The amounts of iron, expressed in milligrams, absorbed per ten plants of the copper series are reported in Table 16 and graphed against the amount of copper in the solution in Figure 114.

Although the amounts of iron absorbed per plant did not show any clear relationship with the amounts of copper in the solution, its variations were followed by the dry weight of the

plants. This data gives a great weight to the close relationship of iron to dry matter, which had been obtained during the first experiment on both iron and copper series. Relationships with ascorbic acid will be discussed later.

## (2) Amount of Iron per 100 Grams of Fresh Material

(a) Effect of iron. The milligrams of iron per 100 grams of fresh plant material appear in Table 17 and Figure 89.

Very clearly the concentrations of iron increased in the fresh tissue of the plants grown with increasing amounts of iron in the solution. This relationship is similar to the one obtained during the first experiment but is much more significant.

(b) Effect of copper. The data indicating the milligrams of iron per 100 grams of fresh plant material of the copper series are shown in Table 18 and Figure 126.

As in the first experiment the amounts of iron per 100 grams of fresh material were quite variable from one culture to the other and a strong positive relationship correlated it with dry weight.

### (3) Amount of Iron per 100 Grams of Dry Matter

(a) Effect of iron. The data for the amount of iron in milligrams per 100 grams of dry material in the iron series are assembled in Table 19 and in Figure 106.

With increasing increments of iron in the nutrient solution were obtained increasing amount of iron in the dry matter. At the same time, the percentage of water in the tissues was increasing as can be seen in the figure 111 graphing the grams of fresh material corresponding to 100 grams of dry matter for the different cultures of the iron series, against the amount of iron in the solution.

(b) Effect of copper. The amount of iron in milligrams per 100 grams of dry plant material are presented in Table 20 and Figure 135.

As the amount of copper varies in the solution, the amount of iron found in the dry plant material changed but did not follow any clear relationship. During the first experiment, less iron was found in the tissues grown on higher copper levels, but this tendency did not show again during this experiment.

## b. Ascorbic Acid

### (1) Description

As for the first experiment, a study of the stability of the ascorbic acid in the plant after harvest was undertaken. It was judged from the results of the first experiment that the successive determinations should be done over a shorter period of time.

The first determination was done at harvest, the second just after drying in a forced air oven at  $60^{\circ}$  C for three days, the third seven days later and the last fourteen days after drying. The samples of plant material after drying were kept at constant temperature in an incubator at  $30^{\circ}$  C.

### (2) Amount of Ascorbic Acid per Plant

(a) Effect of iron. The data for the amounts of ascorbic acid at harvest, after drying, seven days and fourteen days after drying per ten plants of the iron series, are presented in Table 15 and Figures 78, 79, 80, and 81.

The amount of ascorbic acid per plant, at harvest, was inversely proportional to the amount of iron taken up by the plant, except for the value of ascorbic acid obtained per plant grown without iron. This value was the lowest one and out of the general line of the other values.

A very special behaviour was shown by the amount of ascorbic acid left after drying; indeed the relationship observed at harvest between the amount of ascorbic acid per plant and the amount of iron in the corresponding nutrient solution shifted to the opposite sign: more ascorbic acid remained in the plants grown on the highest levels of iron. This relationship held true with amount of ascorbic acid found seven and fourteen days after drying..

Significant positive correlations were obtained between the amount of ascorbic acid remaining after drying, seven days and fourteen days later and the amount of iron in the plants, but in all the cases the value for the plants grown without iron should be considered apart (Figures 86, 87, and 88).

(b) Effect of copper. The data expressing the amount of ascorbic acid at harvest, after drying, seven days and fourteen days after drying per ten plant grown at various levels of copper are assembled in Table 16 and are graphed against the amount of copper in the solution in Figures 115, 116, 117, and 118.

When the amounts of ascorbic acid at harvest and at the different determinations after drying are observed, no apparent relationship with the amount of copper in the solution can be drawn.

However, a positive correlation is apparent between the values of ascorbic acid of these plants at harvest and the amounts of iron found in the plants. This relationship can be seen in Figure 122.

After drying, when the amount of ascorbic acid left is plotted against the amount of iron in the plant a negative correlation appears. But after fourteen days this relation has disappeared.

### (3) Amount of Ascorbic Acid per 100 Grams of Fresh Matter

(a) Effect of iron. The average milligrams of ascorbic acid per 100 grams of fresh plant material grown in the iron series and determined at harvest, after drying, seven days and fourteen days after drying are recorded in Table 17 and Figures 90; 91, 92, and 93.

When the results for ascorbic acid are calculated on a basis per 100 grams of fresh tissue, the correlations which appeared between iron and ascorbic acid per plant stayed almost unchanged. Indeed, the amounts of ascorbic acid per 100 grams of fresh matter at harvest was inversely proportional to the concentrations of iron in the solution and in the plant, Figure 90 and 95. The shifting from a negative to a positive correlation between ascorbic acid and the concentration of iron in the fresh tissue, occurred after drying and more clearly seven days after drying,



as had been observed before, when the data were calculated on a per plant basis. These relations may be seen in Figures 97, 98, and 99.

A strong positive correlation was found as seen in Figure 96 between the concentration of dry matter in the fresh tissue and the amount of ascorbic acid in that tissue at harvest.

(b) Effect of copper. The data in milligrams per 100 grams of fresh matter of ascorbic acid at harvest, after drying, seven days and fourteen days after drying, of the copper series appear in Table 18 and in Figures 127, 128, 129, and 130.

No apparent relationship was indicated between the amount of copper in the solution and the amount of ascorbic acid found at harvest in 100 grams of fresh tissue. However a significant positive relationship is obtained, when the amount of ascorbic acid at harvest is graphed against the amount of iron in 100 grams of fresh tissue; this correlation had already been obtained when the values were calculated on a per plant basis.

A negative correlation appears between the ascorbic acid left after drying and the amount of iron in the tissue, this correlation becomes not so apparent after seven or fourteen days of drying, Figures 102, 103, and 104.

In both copper and iron series a positive correlation was obtained with the concentrations of ascorbic acid at harvest and the dry material in the fresh tissue.

(4) Amount of Ascorbic Acid per 100 Grams of Dry Material

(a) Effect of iron. The amounts in milligrams of ascorbic acid per 100 grams of dry plant material grown on the iron series, determined at harvest, just after drying, seven days and fourteen days after drying are assembled in Table 19 and graphed against the amount of iron in the solution in Figures 107, 108, 109, and 110.

To an increase in the amount of iron in the solution corresponded at harvest a decrease of ascorbic acid in the dry material and a negative correlation between the amount of iron in the dry matter and the ascorbic acid can be shown in Figure 112. The values for the plants grown without iron should be considered as a special case, indeed at harvest and after drying these values behaved in the opposite way to all the other values.

Seven days after harvest, the plant material with high amount of iron had the greatest amount of ascorbic acid left as is indicated in Figure 113 and the same relationship was kept fourteen days after drying.

(b) Effect of copper. The data expressing in milligrams per 100 grams of dry material, the amount of ascorbic acid at harvest, after drying, seven and fourteen days after drying of the plants grown on the copper series are presented in Table 20 and Figures 136, 137, 138, and 139.

The amounts of copper in the nutrient solution did not show any correlation with the amount of ascorbic acid at harvest calculated on a dry weight basis. However a slight positive correlation was found between the amount of iron in the dry matter and the ascorbic acid at harvest, this relationship is presented in Figure 132. Seven days after drying no apparent relationship can be drawn neither with copper nor with iron as can be seen in Figures 134 and 138.

(5) Percentage of the Original Amount of Ascorbic Acid Found at Each Determination

(a) Effect of iron. The data showing the percentage of the original amounts of ascorbic acid found in the iron series after drying, seven days and fourteen days after drying are presented in Table 21 and Figure 141.

From Figure 141 it is apparent that, with the exception of the "no iron" value, the percentages of the original amounts of ascorbic acid were at each determination positively correlated to

the amount of iron in the solution. From Figures 86 and 87, it can be seen that this positive relationship holds true when the amount of iron per ten plants is considered.

The greatest rate of oxidation of the ascorbic acid occurred during the drying process and at a higher rate in the plants containing less iron: In the plant grown on the two parts per million level, 65 per cent of the ascorbic acid were lost, when only 49 per cent were lost in the plants grown on the 16 parts per million level.

(b) Effect of copper. Table 22 and Figure 142 report the values of the percentages of the original amount of ascorbic acid found in the copper series after drying, seven days and fourteen days after drying.

An observation of the Figure 142 does not lead to any apparent relationship between the percentage of ascorbic acid left, and the amount of copper in the solution, but if the percentages values are graphed against the amount of iron in 100 grams of fresh tissue, a very significant negative correlation appears as can be seen in Figure 105. In this case the influence of iron on the catalysis of ascorbic acid in the drying material is shown very definitely.

#### D. Discussion on the Second Experiment

To reproduce the environmental factors is almost impossible and is the major cause to the great difficulty in duplicating quantitative results in biological experimentation. Because of necessity this second experiment was not started at the same time of the year as the first one and the plants were harvested at a younger stage; these reasons would explain the great differences between the quantitative results obtained during the two experiments, but also will give more significance to the comparable tendencies.

As far as iron is concerned, this second experiment was more conclusive than the first one, because according to the experimental results the absorption of this element was directly proportional to the amount of iron added in the solution. This trend was the same as that reported by Lyon and Beeson (1948) in turnips and tomatoes grown at different levels of iron, these workers indeed observed a significant difference in the iron content of the vegetative plant parts at the successive iron treatments. These authors also reported the rather limited power of plants to accumulate iron, in fact to a thirty-fold increase in the concentration of iron supplied to turnips was associated less than a two-fold increase in the iron concentration of the leaves. The results of the present experiment showed that by a comparison of the

results obtained from wheat plants supplied with two parts per million and those supplied with 18 parts per million of iron, less than a two-fold increase in iron concentration occurred.

The amounts of ascorbic acid found at harvest in the plants grown on the iron series, except for the one grown without iron, were inversely proportional to the amount of iron in the plant tissue. This tendency which was not shown at the first experiment should however be considered as specially important because of its similarity with the one obtained by Wynd (1950a) in oats grown in Texas. On the other hand, in the copper series, where the amount of iron was constant in the solution, the amount of ascorbic acid found at harvest showed a positive relation with the amount of iron in the fresh tissue. These two opposite tendencies suggest that iron and copper have a special interrelationship effect on the ascorbic acid equilibrium in the plants.

Among the results which showed the same trends during both experiments was the positive relationship between the amount of iron absorbed by the plants and the yield of dry matter of these plants. Although this study is not directly related to this phase of the effect of iron, it should be emphasized that this relationship occurred under different environmental conditions and also in both copper and iron series.

The most interesting result obtained during this second experiment was the duplication of the tendency observed in the first experiment that the plants from the iron series showed a significant increase of the stability of their ascorbic acid with increase of iron in the solution. It was also observed that a positive correlation occurred between increase of copper in the solution and stability of ascorbic acid in the plants during drying.

Iron was found, in this experiment, to be directly related to the ascorbic acid in two different and even opposite ways. In the iron series a negative correlation was found between the percentage of loss of ascorbic acid during drying and the actual amount of iron in the fresh tissue. On the other hand, in the copper series a positive correlation was obtained when the percentage of loss of ascorbic acid was compared to the amount of iron in the fresh tissue.

These two opposite results do not contradict themselves, because they were obtained in two different sets of conditions, indeed in the first the amount of iron in the solution was increasing and the copper maintained constant and in the second the amount of iron was left constant in the solution but the levels of copper were increased.

It is worth pointing out that at harvest in the iron series the amount of ascorbic acid found was inversely proportional to

the amount of iron in the fresh tissue and that during drying the greater stability of the ascorbic acid occurred in the plants with the highest amount of iron. On the opposite, in the copper series, the amount of ascorbic acid was directly proportional to the amount of iron in the plant and during drying the greater stability of the ascorbic acid occurred in the plant with the lowest amount of iron in the tissue.

Because of the consistency of the trend of the influence of higher levels of iron and copper on the stability of ascorbic acid during the two first experiments, it would be very desirable to know if these tendencies persist in the range of toxicity of these elements. There would be also a need for a more complete analysis of the effects caused by the different treatments on the amount of the other elements absorbed and in particular on the amounts of copper taken up by the plants.

#### E. Summary of the Second Experiment

1. Wheat plants were grown in gravel on two series of cultures whose basic medium was a complete three salt nutrient solution where iron and copper were the only varying factors. The iron series ranged from no iron to 18 parts per million, each culture varying from the one below by an increment of two parts per million. The copper series ranged from no copper to 0.18 parts



per million, each culture varying from the one below by an increment of 0.02 parts per million of copper. This experiment was designed to overlap with the first experiment and to observe if the same tendencies would persist for a wider range of concentrations of these two elements on the synthesis and stability of ascorbic acid.

2. The plants were harvested just before jointing stage. Ascorbic acid was determined on the fresh tissues. Iron was determined on oven dry samples of the tissues. Ascorbic acid was determined again just after drying, seven days after drying and fourteen days after drying, the dried material being kept at constant temperature at 30° C. The data were calculated on a basis per plant, per 100 grams fresh matter and per 100 grams dry matter. Fresh and dry weight of the cultures were recorded.

3. The fresh weight of the plants in the iron series followed the same tendency as during the first experiment: It increased with higher iron concentration up to six parts per million in the solution; the dry weight paralleled the trend of the fresh weight. In the copper series the fresh weight increased up to the last culture of 0.18 parts per million of copper but the dry weight varied widely and was found to correlate positively with the iron absorbed per plant.

4. As the iron concentrations increased in the solution, a corresponding increase of iron absorbed by the plant was observed which was also found in the concentration of this element in the fresh and dry tissues.

5. The amount of ascorbic acid found at harvest in the plants of the iron series, except for the one grown without iron, was inversely proportional to the amount of iron in the solution and also to the amount of iron absorbed by the plants.

6. The rate of loss of ascorbic acid in the plants of the iron series during drying was inversely correlated to the amount of iron in the plant and in the solution.

7. In the copper series, a positive correlation could be drawn between the amount of ascorbic acid at harvest and the total amount of iron per plant and also the percentage of iron in the dry matter.

8. The rate of loss of ascorbic acid was irregular in the plants of the copper series and could not be correlated to the amount of copper in the solution but was positively related to the concentration of iron in the fresh tissue.

## V. EXPERIMENT 3

### A. Purpose

It was found in the two first experiments that to an increase of iron and copper in the nutrient solutions corresponded an increase in the stability of the ascorbic acid during the drying of the plants. Since these tendencies persisted over the range of iron and copper where these elements showed a stimulating effect on growth, it was proposed to investigate the effect of these same elements up to toxic levels.

It was also deemed useful to follow the effect of increasing amounts of iron and copper up to the toxic levels on the absorption of the other major elements, nitrogen, phosphorus, potassium, calcium, and magnesium. The symptoms of toxicity and the possible effects on the ascorbic acid would then be connected with the observed status of the other elements.

The use of larger increments from one culture to the other would permit again an overlapping of the previous concentrations and also reach toxic levels of copper and iron.

The same plants and same basic nutrient solution would also allow a comparison of the effect obtained with those previously established.

## B. Experimental Conditions

### 1. Iron and Copper Levels in the Nutrient Solution

The following amounts of iron and copper were added to the same complete three salts solutions as used in the two first experiments:

| Concentration of Iron and Copper in the Nutrient Solutions<br>of the Third Experiment |                              |                              |                              |
|---------------------------------------------------------------------------------------|------------------------------|------------------------------|------------------------------|
| Iron Series                                                                           |                              | Copper Series                |                              |
| ppm of Fe in<br>the Solution                                                          | ppm of Cu in<br>the Solution | ppm of Fe in<br>the Solution | ppm of Cu in<br>the Solution |
| 0.0                                                                                   | 0.02                         | 1.0                          | 0.0                          |
| 3.0                                                                                   | 0.02                         | 1.0                          | 0.3                          |
| 6.0                                                                                   | 0.02                         | 1.0                          | 0.6                          |
| 9.0                                                                                   | 0.02                         | 1.0                          | 0.9                          |
| 12.0                                                                                  | 0.02                         | 1.0                          | 1.2                          |
| 15.0                                                                                  | 0.02                         | 1.0                          | 1.5                          |
| 18.0                                                                                  | 0.02                         | 1.0                          | 1.8                          |
| 21.0                                                                                  | 0.02                         | 1.0                          | 2.1                          |
| 24.0                                                                                  | 0.02                         | 1.0                          | 2.4                          |
| 27.0                                                                                  | 0.02                         | 1.0                          | 2.7                          |

Iron and copper were added in the same form as in the two first experiments from a stock solution of ferric sulfate and cupric sulfate.

## 2. Plant Material

On March 2, 1949, forty seeds of wheat Illinois No. 1-128 were sown in the quartz gravel in each pot. The coleoptiles appeared on March 6, four days after planting. The plants were harvested on April 15, forty-five days after planting.

### C. Experimental Results

#### 1. Observations on the Plants

##### a. Description of the Plants

On March 16, when the plants were 15 days old, noticeable differences could be observed between the various cultures on both series.

In the copper series a toxicity could be noticed at 0.6 parts per million which increased with higher levels of copper. In the cultures where the level of copper exceeded 0.6 parts per million, the first leaf formed remained green, but the new leaves became more and more chlorotic with increasing levels of copper. At harvest the plants grown on 2.7 parts per million of copper were very much stunted in growth and were starting to perish.

Chlorosis started to appear 15 days after seeding on the plants grown on the cultures where no iron had been supplied. In

the remaining of the iron series, the visible quality of the plants shown by size and color of leaves increased with higher concentrations of iron up to 15 parts per million of iron in the nutrient solution. On the higher levels of iron, the general appearance of the plants did not change.

#### b. Fresh Weight per Plant

##### (1) Effect of Iron

The data indicating the fresh weight per ten plants expressed in grams are given in Table 23 and are graphed against the amount of iron in the nutrient solution in Figure 151.

The plants grown on the nutrient solution where no iron had been added were much smaller than those of the remaining of the series. The fresh weight of the other plants showed a general increase with increase of iron in the nutrient solution but the differences in weight between two consecutive cultures were not very significant.

##### (2) Effect of Copper

The data for the average fresh weight per ten plants of the copper series expressed in grams are assembled in Table 25 and are graphed against the amounts of copper in the nutrient solution in Figure 193.

The tendency shown by the fresh weights of the plants of the copper series was much more significant than the one obtained with iron. Up to 0.6 parts per million, no definite changes occurred in the weight of the plants but then with increasing concentration of copper, an abrupt decline was observed. Indeed the plants grown in the solution with 1.8 parts per million of copper weighed about 50 per cent less than those grown on 0.6 parts per million and at 2.7 parts per million they weighed about 80 per cent less than those grown on 0.6 parts per million.

### c. Dry Weight per Plant

#### (1) Effect of Iron

The average dry weight per 10 plants of the iron series are arranged in Table 24 and graphed against the amounts of iron in the nutrient solution in Figure 144.

From none to six parts per million of iron in the nutrient solution, the dry weight of the plants increased, but with higher concentration the dry weight stayed about constant and no special trend could be defined.

#### (2) Effect of Copper

The data for the average dry weight per ten plants expressed

in grams are shown in Table 26 and are graphed against the amounts of copper in nutrient solution in Figure 185.

To an increase of copper in the nutrient solution of 0.6 to 2.7 parts per million corresponded a decrease in the dry weight of the wheat plants; these levels were toxic to the plants and were shown to produce chlorosis in the new leaves formed. In a general way, the dry weight followed the same tendency as the fresh weight.

## 2. Chemical Analyses of the Plants

The effects of increasing the amount of iron and copper in the nutrient solution on the chemical composition of the wheat plants was determined by the analyses of the experimental samples for iron and copper and the other major chemical components: nitrogen, phosphorus, potassium, calcium, and magnesium, as well as ascorbic acid.

### a. Iron

#### (1) Amounts of Iron per Plant

(a) Effect of increasing the amount of iron in the nutrient solution on the iron content of the plants. The amounts of iron expressed in milligrams, accumulated per plants of the iron series are reported in Table 24 and graphed against the amount of iron in Figure 145.



As in the two first experiments, to an increase of iron in the solution corresponded an increase in the total amount of iron found per plant. This increase did not follow a straight curve but was greater in the low concentrations of iron in the nutrient solution up to nine parts per million and then flattened. When the values for the total amount of iron per plant were compared to the values for the total amounts of the other components, positive correlations with the following components were observed: phosphorus, nitrogen, potassium, magnesium, and also the dry weight and the fresh weight. The other relations dealing with ascorbic acid will be considered later. These numerous positive correlations show that an increase of iron present in the solution stimulated growth and the absorption of the other elements. No levels had been reached where the amount of iron seemed detrimental.

(b) Effect of increasing the amount of copper in the nutrient solution on the accumulation of iron by the plant. The milligrams of iron accumulated per ten plants are presented in Table 26 and graphed against the amount of copper in the nutrient solution in Figure 186.

Above 0.6 parts per million of copper in the solution, to an increase of copper corresponded a decrease in the total amount of iron absorbed per plant. This decrease was followed by all the

other components analyzed for in the plant with the exception of calcium. This over-all effect of copper is understood by the fact that above 0.6 parts per million in the nutrient solution, the concentrations were more and more toxic to the plants and checked growth correspondingly.

## (2) Amounts of Iron per 100 Grams of Fresh Material

(a) Effect of iron. The data indicating the milligrams of iron per 100 grams of fresh plant material of the iron series are shown in Table 28 and Figure 159.

The concentrations of iron in the plant tissue increased with higher amounts of iron in the solution. This tendency was similar to the one obtained during the two first experiments, but above 18 parts per million of iron in the nutrient solution to the following increases of iron in the nutrient solution corresponded a decrease in the concentration of iron in the plant material. Positive correlations between the concentrations of iron in the fresh tissue and nitrogen, potassium, and dry matter were observed.

(b) Effect of copper. The milligrams of iron per 100 grams of fresh plant material of the copper series appear in Table 30 and are graphed against the amount of copper in the solution in Figure 201.

With the increases of copper in the nutrient solution up to 1.5 parts per million corresponded a decrease in the amounts of iron in the tissue. After this level the concentration of iron in the tissue started to increase up to 2.7 parts per million of copper in the nutrient solution. This tendency was not followed by any of the other components and for the first time no apparent correlation was obtained between the iron of the tissue and the amount of dry weight.

### (3) Amount of Iron per 100 Grams of Dry Matter

(a) Effect of iron. The amounts of iron in milligrams per 100 grams of dry plant material are presented in Table 31 and Figure 170.

Not much differences were observed in the concentration of iron found in the dry tissues of the plants grown on the various levels of iron, however a slight upward tendency can be detected up to 24 parts per million of iron. The value for the "no iron" plants is out of the general line and ought to be disregarded for this consideration. No apparent relation with other components were found.

(b) Effect of copper. The data for the amounts of iron per 100 grams of the dry material in the copper series are assembled in Table 32 and in Figure 208.

The amounts of iron found in the dry tissue, was inversely related to the amount of copper in the nutrient solution. This relationship was observed during the first experiment but did not appear at the second. No evident correlation could be found with the other components.

## b. Copper

### (1) Description

It was of interest for this study to obtain information on the accumulative power of the wheat plants toward the copper present in increasing amounts in the solution. Analyses for copper were made only on the plants of the copper series where significant differences in the amounts of copper between plants could be obtained.

### (2) Amount of Copper per Plant

The milligrams of copper absorbed per plant grown on nutrient solutions containing increasing amounts of this element are presented in Table 25 and graphed against the amount of copper in the solution in Figure 192.

Up to 0.6 parts per million of copper in the nutrient solution corresponded a sharp increase in the total amount of copper accumulated per plant. After this level, which corresponded to

the beginning of toxicity symptoms,, the total amount of copper found per plant decreased sharply in an inverse relationship with the levels of copper in the nutrient solution. This result might be explained by the effect of toxic levels of copper on the total growth, which then did not allow the plant to absorb more of this element. When the data for the other components are calculated on a per plant basis, the same decreasing tendency is observed.

### (3) Amounts of Copper per 100 Grams of Fresh Matter

The amounts of copper in milligrams per 100 grams of fresh tissue of the copper series are reported in Table 30 and Figure 202.

A strong positive correlation was found between the concentrations of copper in the nutrient solution and the amounts of copper found in the fresh tissue. Indeed the concentration of copper in the fresh tissue of plants grown on the 2.7 parts per million of copper was 3.4 times greater than its concentration in the plant tissue grown without copper.

It is interesting to note that above 1.2 parts per million of copper in the nutrient solution corresponded an apparent and general change of rate of accumulation of the following elements: phosphorus, calcium, and magnesium (Figures 195, 198, and 199).

#### (4) Amounts of Copper per 100 Grams of Dry Matter

The data for the amounts of copper in milligrams per 100 grams of dry material in the copper series are assembled in Table 32 and graphed against the amount of copper in the solution in Figure 214.

The same increasing tendency in the concentration of copper as in the fresh matter, occurred in the dry tissue of plants grown on increasing levels of copper. However, when these values are considered, it is observed that the rate of accumulation of copper on a dry matter basis was not as great at the higher levels of supply as at the lower levels. This same observation had been made by Lyon and Beeson (1948) for other microelements such as molybdenum, manganese and zinc.

As was observed with the data on a fresh weight basis, a change in the rate of absorption of phosphorus, calcium and magnesium and also potassium and magnesium was apparent, when the values were calculated on a dry matter basis, for the plants grown above the 1.2 parts per million of copper level (Figures 209, 212, 210, and 213).

### c. Ascorbic Acid

#### (1) Description

It was observed during the two first experiments that the amounts of ascorbic acid decreased mostly during the drying process. For this reason, it was decided to follow the loss of ascorbic acid during the drying of the plant material. The original amounts of ascorbic acid at harvest were first determined, then the plants were left in trays in the laboratory at room temperature and after intervals, portions of the drying samples were taken and analyzed. For the iron series, the first determinations were done at harvest, the second fourteen hours later, the third 43 hours after harvest, then 62, 110, and 157 hours after harvest. For the copper series the first determinations were done at harvest, the second 13 hours later, the third 30 hours after harvest, then 52, 100, and 147 hours after harvest.

The amounts of ascorbic acid found at harvest were calculated on a per plant, per 100 grams fresh matter and 100 grams dry matter basis in order to be able to compare these results with those of the other components analyzed. On the other hand, the amounts of ascorbic acid left at the various intervals of drying were only calculated on a 100 grams fresh weight basis because the rate of oxidation of the ascorbic acid is independent of the way

the amount is calculated and comparable results are obtained when the data are computed as the percentage of the original amount found at harvest. A special paragraph will consider the percentages of the original amount of ascorbic acid left at each determination.

## (2) Amounts of Ascorbic Acid per Plant

(a) Effect of iron. The data expressing the amounts of ascorbic acid at harvest per ten plants grown at various levels of iron are assembled in Table 23 and are graphed against the amount of iron in the solution in Figure 143.

The general tendency of the total amount of ascorbic acid in the plants, grown on increasing amounts of iron, was to increase; although this trend was apparent, the differences between average values of ascorbic acid were only of few milligrams per ten plants. This positive relationship do not coincide with the results obtained during the second experiment where, on the opposite, a negative relationship had been obtained.

When the values of ascorbic acid at harvest are compared to the other components a positive relation can be observed with the total fresh and dry weight as well as with the total amount of iron, phosphorus, nitrogen and potassium per plant.



(b) Effect of copper. The data for the amount of ascorbic acid at harvest per ten plants of the copper series are presented in Table 25 and Figure 184.

The more copper was added to the nutrient solution, the less total ascorbic acid was obtained per plant. The main cause of this decrease in the amount of ascorbic acid was the decreasing effect of copper on the growth of the plants. Positive correlations between ascorbic acid and other components of the plants were obtained with the fresh and dry weight, the total amounts of iron, phosphorus, potassium, magnesium, and in the plants grown on solutions containing more than 0.6 parts per million of copper with the total amount of copper itself.

### (3) Amount of Ascorbic Acid per 100 Grams of Fresh Matter

(a) Effect of iron. The data expressed in milligrams of ascorbic acid per 100 grams of fresh matter at harvest appear in Table 28 and the amounts of ascorbic acid at harvest and at the various times during drying are graphed against the amounts of iron in the solution in Figure 152 and from Figures 160 to 164 inclusive.

The concentrations of ascorbic acid in the fresh tissue at harvest showed a decreasing tendency with increasing amounts of iron in the nutrient solution. Although less apparent, this tendency

was similar to the one recorded during the second experiment. On the other hand, a positive correlation is observed between the concentration of ascorbic acid found in the drying material 15 hours after harvest and the amount of iron in the nutrient solution.

When the values for the amounts of ascorbic acid found at harvest are plotted against the amounts of iron in the fresh tissue a negative correlation is observed (Figure 165). On the other hand, when the values for the amount of ascorbic acid found at the first determination 14 hours after harvest are plotted against the amount of iron in the fresh tissue a positive correlation appears. This change from a negative to a positive correlation during the first hours of drying had already been observed during the second experiment. These relations may be seen in Figure 166.

(b) Effect of copper. The average milligrams of ascorbic acid per 100 grams of fresh plant material grown in the copper series and determined at harvest are recorded in Table 30 and the amounts of ascorbic acid at harvest and at the various times during drying are graphed against the amount of copper in the nutrient solution in Figure 194 and from Figures 203 to 207, inclusive.

Up to a level of 0.9 parts per million of copper in the nutrient solution, the concentrations of ascorbic acid in the corresponding fresh material at harvest had a tendency to decrease;

then this tendency changed to a positive direction with higher amounts of copper in the solution. These tendencies are comparable to those observed for the concentrations of phosphorus, calcium, and magnesium in the fresh material (Figures 195, 198, and 199).

In the plant tissue grown on the low copper concentrations, the ascorbic acid was lost at a faster rate than in the tissue grown on the high copper level during the 13 first hours after harvest. A positive correlation is observed, when the concentrations of ascorbic acid at that determination are graphed against the amounts of copper in the solution (Figure 203).

After 30 hours, the values for ascorbic acid in all the cultures became almost similar (Figure 204).

When the concentrations of ascorbic acid at harvest and 13 hours after harvest are plotted against the amounts of copper in the fresh tissue, as in Figures 167 and 168, it is found that to the higher amount of copper correspond at both determinations the higher amount of ascorbic acid: A positive relationship was obtained in both cases between ascorbic acid and copper. These results can be compared to the one obtained by Lyon and Beeson (1948) with tomatoes where significantly higher amounts of ascorbic acid resulted from increase in the copper concentration of the nutrient medium.

In the plants of the copper series, no apparent correlation between the concentration of ascorbic acid and the amount of iron in the tissue can be drawn as in the plant tissues from the second experiment (Figure 169).

#### (4) Percentages of the Original Amounts of Ascorbic Acid Found at Each Determination

(a) Effect of iron. The values for the percentages of the original amount of ascorbic acid found in the iron series 14, 43, 62, 110, and 157 hours after harvest, are reported in Table 33, graphed against the amount of iron in the nutrient solution in Figure 229, and graphed against the amount of iron in 100 grams of fresh matter in Figures 176, 177, 178, and 179.

As in the two first experiments, the amount of ascorbic acid lost during drying was smaller in the plants grown on the solution with a higher amount of iron. In fact, after 14 hours, 53.6 per cent of the original amount of ascorbic acid was lost in the plant grown on three parts per million of iron, while only 22 per cent were lost from the plants grown on 24 parts per million of iron. A positive correlation is obtained when the amounts of iron in the fresh tissue are graphed against the percentages of the original amount of ascorbic acid. This correlation is very apparent 14 hours and 43 hours after harvest and then starts to disappear after

62 hours. At the fourth determination made 110 hours after harvest, no correlation between the percentage of ascorbic acid and the amount of iron in the fresh tissue existed anymore. These relationships can be followed in Figures 176, 177, 178, and 179.

It was observed that during the drying of the plants at room temperature, the loss of water from the plant material was of the same relative magnitude as the loss in ascorbic acid. This relationship can be noted when Figure 228 and Figure 229 are compared. When the percentages of the original amounts of water left after 43 hours are graphed against the amount of iron in the fresh tissue, a positive correlation is obtained as seen from Figure 181. This relationship is apparent 43 hours after harvest and starts to disappear after 62 hours as in the case of the relationship between iron in the fresh tissue and the percentage loss of ascorbic acid (Figures 180, 181, 182, and 183).

(b) Effect of copper. The data for the percentages of the original amount of ascorbic acid found at each determination in the plants grown on the copper cultures are listed in Table 34 and Figure 227. Determinations of ascorbic acid were made at harvest, 13, 30, 52, 100, and 147 hours after harvest.

During the first 13 hours after harvest, the percentages of the original amount of ascorbic acid lost was inversely

proportional to the amount of copper in the nutrient solution. Indeed, 43.4 per cent of the original amount of ascorbic acid had been lost from the "no copper" culture though only ten per cent was lost in the plant grown on the nutrient solution supplied with 2.7 parts per million of copper. It was also noted that the general rate of loss of ascorbic acid in the plants grown on the copper series was slower during the first 15 hours than in the plants of the iron series.

When the percentage values obtained for the plants which were left 30 hours are considered, it is found that the positive relation between ascorbic acid stability and the amount of copper in the nutrient solution disappeared and even showed a slight negative tendency. At the next determinations 52, 100, and 147 hours after harvest, no relationship was noted any more, between copper in the nutrient solution and the percentage of the original amount of ascorbic acid.

When the amounts of copper in 100 grams of fresh material are plotted against the percentages of the original amount of ascorbic acid left after 13 hours of drying a significant positive relationship appears, but which loses its significance when the values at 30 hours are graphed. These relationships can be seen in Figure 219 and 220.

No relationship is apparent after 13 and 30 hours between the rate of loss of ascorbic acid and the amount of iron found in the fresh tissue as shown in Figures 221 and 222.

It was noted that during the drying of the plants in the case of the iron series and of the copper series, the loss of water from the plant material was of the same relative magnitude as the loss in ascorbic acid. This relationship can be observed when Figures 226 and 227 are compared. Although the relative magnitudes of the losses were similar, their rates were specific. The rate of water loss from the plants grown on the copper series was proportional to the amount of copper in the nutrient solutions on which they grew, as shown in Figure 226. This rate was also proportional to the amount of copper in the fresh tissue (Figures 215 and 216). No relationship could be obtained, however, between the rate of water loss and the amount of iron in the fresh tissue of the plants of the copper series as seen in Figures 217 and 218.

#### d. Other Chemical Constituents

The nitrogen, potassium, magnesium, calcium, and phosphorus contents of the plants have been calculated on a basis per ten plants, per 100 grams of fresh material and per 100 grams of dry plant material. No further discussion will be undertaken about these constituents since any relationship between them and

iron, copper and ascorbic acid have already been discussed.

The following table will mention the places where the data can be found:

| Constituents | Amounts per<br>Ten<br>Plants |         | Amounts per<br>100 g. Fresh<br>Material |         | Amounts per<br>100 g. Dry<br>Material |         |
|--------------|------------------------------|---------|-----------------------------------------|---------|---------------------------------------|---------|
|              | Tables                       | Figures | Tables                                  | Figures | Tables                                | Figures |
| Nitrogen     | 24                           | 148     | 27                                      | 155     | 31                                    | 173     |
|              | and                          | and     | and                                     | and     | and                                   | and     |
|              | 26                           | 189     | 29                                      | 197     | 32                                    | 211     |
| Phosphorus   | 24                           | 146     | 27                                      | 153     | 31                                    | 171     |
|              | and                          | and     | and                                     | and     | and                                   | and     |
|              | 26                           | 187     | 29                                      | 195     | 32                                    | 209     |
| Potassium    | 24                           | 147     | 27                                      | 154     | 31                                    | 172     |
|              | and                          | and     | and                                     | and     | and                                   | and     |
|              | 26                           | 188     | 29                                      | 196     | 32                                    | 210     |
| Calcium      | 23                           | 149     | 27                                      | 156     | 31                                    | 174     |
|              | and                          | and     | and                                     | and     | and                                   | and     |
|              | 25                           | 190     | 29                                      | 198     | 32                                    | 212     |
| Magnesium    | 23                           | 150     | 27                                      | 157     | 31                                    | 175     |
|              | and                          | and     | and                                     | and     | and                                   | and     |
|              | 25                           | 191     | 29                                      | 199     | 32                                    | 213     |

#### D. Discussion on the Third Experiment

The main object of this third experiment was to study the effects of increasing amounts of copper and iron up to toxic levels,



on the synthesis and stability of ascorbic acid in wheat plants.

This experiment was only partly successful because if it was possible to reach the level of copper toxicity, the plants of the iron series, on the other hand, did not show any toxic symptoms when grown on the highest amounts of iron supplied.

As far as the effects of copper and iron on the ascorbic acid stability of drying plants are concerned, the results obtained confirmed those reported during the first and second experiments. Indeed, to high concentrations of iron in the solution corresponded a greater stability of its ascorbic acid and in the copper series to high copper concentrations corresponded a low rate of loss of ascorbic acid. The fact that this observation was noted with plants harvested at different times of the year and dried under different conditions give to it a great significance.

If the concentrations of iron in the tissues of the plants of the iron series were positively related to the stability of the ascorbic acid during this third experiment, it should however be recalled that this correlation was not as apparent in the two first experiments. It should also be emphasized that in the plants of the second experiment in the copper series, a negative correlation was obtained between the iron concentration of the fresh tissue and the ascorbic acid stability; in the third experiment no clear relationship was then apparent. The facts just mentioned suggest that

iron plays an important role in the stability of ascorbic acid in the plant, but its mode of action is not known. Because no consistent relation appears between the actual concentration of iron in the tissue and the ascorbic acid the specific role of iron is rather obscure.

Another fact that should be stressed is that the concentration of iron in the fresh tissue during the three experiments where various levels of this element were used, did not show much variations in its concentration in the tissues, although the total amount of iron per plant varied greatly.

The absorption of the major elements, calcium, magnesium, potassium, phosphorus, and nitrogen, was shown to be affected, as seen from their concentration in the plant tissue, by the various concentrations of iron and copper in the solutions. It was thought to correlate the amounts of these elements and the amount of ascorbic acid at harvest. In the copper series the plants with high amount of ascorbic acid corresponded to those with high concentration of phosphorus, calcium, and magnesium. On the other hand, the same trends did not appear in the iron series where to a high concentration of magnesium, potassium and nitrogen, and to a low concentration of calcium, corresponded a high concentration of ascorbic acid. Relationship between ascorbic acid and macro elements in the plants were worked out by Wynd and Noggle

(1950), who reported that in rye, grown on a field in Canada, the concentrations of ascorbic acid was found to be positively related to the percentage of nitrogen, calcium, magnesium, and iron.

Many authors have studied the effect of macro-elements on the accumulation of ascorbic acid, in plant tissue by varying the levels at which they were supplied to the plants. Watson and Noggle (1947) noted that the minus potassium and minus magnesium treatments gave large increases in the ascorbic acid concentration of oat leaves. Bernstein, Hamner and Parks (1945) found that turnip greens grown in sand cultures without potassium produced plants low in ascorbic acid. Somers and Kelly (1951), infiltrating solutions of various inorganic salts in illuminated leaf discs of turnips and broccoli, found that calcium reduces the accumulation of ascorbic acid. When the results reported by these authors are compared to those obtained during the present investigation, it does not seem that a variation in the supply of an element affect in the same way the accumulation of ascorbic acid in the plant as a similar variations of the concentration of the same element in the plant tissue.

An interesting effect of the high levels of copper was made clear by the significant increased accumulation of the elements phosphorus, calcium, and magnesium in the plant tissue grown on substrate containing more than 1.2 parts per million of copper.

One of the toxic effects of excess copper might be due to the formation of some copper protein which through change in their properties would disturb so much the permeability of the protoplasm that abnormal accumulation of salt may occur and be detrimental to vital functions of the cell. It is known that copper binds readily with proteins as shown by Thompson, Kocher and Fritzsche (1948). It was observed that during the drying of the various samples from the copper series, the water was lost at a greater rate in the samples from the higher copper levels. This specific evaporation rate might be explained by a change in the permeability of the protoplasm due to the presence of copper.

#### E. Summary of the Third Experiment

1, In the same manner as for the first two experiments, wheat plants were grown on gravel on two series of culture solutions varying in their amounts of copper and iron. The iron series ranged from no iron to 27 parts per million, each culture varying from the one below by an increment of three parts per million of iron. The copper series ranged from no copper to 2.7 parts per million, each culture varying by an increment of 0.3 parts per million of copper. This experiment was designed to overlap with the preceding ones and to observe if the same tendencies

in the accumulation and stability of ascorbic acid in the plant tissues would persist at levels where the copper and iron would be toxic.

2. The plants were harvested at jointing stage. Ascorbic acid was determined on the fresh tissues and at various intervals during the drying of the plants at room temperature. Iron, nitrogen, phosphorus, potassium, calcium, and magnesium were determined on oven dry samples. Fresh and dry weight, as well as the rate of water loss during drying, were recorded.

3. The fresh weights of the plants of the iron series were found to increase slightly with increase in the iron level of the solution and no toxic level was reached. In the copper series an increase in weight was obtained in the plants up to 0.6 parts per million in the solution and above this level a toxic effect was revealed by the stunting of the plants. In both iron and copper series, the dry weight paralleled the fresh weights of the plants.

4. To an increase of iron in the solution corresponded an increase in the total amounts of iron in the plants; but on a unit weight of fresh and dry tissue, a decrease in the concentration of iron was observed above the level of 18 parts per million of iron in the substrate. In the copper series, the total amounts of iron

absorbed per plant decreased with increases in the amounts of copper in the nutrient solution, however the concentrations of iron in the fresh matter showed a minimum for the plants grown on 1.5 parts per million of copper, followed by an increase to 2.7 parts per million. The iron content of the dry matter of the plants from the copper series was inversely related to the amounts of copper in the nutrient solution.

5. In the plants of the copper series the concentrations of copper in the dry and fresh matter increased correspondingly to the higher levels of copper in the solutions.

6. The concentrations of ascorbic acid in the fresh material at harvest showed a negative relation to the amount of iron in the solution. In the copper series, the amounts of ascorbic acid per 100 grams of fresh matter at harvest showed a decrease up to 0.9 parts per million in the solution and then increased with higher copper levels to higher concentrations of ascorbic acid than in the low copper levels.

7. A positive correlation was obtained between the amounts of iron in the fresh tissue and the stability of the ascorbic acid in the drying tissue after 15 and 43 hours of drying. In the copper series a positive correlation was shown between the concentrations

of copper in the tissue and the stability of its ascorbic acid after 15 hours of drying.

## VI. EXPERIMENT 4

### A. Purpose

In the three first experiments the tops of wheat plants grown on widely different concentrations of iron were observed to accumulate very limited and rather constant amounts of iron. In a similar way, from the data of various workers, it can be seen that the accumulative power of plants for iron is rather limited. For example, Biddulph (1947), studying the iron accumulation in leaves of Red Kidney beans grown on nutrient solution with various forms and levels of iron, found that the concentration of this element in the stem, heart-shaped leaves and trifoliate leaves remained comparatively uniform under the various conditions. Sideris (1949) found in pineapple that, although iron and manganese increase in the tissues with higher concentrations of these elements in the culture solutions, the iron accumulates in the leaves and stem to a much lesser extent than manganese. Lyon and Beeson (1948) observed the same relationship in turnips and tomatoes.

Besides the restricted power of plants to accumulate iron in stems and leaves, another reason for the relatively low concentration of this element, even in the plant tissues grown on solutions where high amounts of iron have been added, might be an actual



unavailability of iron to the roots. Indeed, a great difficulty in supplying iron in water culture might be due, as has been pointed out by Biddulph (1947), to a rapid formation of insoluble complex with certain salts of the nutrient media. It is also known, that at high pH, iron easily precipitates as the hydroxide and if phosphate ions are present, as the phosphate salt. In most of the experiments previously reported, the iron levels represent the total iron added when the solution was made up. Unless specifically emphasized by the authors, it is reasonable to believe that in the literature the level of this element in soluble form in the media is at a much lower concentration than expected from the initial amount added.

In order to obtain substantial and significant information on the relationship of iron to ascorbic acid in plants, it seemed of the greatest interest to obtain plants with various and widely different amounts of iron in their tissues. The following steps were then proposed to investigate the iron accumulation in wheat plants by supplying solutions of controlled iron levels: In order to prevent the precipitation of the iron, a pH value of 4, lower than previously used, was maintained in the nutrient solutions and the iron salt was added to a carbouy not containing the phosphate ions. The complete nutrients, was then supplied to the plants by connecting, at intervals, the culture-pots to the iron containing-carbouy and to the phosphate containing carbouy. According to Biddulph (1947),

ferric citrate seem to give satisfaction in supplying iron to plants at pH 4 and this form of iron was utilized. At short intervals, the iron concentrations of the solutions were checked and more iron added when necessary. At the end of the growth period, the plants were harvested and various information obtained on their chemical compositions, as done in the previous experiments. It seemed useful to investigate at the same time the relative accumulation of iron in the roots, because a possible explanation of the rather constant concentrations of iron in the tops might be due to the precipitation of this element in the roots and its subsequent unavailability to the rest of the plant. Data on the ascorbic acid status in the roots were also collected because of the rather limited amount of information on this compound in that part of the plants.

## B. Experimental Conditions

### 1. The Nutrient Solution

The nutrient solution for each culture was contained in two carboys of a volume of 18 liters.

In one carboy was added the potassium dihydrogen phosphate and the calcium nitrate at the same concentrations as for the previous experiments and in the second carboy was placed the magnesium sulfate and the minor elements including 0.02 parts per million of copper and various levels of iron as described later.

The carboy containing the potassium dihydrogen phosphate and the calcium nitrate was connected to the pumping device during the day and the other carboy during the night.

The only controlled varying factor was the iron supplied at the following levels; 0.0, 0.5, 1.0, 5.0 and 10 parts per million. Two pairs of cultures were grown on each level of iron.

The pH of the solution was adjusted at 4 and was checked every four days and brought back to pH 4 by addition of 0.25 N sulfuric acid.

The stock solution of iron was prepared by dissolving 8.6 grams of ferric citrate with distilled water to a volume of 500 milliliters. This solution was then filtered and analyzed for its iron content against a standard iron solution.

The iron in the solutions was determined at intervals and additional amounts were added to bring the nutrient solution to the desired iron-level.

## 2. Plant Material

Fifty seeds of wheat Illinois No. 1-128 were seeded in each pot evenly spaced and covered with one-half inch of gravel on March 12, 1950, and were harvested 38 days later at the beginning of the jointing stage on April 19, 1950. At the time of harvest three samples of three grams of leaf blades were taken at random in the

four culture pots of each level and their ascorbic acid content determined. Twenty-five plants were then harvested from each pot and the fresh weight of the plant tops were recorded. The plants were then placed in an air forced oven at 60° C to be dried. The roots of the plants were washed free of gravel and the water blotted from the tissues. Two samples of twenty grams of roots were used for the determination of ascorbic acid. The remaining of the root material was placed in an oven at 60° C to be dried at the same time as the tops. The dried material was kept for further analysis.

### C. Experimental Results

#### 1. Observations on the Plants

##### a. Description of the Plants at Time of Harvest

All the plants at harvest time showed the first joint and many had the second joint.

The plants grown on the "no iron" cultures were smaller than the other plants of the series but did not show any chlorosis. Not much difference could be visually noted between each of the other levels, although the plants grown on the nutrient solution with one part per million of iron looked somewhat bigger and healthier than all the others.

#### b. Fresh Weight per Plant

The data indicating the average of the fresh weight of the tops of ten plants expressed in grams are assembled in Table 37 and graphed against the amount of iron in the nutrient solution in Figure 236.

An increase in the fresh weight of the plants was found from the plants grown on the no-iron cultures to a maximum for those grown on the one part per million iron level; the weights of the plants of the next cultures were somewhat lower although no toxic symptom was apparent.

#### c. Dry Weight per Plant

The average dry weight per ten plants are arranged in Table 37 and graphed against the amount of iron in the solution in Figure 237.

The dry weights of the plants paralleled those of the fresh weight reaching a maximum for the plants grown on one part per million of iron.

## 2. Chemical Analyses of the Plants

### a. Iron

#### (1) Amounts of Iron per Plant

The amounts of iron expressed in milligrams accumulated in the aerial part of the plants are presented in Table 37 and in Figure 233.

No correlation could be drawn between the amount of iron found per plant top and the level of iron in the solutions.

The least amount of iron was found in the plants grown on the nutrient solution where no iron has been added, however, the total amount of iron found in these plants was almost as high as in the plants grown on the ten parts per million iron level. This fact might be explained by the low pH of the solution which permitted the iron impurities of the gravel to be dissolved and rendered more iron available to the plants. This would also explain why no chlorosis was observed in the no-iron cultures.

#### (2) Amounts of Iron per 100 Grams of Fresh Tissue

The milligrams of iron per 100 grams of fresh plant tissues appear in Table 38 and in Figure 238.

The concentrations of iron in the fresh matter increased very slightly from the cultures with no iron to those with five parts per million of iron but the differences cannot be considered as significant.

### (3) Amount of Iron per 100 Grams of Dry Material

(a) In the wheat tops. The data for the amount of iron per 100 grams of dry plant material are assembled in Table 39 and in Figure 243.

No special trend in the concentration of iron in the dry material can be defined from the observation of the data mentioned above.

(b) In the wheat roots. The amounts of iron in milligrams per 100 grams of dry root material are presented in Table 39 and Figure 246.

A very significant increase was found in the concentration of iron in the roots of the plants grown from the no iron level to those grown on the ten parts per million of iron level. Indeed a 699 per cent increase was obtained between the concentrations of iron found in the roots grown on those two conditions. The values obtained for the different iron concentrations in the dry root tissues are positively related to the iron levels of the nutrient solutions.

## b. Manganese

### (1) Amounts of Manganese per Plant

The data for the amounts of manganese per ten plants are assembled in Table 37 and in Figure 234.

The amounts of manganese found in the plant tops grown on the different levels of iron was about the same for all the cultures and no apparent depressing effect of the higher levels of iron was observed.

### (2) Amounts of Manganese per 100 Grams of Fresh Material

The amounts of manganese in milligrams per 100 grams of fresh tissue are reported in Table 38 and Figure 239.

When the concentrations of manganese in the fresh tissue are considered an apparent lessening is observed from the plants grown on no iron to those grown on ten parts per million of iron.

### (3) Amounts of Manganese per 100 Grams of Dry Matter

(a) In the wheat tops. The data for the amounts of manganese in milligrams per 100 grams of dry material are assembled in Table 39 and Figure 244.

In the same manner as for the concentration of manganese in the fresh tissue, a significant decrease of its concentration in



the dry material was observed in the plants tops in relation to an increase in the iron level in the nutrient solution.

(b) In the wheat roots. The milligrams of manganese per 100 grams of dry root material appear in Table 39 and in Figure 247.

The opposite trend to the one found in the plant tops was observed in the wheat roots as far as the concentrations of manganese in relation to the amount of iron in the nutrient solution are concerned. In fact the concentrations of manganese in the roots grown on ten parts per million of iron was a little more than twice its concentration in the roots grown on no iron.

### c. Ascorbic Acid

#### (1) Amounts of Ascorbic Acid per Plant

The data for the average amounts of ascorbic acid expressed in milligrams per ten plants are presented in Table 37 and Figure 235.

The highest amount of ascorbic acid was found both in the plants grown on the nutrient solution deprived of iron and on the solution with five parts per million of iron. The values for the cultures grown on 0.5, one and ten parts per million of iron were somewhat lower and did not show significant differences between

each other. The variations in the total amounts of ascorbic acid could not be related to any of the other components analyzed.

(2) Amount of Ascorbic Acid per 100 Grams of Fresh Material

(a) In the wheat top. The concentrations of ascorbic acid per 100 grams of fresh material from the aerial part of wheat are presented in Table 38 and Figure 240.

The highest concentration of ascorbic acid was found in the plants from the "no iron" cultures. The differences between the concentrations of ascorbic acid in the fresh material of the plants tops of the other cultures are significant but could not be correlated with the components analyzed.

(b) In the wheat root. The milligrams of ascorbic acid per 100 grams of fresh root materials are given in Table 38 and Figure 242.

It was observed that the concentrations of ascorbic acid in the wheat roots were very low; in fact, the average concentration of ascorbic acid in the wheat tops of all the cultures was 16 times higher than the average concentration in the wheat roots. The differences between the concentrations of ascorbic acid in the roots grown at the various levels of iron were only of few milligrams and cannot be considered significant.



### (3) Amount of Ascorbic Acid per 100 Grams of Dry Material

The data indicating the milligrams of ascorbic acid per 100 grams of dry matter are shown in Table 39 and Figure 245.

The concentrations of ascorbic acid in the dry matter of the wheat tops shows the same trends as those found in the fresh tissue and the same observations can be drawn.

#### D. Discussion on the Fourth Experiment

Although the concentrations of iron were kept at the desired levels during the time of the experiment, no significant gradient was found in the concentrations of this element in the tissue from the aerial parts of the plants. The restricted accumulative power of wheat plants for iron is hence confirmed by this experiment. On the other hand, the plant roots behave quite differently and a positive relationship was observed between the accumulation of iron in the roots and its concentration in the nutrient media.

The fact that the amounts of iron were found to vary widely in the roots but did not correlate with the amounts in the tops would tend to show that after its absorption, iron is precipitated in the roots and so rendered unavailable to the rest of the plant.

The possible adsorption of colloidal ferric hydroxide on the external part of the roots surfaces might also be an explanation

to the great amount of this element found in the roots grown on the high iron-level medium.

Sideris (1950), studying iron absorption in pineapple, with radioactive iron<sup>59</sup>, found high counts for the root material in comparison to the leaves and suggested that most of the iron<sup>59</sup> in the roots was not absorbed into the tissues but presumably precipitated in the exodermal layer of cells. A great accumulation of iron in the roots of Red Kidney beans was also noted by Biddulph (1947) in comparison to the concentration of this element in the stem and leaves.

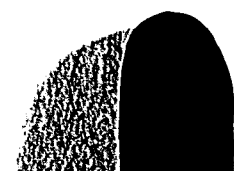
It is interesting to note that, although the plants of the no-iron cultures were smaller in size than those of the other cultures, they did not show any chlorosis. This fact would suggest that at pH 4 the so-called "active iron" needed in very small amounts for chlorophyll formation is available from the impurities of the solution but iron is yet a limiting factor for other growth processes requiring a greater amount of it. In a series of experiments, Stromme (1951), growing wheat at different pHs, observed also that at pH 4 no apparent chlorosis developed in the plants grown without iron. The same result was obtained by Biddulph (1947) in Red Kidney beans. This author suggested that at pH 4, iron "remains mobile and capable of movement to the newly formed stem tips and leaves and that an extremely low iron supply will

suffice providing conditions are such that the iron will not become immobilized within the tissues'' (p. 93).

Manganese should be considered among the factors which might be responsible for a gradient in the concentration of iron in the plants. Indeed, the translocation of iron in pineapple has been reported by Sideris (1950) to be affected by the presence of manganese; in the same manner, when the data reported by Somers and Shive (1942) are consulted, it can be observed that to a higher iron-level in the nutrient solution corresponded a lower absorption of manganese in the soybean than at lower iron-levels.

In the present experiment, the level of manganese supplied to all the cultures was constant, but the accumulation of this element in the plant parts was found to vary widely. In the dried material from the plant tops, a negative relationship was obtained with the levels of iron in the solutions, but on the contrary a positive relationship was found in the roots.

The fact also, that leaves and stems showed a much greater power of accumulation for manganese than the roots was made clear by the data of the present study. Iron interference in the translocation of manganese can hardly explain the fact reported above, because, although the roots had high amounts of iron in comparison to the stems no correlation could be drawn between iron and manganese concentrations in both roots and stems. It should be noted



that no information on the influence of the concentration of iron in the roots on the manganese concentration was found in the literature.

To obtain more information on the manganese status in the plants is of interest because this element has been claimed to be involved in the ascorbic acid synthesis. Indeed, Rudra (1939) reported that when a solution of mannose containing small amounts of manganese was injected in rat liver, increase in ascorbic acid content could be detected. Hester (1941) claimed that an addition of soluble manganese to soils deficient in this element resulted in an increased ascorbic acid concentration in the tomatoes grown on this soil; but Lyon and Beeson (1948) could not obtain any significant effect of manganese on the ascorbic acid content of tomatoes grown on various solution.

From the present experiment no apparent relation between manganese concentration in the tissue and its ascorbic acid content can be drawn; it should, however, be pointed out that in wheat top the highest ascorbic acid concentration was found in the plants of the "no-iron" level and which contained the highest concentration of manganese in the plant material.

Very significant differences were found between the concentrations of ascorbic acid in the plant tops and the ones in the roots. This result confirmed the data reported by Reid (1937), who

found that in cowpea, 75 to 85 per cent of the ascorbic acid is found in the leaf blades. Reid's results are given in the following table:

| Localization of Ascorbic Acid in the Cowpea Plant |                     |               |                                    |
|---------------------------------------------------|---------------------|---------------|------------------------------------|
|                                                   | Mgms<br>per<br>Gram | Total<br>Mgms | Percentage<br>of Total<br>Quantity |
| Leaves                                            |                     |               |                                    |
| Blades                                            | 0.818               | 21.486        | 80                                 |
| Petioles                                          | 0.177               | 1.716         | 20                                 |
| Leaf buds                                         | 0.612               | 0.164         |                                    |
| Blossom buds                                      | 0.560               | 0.084         |                                    |
| Stems                                             | 0.141               | 1.494         |                                    |
| Roots                                             | 0.125               | 1.788         |                                    |
|                                                   |                     | 26.732        |                                    |

It was suggested then by Reid that higher accumulation of ascorbic acid corresponded to tissues with higher cellular activity, however, in plants it is reasonable to say that root tissue exert as much activity as the aerial part. The best explanation for the great difference in concentrations of ascorbic acid between leaf and root is that ascorbic acid is mostly synthesized in the green aerial part of the plant.

### E. Summary of the Fourth Experiment

1. Wheat plants were grown on nutrient solutions of controlled iron level; the iron being supplied in the citrate form from a solution free of phosphorus and at a pH of 4 in order to lessen its precipitation. The concentrations of the iron in the various solutions were: none, 0.5, 1.5 and ten parts per million.

2. The plants were harvested at the beginning of the jointing stage. Ascorbic acid was determined on the fresh tissues. The determinations of iron and manganese were carried on the oven dried samples. Fresh and dry weight were recorded.

3. The fresh and dry weight of the plants showed a maximum for those grown on one part per million of iron. Neither chlorosis nor toxic symptoms were apparent on the plants from the extreme cultures.

4. The highest amount of iron accumulated in the aerial part of the plants was in those from the cultures supplied with one part per million of iron, corresponding to the highest fresh and dry weight. On the other hand, no apparent trend in the concentration of iron in the tissue of the tops could be noted. In the dry root material, the concentration of iron was positively related to the amount of iron in the solution.



5. No clear relationship could be drawn between manganese and iron or manganese and ascorbic acid. The most striking result obtained from the manganese data was the comparatively higher accumulative power of stems and leaves for this element than the plant roots.

6. No relationship could be drawn between ascorbic acid and the concentrations of iron or manganese in the solutions or in the plant tissues. On the other hand, a very significant difference in the concentration of ascorbic acid was found between the leaves and the roots, the concentrations of ascorbic acid in the roots being almost nil.

## VII. EXPERIMENT 5

### A. Purpose

All the previous experiments had been conducted with wheat plants and their responses toward iron and copper studied in relation to ascorbic acid. It was thought necessary to study some other plant material to find out if the results already obtained could be generalized.

The first object of the study was to find out how different levels of iron might affect the concentration of ascorbic acid found at harvest in such other plants as tomatoes, for which the nutritional requirements have already been much studied.

Because of the interrelationship between iron and manganese, the status of the latter in the plant was also studied.

In order to keep the iron levels as constant as possible the nutrient solution was kept at a pH value of 4 and the two carboys system was used as in the preceding experiment, to prevent the iron to be in the same solution as the phosphate salt.

It was also proposed to study the possible gradient in the concentrations of ascorbic acid in the leaves from the top and the base of the plants and relate any difference found with the data available. It was thought that by studying variations in the different



parts of the same plants, one of the great advantage was that the effect of uncontrollable environmental factors would then be reduced to the minimum.

## B. Experimental Conditions

### 1. The Nutrient Solution

The same nutrient solution as in the last experiment with wheat was used to grow the tomato plants. The same levels of iron were chosen and as before the carboy containing the potassium acid phosphate and the calcium nitrate was connected to the pumping device during the day and the other carboy during the night.

The pH of the nutrient solution was also adjusted at a pH of 4. Since a possibility of iron contamination had been attributed to the quartz gravel, a hand magnet was passed through the latter during the washing; it was possible to get rid in this manner of small particles of iron which might have released enough iron for growth at a low pH.

### 2. The Plant Material

The tomato plants used for this experiment had first been sown in a flat filled with soil. When they were one month old, on May 5, 1950, four plants were transplanted per pots. As in the

previous experiment two pairs of cultures were grown on each iron level. Before transplantation to the quartz gravel the roots of the tomato plants had been washed in tap and distilled water..

At the time of harvest, 45 days after transplanting, on June 19, 1950, two kinds of samples were taken. From each culture a composite sample of the four leaves from the tip of each plant was taken which later will be mentioned as "terminal leaves" and four leaves from the base of each plants of each culture which later will be referred as "basal leaves."

### C. Experimental Results

#### 1. Observations on the Plants

##### a. Description of the Plants at Time of Harvest

At time of harvest not much difference could visually be seen between the plants grown on the various levels of iron. The plants grown on the "no iron" level were of smaller size than those grown on the other iron levels but did not show any chlorosis. The highest plants were those grown on the five parts per million iron level. The average height of all the plants at harvest was about seventy centimeters.

## b. Percentages of Dry Matter in the Leaves

The values for the percentages of dry matter in the basal and terminal leaves of the tomato plants are presented in Table 40 and Figure 250.

Variations in the percentages of dry matter of the leaves were observed from one level of iron to the other but no definite trend can be noted.

Consistently and significantly, a higher percentage of dry matter was found in the terminal leaves than in the basal leaves.

## 2. Chemical Analyses of the Plant

### a. Iron

#### (1) Amounts of Iron per 100 Grams of Fresh Plant Material

The amounts of iron per 100 grams of fresh plant material in the basal and terminal leaves appear in Table 40 and in Figure 248.

In the basal leaves a negative relationship was observed between the concentration of iron in the fresh tissues and its concentration in the nutrient solution.

In the terminal leaves, no significant trend in the iron concentration was noted.

In all the cultures the iron concentrations in the fresh tissue of the basal leaves were significantly higher than in the terminal leaves.

## (2) Amounts of Iron per 100 Grams of Dry Matter

The concentrations of iron in milligrams per 100 grams of dry matter in the basal and terminal leaves are presented in Table 41 and in Figure 252.

The negative relationship found between the iron concentrations in the fresh tissue of the basal leaves and the concentrations of iron in the nutrient solution hold true but to a lesser extent for the concentrations of iron in the dry matter.

No great variations were reported in the amount of iron found in the dry material of the terminal leaves.

In all the cultures, the iron concentrations in the dry material of the basal leaves were significantly higher than those of the terminal leaves.

## b. Manganese

### (1) Amounts of Manganese per 100 Grams of Fresh Material

The amounts of manganese in milligrams per 100 grams of fresh tissue of the basal and terminal leaves are reported in Table 40 and Figure 249.

Variations in the concentrations of the manganese was observed in the fresh tissue of both basal and terminal leaves but no trend could be related to the various levels of the iron in the nutrient solutions.

In all the cases, except for the plants grown on ten parts per million of iron, the concentration of manganese in the fresh tissue was higher in the basal than in the terminal leaves.

## (2) Amounts of Manganese per 100 Grams of Dry Material

The concentrations of manganese in milligrams per 100 grams of dry matter in the basal and terminal leaves are assembled in Table 41 and Figure 253.

No apparent relationship between the amounts of iron in the nutrient solutions and the concentration of manganese in the dry tissue of the basal leaves was observed.

However, in the terminal leaves, if the ones grown on "no-iron" are let aside, a positive relationship is observed between the manganese concentrations in the dry tissue and the levels of iron in the nutrient solution.

In all the cases, more manganese was found in the dried basal leaves than in the dried terminal leaves.

### c. Ascorbic Acid

#### (1) Amounts of Ascorbic Acid per 100 Grams of Fresh Material

The milligrams of ascorbic acid per 100 grams of fresh material in the basal and terminal leaves of the tomato plants are presented in Table 40 and Figure 251.

In the basal leaves, the amounts of ascorbic acid did not show any relationship with the concentrations of iron in the solution on which the plants grew. On the other hand, in the terminal leaves a positive correlation was observed with the concentration of iron in the solution.

If the data for the amounts of ascorbic acid in the fresh matter of the basal and terminal leaves are considered separately no correlation is apparent with the dry matter, iron or manganese per unit of fresh weight. However, when the results are pooled a negative relationship appears between the iron in the fresh tissue and the ascorbic acid (Figure 257) while a positive correlation is shown between ascorbic acid and the percentage of dry matter (Figure 259). No correlation was shown between manganese and ascorbic acid in the fresh tissue (Figure 258).



## (2) Amounts of Ascorbic Acid per 100 Grams of Dry Material

The milligrams of ascorbic acid per 100 grams of dry material in the basal and terminal leaves are given in Table 41 and Figure 254.

When the concentrations of ascorbic acid are calculated on a dry matter basis, no relation can be found in the basal leaves with the other data, but a positive correlation is apparent between the concentration of ascorbic acid in the dry material of the terminal leaves and the level of iron on which the plants grew.

However, as can be seen in Figures 255 and 256, no relationship can be drawn between the concentrations of iron or manganese and the amounts of ascorbic acid in 100 grams of dry material.

### D. Discussion on the Fifth Experiment

A significant increase in the amounts of ascorbic acid in the terminal leaves of the tomato plants was reported to correspond to an increase in the level of iron in the solution. However, this result might be misleading as to the effect of iron on the ascorbic acid status in the plant tissue: Indeed, a negative relationship was found between the levels of iron in the nutrient solution and the concentrations of iron in the fresh tissue. In consequence, in the

present experiment the possible effect of the iron on the ascorbic acid content of the plant at harvest was a depressing one. This relationship is not apparent, when the data for the basal leaves are considered, although when all the data are pooled, an over-all negative relationship can be seen between iron concentration and ascorbic acid in the fresh matter.

A positive correlation between the ascorbic acid concentration in the fresh leaves and the dry matter was obtained when all the data for basal and terminal leaves were pooled. A similar positive relationship has been found in tomato by Aberg (1948), and in oat by Wynd (1946). A high correlation between accumulation of dry matter and ascorbic acid is also reported by Somers and Kelly (1951) in turnip and broccoli leaf discs.

It should be noted that high ascorbic acid values were found in the terminal leaves and that these leaves had a high dry weight content. If the present data are segregated into basal and terminal leaves the relationship between ascorbic acid and dry weight no longer holds true. It is suggested from this fact that the higher activity of the young terminal leaves corresponded to a tissue with a relatively high dry weight which had a greater power of synthesis for ascorbic acid not necessarily related to the dry matter. This idea was put forth by Reid (1937), who observed that higher concentrations of ascorbic acid were found in parts of tissue with greater activity, as shown in the following table:

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### Localization of Ascorbic Acid in Roots and Stems

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Milligrams per Gram

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|                      |       |
|----------------------|-------|
| Roots                |       |
| Tips 1/2" long       | 0.475 |
| Adjoining segment 1" | 0.171 |
| Remaining portion    | 0.115 |
| Stem                 |       |
| Tips 1"              | 0.169 |
| Middle               | 0.018 |
| Basal                | 0.056 |

---

A positive relationship between the age of the tissue and its ascorbic acid content was observed by Bernstein, Hamner and Parks (1945) as presented in the following table:

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### Effect of Leaf Age on Ascorbic Acid Concentration (mg./100 g. fresh weight). Five Plants Harvested from Sand Culture Balanced Nutrient.

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| Position of Leaves | Average of 5 Plants |
|--------------------|---------------------|
| Oldest             | 192.8               |
| Middle             | 204.3               |
| Youngest           | 227.9               |

---

On the other hand, a significant gradient in the concentrations of iron and manganese was also found between the terminal

and basal leaves of the tomato plants; this relation was consistently found in all the cultures. A similar tendency of a higher amount of iron and manganese in the basal than in the terminal leaves was reported by Lyon, Beeson and Ellis (1943) as shown in the table given below:

| Iron and Manganese Analyses of Tomato Plants<br>Grown in Complete Culture Solution |                                  |                                       |
|------------------------------------------------------------------------------------|----------------------------------|---------------------------------------|
| Plant Part                                                                         | Ppm of Iron in<br>the Dry Matter | Ppm of Manganese<br>in the Dry Matter |
| Top one-third of the plant                                                         | 175 $\pm$ 14.9                   | 69.7 $\pm$ 9.08                       |
| Middle one-third of the<br>plant                                                   | 199 $\pm$ 9.0                    | 248.3 $\pm$ 29.95                     |
| Lower one-third of the<br>plant                                                    | 229 $\pm$ 10.8                   | 398.0 $\pm$ 23.79                     |

It is observed that the basal leaves which had a relatively high iron and manganese content were found to have a rather low ascorbic acid concentration; the opposite being true for the terminal leaves. It cannot be said that the internal concentration of these elements affected directly the ascorbic acid status because no consistent relationship could be drawn between these factors.

### E. Summary of the Fifth Experiment

1. Tomato plants were grown on nutrient solutions of controlled iron levels, at pH 4, in the same manner as the wheat plants of the fourth experiment.

2. Terminal and basal leaves were harvested when the plants were starting to flower. Ascorbic acid was determined on the fresh tissues. The determinations of iron and manganese were carried on corresponding oven dried samples. The percentages of dry matter were recorded.

3. An increase in growth of the plants was observed corresponding to an increase in the concentration of iron in the solution up to five parts per million. Although, smaller in size, the plants deprived of iron did not show any chlorotic symptoms. The percentage of dry matter was consistently higher in terminal than basal leaves.

4. The concentration of iron in the fresh tissue showed a negative relationship with the amount of iron in the nutrient solution but this tendency did not hold on a dry matter basis. In all cases, a significantly higher amount of iron was found in the basal leaves.

5. The manganese values of the basal leaves did not show any special trend but in the terminal leaves the amounts of this element was found to increase with higher iron levels in the nutrient solution.

6. The amounts of ascorbic acid in the basal leaves varied only slightly with the iron levels of the nutrient solution but its concentration in the terminal leaves showed a positive relation to the iron in the nutrient solution. In the fresh matter of the plants a negative relationship was found between iron and ascorbic acid and also a positive relationship between dry matter and ascorbic acid when all the data from basal and terminal leaves were pooled. These relations did not hold true, however, when the values were calculated on a dry basis.

## VIII. EXPERIMENT 6

### A. Purpose

It was established in the preceding experiment with tomato plants that definite gradients in the concentration of ascorbic acid, iron, and manganese existed in the different parts of the plant. Not enough data were then available to find out if the internal concentration of each of the above components had an influence on the other.

It was then planned to grow tomato plants on various levels of iron and copper and to carry chemical analyses on the plants at different times of the growing season and from different parts of the plant. This way of attack permitted to obtain information on the consistency of the gradients in the concentrations of the various components during growth.

Because of the rather unexpected iron concentration found in the plants of the fifth experiment grown without iron, it was suggested to plant these tomatoes on quartz sand instead of soil and to transplant them later to the culture pots.

The procedure of supplying the nutrient solution in two carboys was not used because it did not give entire satisfaction as far as iron availability is concerned. Ferric citrate was supplied

directly to the complete solution where citric acid was added to maintain the iron in solution.

## B. Experimental Conditions

### 1. The Nutrient Solution

The same three salts solution as used in the first experiment with wheat plants was made up with various concentrations of iron and two levels of copper as follows:

| Concentrations of Iron in the Nutrient Solutions<br>of the Sixth Experiment |                            |
|-----------------------------------------------------------------------------|----------------------------|
| No Copper Series                                                            | 0.04 ppm. of Copper Series |
| No iron                                                                     | 0.0 ppm Fe                 |
| 0.5 ppm Fe                                                                  | 0.5                        |
| 1.0                                                                         | 1.0                        |
| 5.0                                                                         | 5.0                        |
| 10.0                                                                        | 10.0                       |

These two series of cultures will be referred to later as the "no copper-series" and the "0.04 parts per million of copper-series."



Ferric citrate was added from a stock solution which had been standardized for its iron content. The iron in the solution was determined at intervals and additional amounts of the stock solution were added to bring the solution to the desired iron level.

In order to keep the iron in solution, 20 milliliters of 20 per cent citric acid was added per carboys of 16 liters of solution. The complete nutrient solution was first made up on November 1, 1950, and renewed on November 29, 1950, because of the growth of mold in the solutions. The second time the solutions were made up, 25 milliliters of 20 per cent citric acid were added. During the whole experiment the pH of the nutrient solutions varied from 5.3 to 6.0.

Before starting the experiment, the quartz gravel was passed through an electro-magnet to get rid of iron dusts and particles still found in the gravel.

To each level of iron corresponded a duplicate pair of cultures.

## 2. The Plant Material

On September 28, 1950, seeds of tomatoes, variety Rutgers Ferry Morse Detroit No. 290 stock D7068 were sown in vermiculite. Five plants were transplanted in the quartz gravel per culture pots when the plants were 35 days old on November 2, 1950.

The day after transplanting, the cotyledons of each plant were cut off. When the plants were 58 days old, one plant per pot was harvested. At that time, samples of the whole plants were used to determine the ascorbic acid, iron, and dry matter content. The second harvest was made when the plants were 78 days old and then two plants per pots were taken. Because the plants were rather large, only the leaves were used for the different analyses of ascorbic acid, iron, and manganese. For these samples a differentiation was made between the four upper leaves referred later as "terminal leaves" and the ones below as "basal leaves." In the same manner, a third harvest was made when the plants were 97 days old on January 3, 1951.

### C. Experimental Results

#### 1. Observations on the Plants

##### a. Description of the Plants at Time of Harvest

During the whole growth period, no chlorosis symptoms appeared on the plants grown on the "no-iron levels" of both series. In general the plants grown on the "0.04 parts per million of copper series" were of greater size than those grown on the solutions in which no copper had been added. At all times, all the plants looked healthy and stayed very green. Just before the last

harvest, some plants started to flower; the flowers were then pinched off.

#### b. Percentages of Dry Matter in the Leaves

The amounts of dry matter at the different harvests, expressed as percentages of the fresh matter of the leaves of both copper series are given in Table 45 and Figures 260 to 265, inclusive.

In general the plants grown on the solution where 0.04 parts per million of copper had been added, gave a higher yield of dry matter. This difference was most apparent when the plants were young, indeed the average value of all the plants of the 0.04 ppm Cu series when 58 days old was 16 per cent greater than the average value of the plants grown on no copper. This difference between both series somewhat disappeared at the later harvests.

In almost all the cases when both basal and terminal leaves were sampled, the greatest amount of dry matter was found in the terminal leaves.

A tendency which is shown in the data on dry weights is an increase of the dry matter in the leaves with age and in both copper series. This tendency was more apparent in the "no copper series."

## 2. Chemical Analyses of the Plants

### a. Iron

#### (1) Amounts of Iron per 100 Grams of Fresh Material

The concentrations of iron in milligrams per 100 grams of fresh matter in the leaves harvested when the plants were 58, 78 and 97 days old, from the no copper and the 0.04 parts per million of copper series are listed in Table 42 and Figures 266 to 271, inclusive.

In all the samples, the concentrations of iron in the fresh matter did not show any clear tendencies and no significant correlation can be drawn between the amount of iron in the leaves and its concentration in the nutrient solution.

When comparing both the "no-copper" and the "0.04 parts per million of copper" series, it is observed that the accumulation of iron in the leaves generally greater in the plants grown on the "no copper" series than on the "0.04 parts per million copper series." This observation is most apparent in the 58 days old plants, less in the 78 days old and has disappeared in the 97 days old plants.

In opposition with the results obtained in the previous tomato experiment, a tendency was shown by the terminal leaves to

have a higher concentration of iron, however the differences between basal and terminal leaves were not very significant.

(2) Amounts of Iron per 100 Grams of Dry Material

The data for the amount of iron per 100 grams of dry plant material expressed in milligrams for the leaves harvested from plants 58, 78 and 97 days old from the "no copper" and the "0.04 parts per million of copper" series are assembled in Table 46 and Figures 272 to 277, inclusive.

The same observations as those reported for the concentrations of iron in the fresh matter can also be drawn for the data of iron in the dry matter. Hence no further discussion will be carried.

b. Manganese

(1) Amount of Manganese per 100 Grams of Fresh Material

The concentrations of manganese in milligrams per 100 grams of fresh material in the leaves of 78 and 97 days old tomato plants grown on the "no copper" and the "0.04 parts per million of copper" series are reported in Table 43 and Figures 278 to 281, inclusive.

To increase amounts of iron in the nutrient solutions corresponded a significant decrease in the accumulation of manganese in the leaves.

In agreement with the previous experiment, a greater concentration of manganese was found in the basal leaves of the tomato plants than in the terminal leaves. This relationship was most significant in the 97 days old plants.

In this experiment the copper content of the solution had apparently no effect on the accumulation of manganese in the leaves.

## (2) Amounts of Manganese per 100 Grams of Dry Matter

The values for the amount of manganese in the dry matter corresponding to those given in the preceding paragraph for the fresh matter are presented in Table 47 and Figures 282 to 285, inclusive.

The same observations as those made for the manganese concentrations in the fresh matter can be drawn from the data on the dry matter, in consequence no further discussion will be done.

### c. Ascorbic Acid

#### (1) Amounts of Ascorbic Acid per 100 Grams of Fresh Material

The concentrations of ascorbic acid per 100 grams of fresh material grown on different levels of iron without or with copper are listed in Table 44 and Figures 286 to 291, inclusive.

The concentrations of ascorbic acid varied to a significant extent between samples grown on various iron levels but no significant relationship was apparent between the iron levels in the nutrient solutions and the ascorbic acid in the plant. In all cases and at all the harvests, the amounts of ascorbic acid were much greater in the terminal leaves than in the basal leaves.

In order to find any possible relationship between the plants components analyzed and the ascorbic acid at the different harvests, the values of ascorbic acid in the fresh tissue were graphed respectively against the amounts of iron, of manganese and the percentages of dry matter as seen in Figures 298 to 305, inclusive.

A positive correlation between iron and ascorbic acid in the terminal leaves of the 97 days old plants of both series is shown in Figure 301. When the values for terminal and basal leaves are pooled a positive relationship is observed between ascorbic acid in the fresh tissue and the percentages of dry matter of the 78 days old plants (Figure 304). A positive relationship

is also observed in Figure 305 between the percentages of dry matter of the basal leaves of the 97 days old plants and their ascorbic acid concentrations.

Besides these few observations, no consistent relationship was observed and it was not possible to relate the variations observed in the ascorbic acid concentrations and the factors studied.

## (2) Amounts of Ascorbic Acid per 100 Grams of Dry Matter

The milligrams of ascorbic acid per 100 grams of dry plant material grown on different levels of iron without or with copper are recorded in Table 48 and Figures 292 to 297.

When the values for ascorbic acid are calculated on a dry matter basis, about the same tendencies as shown in its concentration in the fresh matter appeared. Should be mentioned however, that, on a dry weight basis, more ascorbic acid was found in the 58 days old plants grown without copper than on those grown with 0.04 parts per million of copper, but this relation does not hold true at older ages.

The other observations reported for the ascorbic acid in the fresh matter hold true for the values in the dry matter.



#### D. Discussion on the Sixth Experiment

In this experiment, under various stages of growth and from the base to the tip of the plants, gradients of increasing concentrations of ascorbic acid and dry matter and decreasing concentrations of manganese were found and confirmed the findings established and discussed at the end of the fifth experiment.

As in the preceding experiment, the concentration of ascorbic acid in the fresh plant tissue was found to be positively related to the amount of dry matter of the tissue. This relationship seems to be highly significant because it holds true in most cases when the data for terminal and basal leaves are pooled or segregated. On the other hand, the effect of iron and manganese concentrations in the tissue on the ascorbic acid content was not consistent and no generalization can be made.

A lessening in the accumulation of iron in the plant tissue was noted as a special effect of the presence of copper in the nutrient solution. This result confirms those reported by Maquenne and Demoussy (1920) and also by Willis (1936).

It was also observed in the present experiment that to high iron levels in the solution corresponded low amounts of manganese accumulated in the tissues. This observation agrees with a similar relationship observed in wheat tops during the fourth experiment.

In the literature, the usual interaction between iron and manganese reported, is a depressing effect of high amounts of manganese on the accumulation of iron, fact which was observed by Johnson (1917) in pineapple, by Hopkins (1930) in *Chlorella*, and more recently by Sideris (1950).

During the present study, ferric citrate was supplied to the nutrient solutions and citric acid was added to prevent the precipitation of iron; this method gave rather good results as can be observed from the amounts of iron taken up by the plants. One of the disadvantages however is that the presence of citric ion favour the growth of mold in the solution, which then must be changed. The same method of providing iron in combination with citric acid has been reported very recently to be used by Bitcover and Sieling (1951) who noted that a solution containing ten parts per million, with a 4 to 1 molar ratio citric acid to iron, gave the best growth of *Spirodela*.

#### E. Summary of the Sixth Experiment

1. Tomato plants were grown on nutrient solutions of controlled iron-levels with and without copper. The iron was supplied in the same carboys as the other nutrients in the form of ferric citrate supplemented with citric acid.

2. Whole plants were harvested when 58 days old. Terminal and basal leaves were collected on 78 and 97 days old plants. Ascorbic acid was determined on the fresh tissues. The determinations of iron and manganese were carried on corresponding oven dried samples. The percentages of dry matter were recorded.

3. Larger plants were grown on the nutrient solutions supplied with copper; these plants also contained a greater percentage of dry matter. A greater amount of dry matter was found in all the terminal than in the basal leaves. In all the cases, the amounts of dry matter in the tissues increased with age.

4. The concentration of iron in the tissue did not show any significant relationship to the level of iron in the solution. A depressing effect on the intake of iron in the presence of copper in the solution was observed. No significant gradient in the concentration of this element was noted in the different part of the plants.

5. A negative relationship between the concentration of manganese in the tissue and the level of iron in the solution was reported. No effect of copper on the manganese uptake was observed. Generally a higher amount of this element was found in the basal than in the terminal leaves.

6. No significant relationship could be drawn between the iron content of the solution and the concentration of ascorbic acid in the plant tissue. However a positive correlation between the iron in the tissue and its ascorbic acid concentration was observed in the terminal leaves when the plants were 97 days old. A consistent positive relationship between the percentage of dry matter and the concentration of ascorbic acid in the plant tissue was observed at each determination.

## IX. EXPERIMENT 7

### A. Purpose

The preceding two experiments established the existence of a significant gradient in the concentrations of ascorbic acid, in the terminal and basal leaves of the tomato plants. However, no consistent relationship between these various concentrations of ascorbic acid and other factors could be drawn, except with the dry matter content of the tissues.

The higher concentration of ascorbic acid in the terminal leaves, might be explained by a greater rate of synthesis or by a greater stability of the ascorbic acid in that plant tissue.

A study of the stability of the ascorbic acid in the drying tissue from different parts of tobacco plants was then investigated. The tobacco plants were chosen as the experimental material because of the large leaves which give a rather uniform material and which easily can be split in two comparative samples along the midrib. On one half of the leaf the ascorbic acid determination was carried on the other half the ash analyses were done.

It was proposed to follow the oxidation of the ascorbic acid in comparative samples from three different parts of the tobacco plants, grown on substrate differing in their iron and copper content.

Relationships between the rate of oxidation and the chemical composition of the tissues were then investigated.

In a preliminary experiment, the variations in concentrations of ascorbic acid, iron and manganese was first determined for each leaves of a tobacco plant.

### B. Preliminary Experiment

In order to find out the possible variations in ascorbic acid, iron, and manganese in tobacco leaves, the following experiment was carried out: All the leaves of a tobacco plant, grown on soil in the greenhouse, were cut in half along the midrib. One half of each leaf was used for the ascorbic acid determination and the other half was used for analyses of iron and manganese; at the same time the percentages of dry matter of each leaf were recorded.

The data compiled on this tobacco plant are listed in Tables 49 and 50 and graphed in Figures 306 to 313, inclusive.

From the base to the top of this tobacco plant, ten leaves were cut and analyzed. As seen from the data, definite gradients in the concentrations of iron, manganese, and ascorbic acid were found, and are discussed below.

The concentration of iron in the fresh material was the highest in the bottom leaf, then decreased to a minimum in the

fifth leaf, 29 centimeters from the soil, and then increased again till the last leaf 62 centimeters from the soil (Figure 306).

The amounts of manganese were found to be of very low concentrations: only one-tenth of the concentration of iron (Figure 301). The gradient in the concentrations of manganese was apparently increasing from the bottom leaf to the tip leaf (Figure 312), although few leaves gave values which were out of the general trend. These variations in the values from the general trend may be explained by the relative small concentration found, which itself is more difficult to detect quantitatively with accuracy.

The concentration of ascorbic acid in each leaf showed a definite gradient in the concentrations from bottom to top (Figures 308 and 313). The least concentration was found in the bottom leaf and then increased up to a maximum at the sixth leaf, 34 centimeters from the soil. In the upper leaves (39 centimeters and above) the concentrations had a tendency to decrease but the differences in amounts between successive leaves were not as great in magnitude than those between the lower leaves,

No significant correlations could be drawn either between the concentrations of ascorbic acid and iron, or manganese, in the fresh matter. However, a slight negative relationship was observed between the values of manganese above 0.2 milligrams per 100 grams of fresh tissue and the corresponding concentrations

of ascorbic acid (Figure 309). Although no significant correlation can be brought out between ascorbic acid and iron, it should be noted that a slight negative trend related these two components (Figure 310).

A significant relationship between the amount of ascorbic acid and the percentages of dry matter was observed in the leaves. Indeed when the values of ascorbic acid in the fresh matter were graphed against the percentages of dry matter, a parabolic curve was obtained, as seen in Figure 311.

From the above reported data, it was concluded that tobacco plants would be adequate to provide plant tissues of varying ascorbic acid, iron, and manganese concentrations.

### C. Experimental Conditions

#### 1. The Nutrient Solution

The nutrient solution recommended by Spencer (1941) was used. The final composition of the solution was as follows:



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Composition of the Nutrient Solution

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| Salt                                                  | Grams per 16 Liters |
|-------------------------------------------------------|---------------------|
| $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$            | 7.9                 |
| $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ | 20.8                |
| $\text{KH}_2\text{PO}_4$                              | 14.0                |
| $(\text{NH}_4)_2\text{SO}_4$                          | 3.5                 |

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The final volume in each carboy was made up to 16 liters with distilled water. Microelements were supplied by adding 10 milliliters per carboy of a stock solution having the following composition:

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Composition of the Stock Solution of Microelements

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| Salt                    | Grams per Liter |
|-------------------------|-----------------|
| $\text{H}_3\text{BO}_3$ | 4.568           |
| $\text{MnSO}_4$         | 2.462           |

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The final concentrations of the micro-elements in the nutrient solutions were as follows:

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Concentration of Micro-elements in the Nutrient Solutions

---

| Element   | Ppm of the Element |
|-----------|--------------------|
| Boron     | 0.5                |
| Manganese | 0.5                |

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In the same manner as in the second experiment with tomato plants, two levels of copper were used: one where no copper was added and the other with 0.08 parts per million of copper. These two series will later be referred to as the "no copper series" and the "copper series." In these two series, existed three different cultures in respect to iron: one where no iron was added, a second where a concentration of four parts per million was made up by adding ferric sulfate and the third where the iron was supplied from a glassy frit, used instead of the quartz gravel. This iron-frit No. A6300A was supplied by the Ferro Enamel Company in Cleveland. The cultures mentioned above will be referred to later as the "No iron," the "Iron-gravel" and the "Iron-Frit" cultures.

## 2. The Plant Material

Seeds of Nicotiana tobacum variety Havana No. 38, were planted in soil on December 7, 1950. The seeds were supplied through the courtesy of Dr. James Johnson of the University of Wisconsin. When the plants were 34 days old, on January 10, they were transplanted in the culture pots. During the transplanting the roots were washed with tap and distilled water.

At the time of harvest 59 days after transplanting, on March 10, 1951, three kinds of samples were taken: the terminal, median and basal leaves, as described below.

From each culture the four terminal leaves were taken to constitute a composite sample of what will be mentioned later as "terminal leaves."

The next three leaves of each plants were collected and put together to form the samples of "median leaves."

At last, going further down on the plants, the three next leaves were used for the "basal leaves" samples.

Each sample was subdivided into six subsamples by cutting each leaf into six parts and distributing each part into each subsample so that a composite of all the leaves parts would constitute each subsample.

One of the subsamples of each culture was immediately used to determine the amounts of ascorbic acid at harvest. Another subsample of each culture was washed with distilled water and put to dry in a forced air oven and was later utilized for the iron and manganese analyses. The remnant subsamples were left in trays to dry at room temperature in the laboratory and were used to determine the ascorbic acid at various time of drying.

#### D. Experimental Results

##### 1. Observations on the Plants

###### a. Description of the Plants

Fourteen days after transplanting, the plants grown on the nutrient solution without iron and with 0.08 parts per million of copper started to show chlorosis, while the plants of the "no iron" culture in the "no copper series" did not show any chlorosis. At harvest the plants of the "no iron" culture in the "copper series" were very stunted and chlorotic, while in the "no copper series" no apparent chlorosis was showing and the plants were almost as big as the one supplied with iron.

The plants of the "Iron-gravel" and "Iron-frit" were at harvest in very healthy condition and the plant of both of these cultures were somewhat larger in the "copper series."

### b. Fresh Weight per Plant

The average fresh weights in grams per plant grown on the various cultures of both series are given in the following table:

| Average Fresh Weights per Plant in Grams |                 |                    |
|------------------------------------------|-----------------|--------------------|
| Cultures                                 | "Copper Series" | "No Copper Series" |
| "Iron-Frit"                              | 592.9           | 522.9              |
| "Iron-gravel"                            | 581.0           | 537.0              |
| "No iron"                                | 332.0           | 500.0              |

It can be seen that at the exception of the "No iron" culture, the plants on the copper series were significantly heavier than the plants grown on the solutions where no copper had been added. This observation agrees with the one made on the appearance of the plants at harvest.

### c. Percentages of Dry Matter in the Leaves

The percentages of dry matter in the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron content of both "copper" and "no copper" series are listed in Table 51 and Figures 314 to 319, inclusive.

In both series the percentages of dry matter showed the same tendencies as far as its concentration in the different kind of leaves is concerned: the terminal leaves having the greatest percentages of dry matter, the basal ones the least and the median leaves being in between in their amounts of dry matter per unit weight.

Comparing the copper series with the "no copper" series, it is found that the percentages of dry matter were generally higher in both "iron-frit" and "iron-gravel" cultures of the copper series than of the "no copper series." However, the opposite was observed in the case of the "no iron" cultures, where a greater percentage of dry matter was found in the terminal and median leaves of the "no copper" series than of the "copper" series. It should be recalled that the plants of the copper series grown without iron were very chlorotic and stunted at the time of harvest, while the plants of the other series on the same culture did not appear abnormal except for their smaller growth in comparison to the plants supplied with iron.

When comparing the different cultures, it is noted that the highest percentage of dry matter was obtained in the frit grown plants and the least in the cultures deprived of iron.

## 2. Chemical Analyses of the Tobacco Plants

### a. Iron

#### (1) Amounts of Iron per 100 Grams of Fresh Material

The concentrations of iron in milligrams per 100 grams of fresh matter in the terminal, median and basal leaves of tobacco plants grown on the "iron-frit," "iron-gravel" and "no iron" cultures of both "no copper" and "copper" series are recorded in Table 51 and Figures 320 to 325, inclusive.

Consistently in the cultures supplied with iron, i.e., the frit and the gravel cultures of both copper series, the median leaves contained the highest concentration of iron. However, it should be reminded that in the tobacco plant grown on soil and used for the preliminary experiment the least concentration of iron had been found in the median leaves. When the plants were deprived of iron, in both series, with or without copper, the greatest concentration of iron was found in the basal leaves and the least in the terminal leaves.

The accumulation of iron in all the leaves seem to have been directly influenced by the presence of copper in the nutrient solution; indeed, in all the leaves of all the plants grown on the

solutions deprived of copper more iron was found than in the series supplied with copper.

(2) Amounts of Iron per 100 Grams of Dry Matter

The data for the amounts of iron per 100 grams of dry plant material of the various leaves of tobacco plants are reported in Table 54 and Figures 326 to 331, inclusive.

The same tendencies as those observed in the concentrations of iron in the fresh tissue hold true for the concentrations of iron in the dry material. It should also be mentioned that in all the cases the concentrations of iron in the dry matter was the least in the terminal leaves. The observation made about the depressing effect of copper on the accumulation of iron in the leaves is even more apparent on a dry matter basis.

b. Manganese

(1) Amounts of manganese per 100 Grams of Fresh Material

The concentrations of manganese in milligrams per 100 grams of fresh material in the terminal, median and basal leaves of tobacco plants grown on the "iron-frit," "iron-gravel" and the "no iron" cultures of both "no copper" and "copper" series are assembled in Table 51 and Figures 332 to 337, inclusive.



In both cultures provided with iron and with or without copper, a gradient in the concentrations of manganese was observed from the basal to the terminal leaves, the basal containing the least and the terminal the most. On the opposite, in both of the "no iron" cultures and especially in the one of the "copper series" a significant higher concentration of manganese was found in the basal leaves, in comparison to the terminal ones which contained the least. In these cultures the plants from the "copper series" had a much higher concentration of manganese than those of the "no copper" series. This relationship between both copper series did not exist in the cultures supplied with iron.

## (2) Amounts of Manganese per 100 Grams of Dry Material

The values for the amounts of manganese in the dry matter corresponding to those given in the preceding paragraph for the fresh matter are presented in Table 54 and Figures 338 to 343, inclusive.

On a dry basis, no significant gradient in the concentrations of manganese in the various leaves of tobacco plants could be observed at the exception of those of the "no iron" cultures. It was found, as on a fresh weight basis that in the "no iron" cultures, the highest concentrations of manganese was obtained in the basal leaves while the least was found in the terminal leaves. The leaves

from the plants of the copper series grown on the "no iron" culture had a much greater amount of manganese per unit weight of tissue than the same culture in the "no copper" series.

### c. Ascorbic Acid

#### (1) Description

The way by which the loss of ascorbic acid in the fresh and drying plant material had been followed in the third experiment on wheat proved to be satisfactory and to yield results in a rather short period of time. The same procedure was used for the study of ascorbic acid loss in the tobacco leaves during drying. As described before, each kind of leaves, the terminal, median, and basal, were divided in comparable composite subsamples which were left to dry in the laboratory. For the samples of the "no copper series," the first determination was done at harvest, the second 24 hours later, the third 48 hours after harvest and then 72, 120 and 144 hours after harvest. For the samples of the "copper series" the first determination was carried at harvest, the second 24 hours later, the third 48 hours after harvest and then 96, 120 and 192 hours after harvest.

The amounts of ascorbic acid found at each determination were calculated on a 100 grams fresh matter basis and the percentage

of the original amount left at each determination was also computed.

## (2) Amounts of Ascorbic Acid per 100 Grams of Fresh Matter

(a) Effect of iron in the presence of copper. The data expressed in milligrams of ascorbic acid per 100 grams of fresh matter at harvest and at the various times of drying appear in Table 53 and Figures 344 to 346, inclusive.

In the plants grown on both cultures supplied with iron the concentrations of ascorbic acid was greater than in the "no iron" culture and in all the cases the following sequence in concentrations of ascorbic acid in the leaves was apparent: Terminal median basal.

No correlation was obtained between the amounts of iron in the fresh material and the amounts of ascorbic acid found at harvest (see Figure 350).

On the other hand, a positive correlation between manganese and ascorbic acid was found, as seen in Figure 352, when the data for both cultures supplied with iron were pooled. On the opposite, a negative relationship is observed in the no iron culture, but this fact should not be generalized because of the few data available for this culture.

During the first 48 hours of drying, a great amount of the original value of ascorbic acid was lost. The rate of loss will be discussed later in the paragraph where the percentages of the original amounts of ascorbic acid at each determination are reported.

(b) Effect of iron in the absence of copper. The average values in milligrams of ascorbic acid per 100 grams of fresh tissue at harvest and at the various times of drying are recorded in Table 52 and Figures 347 to 349.

In most of the leaves of the plants supplied with iron, the concentrations of ascorbic acid were higher than in the culture deprived of iron. In all the cases and in the same manner as in the "copper" series the sequence: terminal      median      basal was found in the concentrations of ascorbic acid in the leaves.

No correlation was obtained between the amount of iron in the fresh material and the amount of ascorbic acid found at harvest (Figure 351).

When the results for the "copper" and the "no copper" series are pooled (Figure 351) and the data for each category of leaf considered separately, positive relationships are then observed between iron and ascorbic acid; a relationship is especially apparent in the case of the terminal leaves.

As far as manganese is concerned in the "no copper" series (Figure 353), a slight positive relationship is obtained. When the data for the "copper" and the "no copper" series are pooled, a more significant positive relationship appears (Figure 353).

A significant positive correlation was obtained when the data for the percentages of dry matter in the leaves of the plants from all the cultures and of both copper series and the amount of ascorbic acid were pooled.

At harvest more ascorbic acid was found in all the categories of leaves of the "no copper" series at the exception of the median leaves of the "iron-gravel" culture and the basal leaves of the "no iron" culture than in the corresponding leaves of the "copper series." During drying, less ascorbic acid was generally detected in the leaves from the plants of the "no copper" series at the exception of those from the culture deprived of iron, as can be observed in Figures 355 to 360, inclusive.

### (3) Percentages of the Original Amounts of Ascorbic Acid Found at Each Determination

(a) Effect of iron in the presence of copper. The values for the percentages of the original amounts of ascorbic acid found in the terminal, median and basal leaves of the various cultures of

the "copper" series are listed in Table 56 and graphed against the time of the various determinations in Figures 361 to 363, inclusive.

A positive relationship between the percentages of the original amounts of ascorbic acid and the amounts of iron per unit weight of fresh matter is apparent in Figures 370 to 373, inclusive, and hold true during all the process of drying. These results agree with those obtained in the previous wheat experiment, where to a greater amount of iron in the fresh tissue corresponded a greater stability of the ascorbic acid.

(b) Effect of iron in the absence of copper. The data for the percentages of the original amounts of ascorbic acid found at each determinations in the different leaves of the "no copper" series are reported in Table 55 and graphed against the time of the various determinations in Figures 364 to 366, inclusive.

In the case of the "no copper" series, a slight positive relationship was found between the concentration of iron in the plant and the percentages of ascorbic acid (Figures 374 to 377) left after 24 hours of drying, however this relationship disappeared at the next determinations.

In order to compare the rate of oxidation of ascorbic acid in the copper and the "no copper" series, the differences between

the percentages found for each series at each determination were calculated and are presented in Table 57 and Figures 367 to 369. From these data it can be seen that in general the rate of oxidation was greater in the leaves of the plants of the copper series supplied with iron in comparison to the corresponding plants of the no copper series. On the other hand, in the plants deprived of iron of the "copper" series, the rate of oxidation of the ascorbic acid was smaller than in the corresponding plants of the "no copper series."

#### E. Discussion on the Seventh Experiment

During this experiment on tobacco plants, many of the facts observed on the effects of iron and copper nutrition on some aspects of the physiology of the plants, were found to be in agreement with those already disclosed during the previous experiments with other plant materials. Greater weight should hence be given to those relationships which occurred under these various conditions.

For example, the depressing effect of the presence of copper in the nutrient solution on the accumulation of iron in the leaves, effect which had already been discussed in the sixth experiment, was shown again to exist in the case of tobacco plants.

It was observed that when the plants were deprived of iron, a higher concentration of manganese was found in the leaves from

the plants of the copper series than from those of the "no copper" series. This fact could be explained by the depressing effect of copper on iron-accumulation, which in turn has a depressing effect on manganese accumulation. This explanation, however, is not completely satisfactory because it was observed that this fact only occurred for the plants deprived of iron; another explanation is that, the plants deprived of iron being larger in the "no copper" series than in the "copper" series, the smaller concentrations of manganese in the former might be due to a dilution effect.

As in the case of the tomato leaves, a positive relationship was obtained between the amounts of ascorbic acid at harvest and the percentages of dry matter in the tissues. This relationship was discussed at the end of the fifth experiment, and more evidence for its general occurrence is given by the results reported during the present experiment.

As far as the iron-ascorbic acid relationship is considered, it should be pointed out that on one hand, when the results for each category of leaf are taken separately, a positive relationship is obtained between the concentrations of ascorbic acid at harvest and the concentrations of iron in the fresh tissues; on the other hand, when the same results are pooled no special correlation can be drawn. It can then be concluded that the iron-ascorbic acid



relationship had a certain importance on the ascorbic acid status in the living plants, but a more fundamental factor seem to be directly related with the age and the activity of the tissues, because of the great influence of the position of the leaves (i.e., at the base, the middle, or the tip of the plant) on their ascorbic acid contents.

The ascorbic acid, in the drying plant tissues was found to be more stable in the plant containing a high than a low concentration of iron. This greater stability was more apparent before fifty per cent of the original amount of ascorbic acid were lost. It should be recalled that this observation had already been made in the third experiment with wheat plants. An interaction between copper and iron, related to ascorbic acid stability is apparent from the present data, indeed the relationship iron-ascorbic acid stability appeared in the plants supplied with 0.08 parts per million of copper while it was less consistent in the plants deprived of copper.

On the other hand, and in agreement with the results obtained in the wheat experiments, it was found that the ascorbic acid from the plants supplied with copper, was lost at a slower rate than those deprived of copper.

What could be said to explain the present facts would only be speculation, but at least, the possible role of iron and copper

on the increase in stability of ascorbic acid in the drying plant tissues should be emphasized.

#### F. Summary of the Seventh Experiment

1. Tobacco plants were grown on nutrient solutions, under three different conditions of iron availability: on gravel without iron, on an iron-frit, on gravel supplied with four parts per million of ferric sulfate in the nutrient solution. These conditions of iron availability were set up at two copper levels: one series without copper, the other with 0.08 parts per million of copper.

2. Terminal, median, and basal leaves were collected when the plants were harvested on 93 days old plants. Ascorbic acid was determined on the fresh tissues and at various intervals during the drying of the plants at room temperature. The determinations of iron and manganese were carried on corresponding oven dried samples. The percentages of dry matter were recorded.

3. A decreasing gradient in the percentages of dry matter was found from the terminal to the basal leaves and except on the "no iron" cultures the percentage of dry matter was greater for the plants from the copper series than for those from the "no copper" series.

4. The amounts of iron per 100 grams of fresh material were found the highest in the median leaves of the cultures supplied with iron of both copper cultures; the plants from the "no iron" cultures showed an increasing gradient in the percentages of iron from terminal to basal leaves. A greater concentration of iron was found in all the leaves from the no copper series. The same results calculated on a per cent dry material basis gave approximately the same tendencies as reported above.

5. The amounts of manganese per 100 grams of fresh material of plant tissue showed an increase from the basal to the terminal leaves of the plants grown on the cultures supplied with iron. When no iron was present in the nutrient solution, the opposite gradient in the concentration was found, i.e., the concentrations in the basal leaves was the greatest and in the terminal leaves the least. When the plants were deprived of iron, a higher concentration of manganese was found in the leaves of the plants from the 0.08 parts per million copper series than of these of the no copper series.

6. In all the plants, from all the cultures, the greatest concentrations of ascorbic acid at harvest were found in the terminal leaves and the least in the basal leaves. In both copper series, the plants from the cultures supplied with iron had the

most ascorbic acid and when the data of both series are pooled and each category of leaf considered a positive correlation is found between the iron and the ascorbic acid. At harvest, more ascorbic acid was found, in most cases in the leaves from the plants of the "no copper" series, but during drying less ascorbic acid was found in this same series.

7. The percentages of the original amounts of ascorbic acid found at each determination, during drying showed a positive relationship with the concentration of iron in the fresh tissues of the plants from the "copper" series. In the plants from the "no copper" series, the same relationship was not so apparent and disappeared after 24 hours of drying. A greater rate of oxidation of the ascorbic acid was found in the leaves from the plants of the "copper" series supplied with iron than in those of the "no copper" series. But, when the plants were deprived of iron, the rate of oxidation was smaller in the leaves from the "copper" series than from those of the "no copper" series.

## X. GENERAL DISCUSSION

The comparative study of the effects of various concentrations of copper and iron, in the nutrient media and in the plant tissues on the concentration and stability of ascorbic acid in plants brought about certain facts which seem worthy of being emphasized at the end of this investigation. Quantitative comparisons between the data obtained by the different experiments have been avoided because the plants, although supplied with nutrient solutions of controlled compositions, were grown at different periods of the year. Consequently the climatic factors known to affect the absorption of the inorganic nutrient and the synthesis of ascorbic acid varied between wide limits. On the other hand, a few general qualitative trends were found in the various experiments.

As a general conclusion, it can be said that although copper and iron are powerful catalysts of the oxidation of ascorbic acid in vitro, the presence of these elements in the plants did not increase the rate of oxidation of the ascorbic acid in the drying plant tissues. Surprisingly, it was generally found that the stability of the ascorbic acid in these tissues was positively related to the amounts of iron and copper in the nutrient solution. At the same time, it was suggested that a possible interaction between iron and

copper was involved in the ascorbic acid system of plants. A positive relationship between the ascorbic acid stability and the iron content of the plant tissue was more apparent in the plants supplied with various amounts of iron than in those supplied with various amounts of copper.

The mechanism involved in the relation between iron, copper, and ascorbic acid in plant are not known, it is possible, however, that copper and iron may form complexes of organic nature after their absorption by the plant. It has been shown in the literature that the protein fractions easily complex with iron and copper. A change in the properties of iron is illustrated by the oxidation-reduction potential of  $\text{Fe}^{++} \rightarrow \text{Fe}^{+++} - e$  which is -0.77 volts, while the potential of the same reaction is -1.14 volts when the iron is complexed with ortho phenanthroline.

It is evident that it is not possible to predict the action of metals such as copper and iron in oxidation-reduction reactions in relation to the ascorbic acid status of plants until detailed knowledge is available concerning the particular metallic compounds involved. But the changes of some properties of the metals when transformed into complex organic compound may explain the increased stability of the ascorbic acid obtained with increases of iron and copper concentrations in the plant tissues.

During this investigation, a significant increase in the gradient of ascorbic acid was observed from the old, basal leaves to the young, terminal leaves of tobacco and tomato plants. Two main factors may be the cause of this change in concentration: (1) a greater stability of the ascorbic acid in the higher part of the plant, and (2) a greater synthetic power of that part of the plant. In order to test the stability of ascorbic acid in the plant tissues, determinations were carried out at different intervals during the drying of the tissues. It was assumed that the stability of the ascorbic acid would then be relatively comparable to that of living tissue. It should, however, be realized how different the conditions can be in both cases and how limited this assumption is; but no present technique offers any information as to the stability of ascorbic acid in living tissues, because its active synthesis will mask the actual status of its stability. It was found from stability studies performed during the drying of different plant parts containing varying amounts of ascorbic acid, that the rate of oxidation was about as great and sometimes greater in the terminal leaves than in the basal leaves. The first factor mentioned as a cause for greater accumulation of ascorbic acid in the terminal parts of the plants was thus eliminated. On the other hand it was observed consistently that the amounts of ascorbic acid found at harvest were positively related to the percentage of dry matter in

the tissue. It is, therefore, possible to suggest that the ascorbic acid content of plant tissues may be positively related to the activity of the cells. The percentage of dry weight has been shown by Reid (1941a) to be correlated, in young tissues, to a greater activity of the cells. This aspect of the problem would then be in agreement with the results of Reid (1941b), who found a correlation between dry weight, ascorbic acid, and the activity of the cells in cowpea seedlings; and with those of Shaw and Pascoe (1949), who reported a rapid synthesis of ascorbic acid in the broad bean during the first days of germination and found the greatest concentration of ascorbic acid in the most active parts of the seedlings.

From this information, it is rather difficult to give any explanation of the results reported earlier in the introduction, which showed a greater concentration of ascorbic acid related to a lower concentration of iron in the tissues. It is possible, however, that under specific conditions, a luxurious growth due to the great activity of dividing and enlarging cell will correspond to a dilution of the total iron present. The large amount of ascorbic acid found could be a cause or an effect of the great activity of this tissue. From the results of analyses, it would then seem that to a small amount of iron corresponded a great amount of ascorbic acid, but this correlation would not be a true cause or effect relationship.



Although the study of the relationship between ascorbic acid, iron, and copper was the main purpose of this investigation, some interaction between copper, iron and manganese were also observed. At this point, a differentiation should be made between the interactions of ions in the nutrient solution from their interactions in the plant tissue. The oxidation-reduction potential, which was shown by Erkama (1949) to have a direct effect on the absorption of heavy metals, is at a state of equilibrium in the nutrient solution; while an equilibrium never exists in the redox systems of the living cells, thus the same interactions will not be apparent. It was found that, in tomato and tobacco plants, the presence of copper in the solution exerted a depressing effect on the accumulation of iron in the leaves and that a high concentration of iron in the solution corresponded to a low accumulation of manganese in the plants. Such an interaction, however did not exist in the leaves, where high amounts of iron were detected in the presence of widely varying amounts of manganese.

## XI. GENERAL SUMMARY AND CONCLUSION

1. Wheat, tomato and tobacco plants were grown on nutrient solutions supplied with various concentrations of copper and iron in order to find out the possible effect of these nutrient on the concentration and on the stability of ascorbic acid in plant tissues.

2. In most cases, a greater stability of ascorbic acid in the drying plant tissues was associated with a greater concentration of copper and iron in the nutrient solution. But no general correlation between the concentration of ascorbic acid at harvest and the concentrations of copper and iron in the nutrient solution was apparent from the data obtained.

3. The possibility of an interaction between copper and iron was suggested because a positive relationship between the amount of iron in the plant and the stability of ascorbic acid was more apparent in plants supplied with various amounts of iron than with various amounts of copper in the nutrient solution.

4. A greater concentration of ascorbic acid was found in the young active tissues than in the old mature tissues. The larger concentration of ascorbic acid was not related to the stability of

this vitamin during the drying of the tissue. It could be concluded, therefore, that the power of synthesis of the tissues to form ascorbic acid is the most important factor governing its concentration in the living plant tissues.

5. The presence of copper in the nutrient solution was found to exert a depressing effect on the accumulation of iron in tomato and tobacco plants. It was also found that to a high concentration of iron in the solution corresponded a low accumulation of manganese in the plants. However, neither of these conditions could be related to the concentration of ascorbic acid in the fresh tissues or to its stability during drying.

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## XII. APPENDIXES. ANALYTICAL PROCEDURES

### 1. Ashing of the samples.

#### REAGENTS

Hydrofluoric acid. Concentrated

Sulfuric acid. Concentrated

Nitric acid. 0.1 N.

#### PROCEDURE

Place one-gram of dry plant material into a platinum crucible.

Place into a muffle oven and increase gradually the temperature until the sample starts smoking. Ash at  $850^{\circ}$  C. for two hours. Let cool. Add 1 milliliter of 1:4 sulfuric acid and fill the crucible half full with hydrofluoric acid.

Evaporate to dryness on hot plate at intermediate heat.

Infra red lamps may be sused to step up the evaporation of the hydrofluoric acid.

Dissolve the ash in 0.1 nitric acid. Make up to volume in 200 milliliters volumetric flask with nitric acid 0.1 N.

## 1. Determination of iron

Iron was determined in aliquots of the ash solution by the procedure of Hummell and Willard (1938)

### REAGENTS

Acetic acid. Approximately 2 N solution. Dilute 114 milliliters of glacial acetic acid to one liter with distilled water.

Hydrochloric acid. 1:1 dilution

Hydrochloric acid. 1:100 dilution

Ammonium citrate. 1 per cent solution

Bromophenol blue indicator solution. 0.4 per cent.

Grind 1 gram of bromophenol blue with 3 milliliters of 0.05 N sodium hydroxide, and dilute to 250 milliliters with water.

### Buffer solutions.

1. Solution of pH 3.5. Mix 6.4 milliliters of 2 N sodium acetate with 93.6 milliliters of 2 N acetic acid, and dilute to 1 liter.

2. Solution of pH 4.5. Mix 43 milliliters of 2 N sodium acetate with 57 milliliters of 2 N acetic acid, and dilute to 1 liter.

### Hydroquinone solution.

Dissolve 1 gram of hydroquinone in 100 milliliters of a buffer solution of pH 4.5. Store in a refrigerator. Discard as soon as color develops.

O-phenanthroline solution.

Dissolve 1 gram of o-phenanthroline monohydrate in distilled water. Warm if necessary to effect solution, and dilute to 200 milliliters.

Sodium acetate. 2 M.

Dissolve 272 grams of sodium acetate trihydrate in distilled water and dilute to one liter.

Iron. Standard solution

Dissolve 1.000 gram of electrolytic iron in 50 milliliters of a ten per cent sulfuric acid solution. Warm if necessary to hasten reaction. Cool, and dilute to one liter with distilled water. One milliliter contains one milligram of iron.

PROCEDURE.

1. Pipet 15 milliliters of the ash solution, representing 0.075 grams of dry plant material, into a 25 milliliters volumetric flask, and a similar aliquot into a 25 milliliters beaker. The aliquot should contain from 0.01 to 0.10 milligrams of iron.
2. Add 5 drops of bromophenol blue indicator to the aliquot in the beaker, and titrate the solution with 2 N sodium acetate until the color matches that of an equal volume of the buffer whose pH is 3.5 and which also contains the same amount of indicator.
3. Add 1 milliliter of the hydroquinone solution, and 2 milliliters of the ortho-phenanthroline reagent to the aliquot in the

volumetric flask.

4. Add the same volume of 2 N sodium acetate that was found necessary to adjust the aliquot in the beaker to a pH of 3.5. If turbidity develops at pH 3.5, add 1 milliliter of the ammonium citrate solution before adding the sodium acetate. Dilute to 25 milliliters, mix and let stand for 1 hour to assure complete color development.
5. Determine the optical density in the Coleman spectrophotometer at a wave length of 510  $m\mu$  and with a PC-4 filter, using a water blank to balance the instrument.

#### CALCULATIONS:

Prepare a calibration curve with standard solutions containing from 0.01 to 0.1 milligrams of iron.

$$\text{parts per million of iron} = \frac{\text{mg. found} \times 1000}{\text{g. sample}}$$

$$\text{per cent of iron} = \frac{\text{mg. found} \times 0.1}{\text{g. sample}}$$

## 2. Determination of calcium

Calcium was determined in aliquots of the ash solution by the semimicro procedure of R. F. Reitemeier (1943)

### REAGENTS

Ammonium oxalate. 4 per cent solution

Sodium hydroxide. 30 per cent solution.

Hydrochloric acid. 1:15 dilution

Perchloric acid. Approximately 4 N solution. Dilute 340 milliliters of 70 per cent perchloric acid to 1 liter.

Sodium oxalate. Standard 0.01 N solution. Dissolve 0.670 grams of sodium oxalate in water and dilute to 1 liter.

Ammonium hexanitrate mixture. Approximately 0.01 N standard solution in perchloric acid. Dissolve 5.76 grams of highest quality ammonium hexnitrate cerate in 250 milliliters of 4 N perchloric acid and dilute to 1 liter. The reagent is then standardized as follows: Pipet 5 or 10 milliliters of fresh standard 0.01 N sodium oxalate into a beaker containing 5 milliliters of 4 N perchloric acid. Add 0.2 milliliters of nitro-ferroin indicator and titrate with the cerate solution to a colorless end point. Determine a blank titration correction on a similar sample lacking the oxalate solution. The normality of the cerate solution is obtained as follows:

$$N = \frac{\text{mls.} \times 0.01}{C - D}$$

where mls = mls. of oxalate used

C = mls. of cerate used to titrate the oxalate

D = mls. of cerate to titrate the blank.

Do not attempt to adjust the normality of the cerate to exactly 0.01 N. Store in a dark bottle, and restandardize every 2 or 3 days.

Nitro-Ferroin indicator. Either nitro-orthophenanthroline ferrous sulfate, or nitro- orthophenanthroline ferrous perchlorate may be used. When the sulphate is used, add 0.1 milliliter to the unknown solution and 0.2 milliliters to the standardization solution. If the perchlorate is used add 0.5 milliliters.

Methyl red indicator. Dissolve 0.1 grams in 100 milliliters of 95 per cent alcohol.

#### PROCEDURE.

1. Pipet 5 milliliters of the ash solution, representing 0.025 grams of the dry plant material, into a conical, 15-milliliters, heavy duty centrifuge tube.
2. Add 1 drop of methyl red indicator solution, and then add 30 per cent sodium hydroxide drop by drop with constant stirring until a yellow color appears. The pH should be 6.3
3. Add 1:15 hydrochloric acid drop by drop until the solution barely turns pink.
4. Add 2 milliliters of the ammonium oxalate, mix and let stand over night. The solution need stand only 2 hours, but when a large number of samples are being determined at one time,

it is convenient to let stand over night at this stage.

5. Centrifuge at 3000 r.p.m. for 10 minutes. Carefully decant the supernatant liquid into a 25-milliliters volumetric flask and preserve for the determination of magnesium.
6. Rinse down the sides of the tube with 5 milliliters of 1:50 ammonium hydroxide blown from a pipette. Break up and stir the precipitate with a thin glass rod and again centrifuge at 3000 r.p.m. for 10 minutes. Decant the supernatant liquid into the 25-milliliters volumetric flask containing the solution preserved for the magnesium determination.
7. Drain the tubes for 10 minutes by inverting them on filter paper.
8. Blow 3 milliliters of the 4 N perchloric acid into the tube.  
When the precipitate has dissolved, transfer the solution to a 25 milliliters volumetric flask, and rinse the tube with 5 milliliters of water.
9. Add 0.1 milliliter of the ferrous nitro-ferroin (or 0.5 milliliter of the perchlorate nitro-ferroin) indicator, and titrate with approximately 0.01 N standard cerate to a colorless end point by means of a micro burette. The blank titration, determined in the same manner is usually about 0.03 milliliters.

CALCULATIONS :

$$\text{per cent calcium} = \frac{(\text{mls. cerate}) (\text{N of cerate}) (2.004)}{(\text{gs. sample})}$$



#### 4. Determination of magnesium

Magnesium was determined in aliquots of the ash solution by the method of Reitemeier, (1943).

#### REAGENTS

Ammonium chloride. 30 per cent solution. Dissolve 30 grams of ammonium chloride in water and dilute to 100 milliliters. Filter before use.

Ammonium phosphate. 5 per cent solution. Dissolve 25 grams of ammonium dihydrogen phosphate,  $\text{NH}_4\text{H}_2\text{PO}_4$ , in water and dilute to 500 milliliters. Filter before use.

Ammonium hydroxide. Concentrated solution.

Ammonium hydroxide wash solution. Mix 20 milliliters of concentrated ammonium hydroxide in 80 milliliters of water, 100 milliliters of 95 per cent ethyl alcohol, and 100 milliliters of ether.

Sulphuric acid. Approximately 1 N. Dilute 27.7 milliliters of concentrated sulphuric acid (specific gravity 1.84) to 1 liter.

Ammonium molybdate. Dissolve 5 grams of ammonium molybdate in 80 milliliters of warm water, add 2.3 milliliters of concentrated sulphuric acid to the cool solution, and dilute to 100 milliliters with water. Prepare fresh every 7 days.

Hydroquinone. 0.5 per cent solution. Dissolve 0.5 grams of hydroquinone in 100 milliliters of water made slightly acid with one drop of concentrated sulphuric acid. Store in the

refrigerator.

Sodium succinate. 20 per cent solution. Dissolve 20 grams of anhydrous succinate in 100 milliliters of water. If hydrated sodium succinate is used, dissolve 33.6 grams in 100 milliliters of water. Store in the refrigerator.

Ammonium hydroxide. 1:1 dilution

Boric acid. Approximately 0.8 M solution. Dissolve 50 grams of boric acid in water and dilute to 1000 milliliters.

Brom thymol blue indicator. Dissolve 0.05 grams of brom thymol blue in 2.4 milliliters of 0.01 N sodium hydroxide and dilute to 175 milliliters with water.

Phenolphthalein indicator. Dissolve 1 gram of phenolphthalein in 100 milliliters of 60 per cent ethyl alcohol.

Magnesium sulphate. Standard solution. Dissolve 0.1014 of magnesium sulphate,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , in 1 liter. Each milliliter contains 0.01 milligram of magnesium.

#### PROCEDURE

1. Dilute the supernatant liquid obtained by centrifuging the calcium as the oxalate to 25 milliliters.
2. Pipet 5 milliliters of this solution, representing 0.005 grams of dry plant material, into a 15 milliliters conical, heavy duty centrifuge tube. If a different aliquot is used, dilute or evaporate to 5 milliliters.
3. Add 1 milliliter of 30 per cent ammonium chloride and 1 milliliter of 5 per cent ammonium phosphate, and 1 drop of phenolphthalein indicator.

4. Heat to  $90^{\circ}$  C. in a water bath, and add concentrated ammonium hydroxide drop by drop, with constant twirling, until a pink color appears. Cool.
5. Add 2 milliliters of concentrated ammonium hydroxide and stir with a thin glass rod. Stopper, and let stand over night.
6. Centrifuge at 1500 r.p.m. for 10 minutes, carefully decant the supernatant liquid, and drain the tube for 10 minutes by inverting it on a filter paper. Wipe the mouth of the tube with a clean cloth.
7. Wash the sides of the tube with 5 milliliters of the ammonium hydroxide was solution, and break up the precipitate with a thin glass rod, and again centrifuge at 1500 r.p.m. for 10 minutes, decant, and drain as before.
8. Repeat the washing, centrifuging, and decanting for the third time.
9. Add 2 milliliters of 1.0 N sulphuric acid, and dilute with water to about 5 milliliters and let stand 5 minutes.
10. Transfer the content of the tube to a 25 milliliters volumetric flask, washing out the tube with 3 or 4 milliliters of water.
11. Add 5 milliliters of the boric acid solution. If the solution is acid, add 5 drops of brom phenol blue indicator, and neutralize by adding ammonium hydroxide dropwise.
12. Add 2 milliliters of the ammonium molybdate solution. Mix.
13. Add 2 milliliters of the hydroquinone solution. Mix.
14. Add 2.5 milliliters of the sodium succinate solution. Mix; make up to 25 milliliters with water, mix, and let stand

for 30 minutes. The color is constant between 0.5 and 4 hours.

15. Measure the optical density of the solution at a wave length of 775  $m\mu$  using a PC-5 Coleman filter.
16. Run a blank determination, using all of the reagents used in determining the unknown.
17. Make a calibration curve by running standards containing from 0.01 to 0.10 milliligrams of magnesium.

CALCULATIONS:

$$\text{per cent Mg} = \frac{\text{mgs. found} \times 0.1}{\text{g. sample}}$$

## 5. Determination of potassium

Potassium was determined in the solution of digested plant material by the colorimetric method of Peech M.(1941).

### REAGENTS

Ethanol. 70 per cent solution. Dilute 500 mls of 95 per cent ethanol with 180 milliliters of water.

Nitroso R-salt. 0.5 per cent solution. Dissolve 0.5 grams of Nitroso R-salt (disodium salt of 1-nitroso-2-hydroxy-3,6-naphthalene-disulfonic acid) in 100 milliliters of water. When not exposed to direct sunlight, the reagent is stable for several weeks.

Sodium cobaltinitrite. 25 per cent solution. Dissolve 25 grams of reagent quality trisodium cobaltinitrite in water, and dilute to 100 milliliters and filter. Cool the solution before use and keep in a refrigerator. Prepare daily the necessary amount of this reagent needed.

Sodium pyrophosphate. 5 per cent solution. Dissolve 5 grams of powdered sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 100 milliliters of water.

Sodium acetate. Approximately 2.4 N solution.

Sulphuric acid. Approximately 0.1 N solution.

Nitric acid. Approximately 0.1 N solution.

Potassium chloride. Standard solution. Dissolve 0.9533 grams of dry potassium chloride in 500 milliliters of water. Each milliliter contains 1 milligram of potassium.

## PROCEDURE

1. Pipet an aliquot of 3 milliliters of digested ash solution, representing 0.015 grams of dry plant material, into a 15 milliliters conical, heavy duty centrifuge tube. If the aliquot is less than 3 milliliters dilute to 3 milliliters with 0.1 N nitric acid. Add 1 milliliter of the sodium cobaltinitrite reagent and mix thoroughly by twirling the tube. Let stand in the refrigerator for 1 hour.
2. Add 4 milliliters of 70 per cent ethanol, stir with a thin glass rod, wash the rod with ethanol, and centrifuge for 15 minutes at 1700 r.p.m. Decant the supernatant liquid and drain the tube for several minutes on filter paper.
3. Add 5 milliliters of 70 per cent ethanol down the walls of the tube, break up the precipitate with a stirring rod, wash the rod with 1 milliliter of alcohol, and again centrifuge for 10 minutes at 1700 r.p.m.
4. Decant the clear solution, allowing the tube to drain for several minutes, and repeat for the third time, the washing with 5 milliliters of 70 per cent alcohol.
5. Dissolve the precipitate in 5 milliliters of 2 N sulphuric acid and place the tube in a water bath at about 70° C. After the precipitate in the bottom of the tube has been dissolved, add 5 to 7 milliliters of water and heat for another 5 to 10 minutes to dissolve any precipitate adhering to the sides of the tube. Cool. Dilute to 10 milliliters, stopper, and mix.

6. Introduce a 1 milliliter aliquot of the solution into a 25 milliliters volumetric flask.
7. Add 1 milliliter of 5 per cent pyrophosphate solution, dilute to a volume of 20 milliliters with water.
8. Add 1 milliliter of 2.5 N sodium acetate solution and mix.
9. Add 2 milliliters of 0.5 per cent solution of Nitroso-R-salt, mix, bring to volume of 25 mls. and mix. After 15 minutes, determine the optical density by the Coleman spectrophotometer, using a wave length of 550  $m\mu$  and a PC-4 filter. A water blank is used in balancing the instrument.
10. Make a standard curve by preparing standard solutions containing from 0.1 to 1.0 milligrams of potassium in the aliquot placed in the centrifuge tube.

CALCULATION:

$$\text{per cent K} = \frac{(\text{mgs. found}) (0.1)}{(\text{gs. sample})}$$

## 6. Determination of phosphorus

Phosphorus was determined on aliquots of the ash solution by the method described by Stoloff, L.S. (1942)

### REAGENTS

Ammonium molybdate-sulphuric acid. Dissolve 5.0 grams of ammonium molybdate in approximately 80 milliliters of warm (about 50° C.) water, add 2.8 milliliters of concentrated sulphuric acid to the cool solution and dilute to 100 milliliters with water. This solution should not be used if a white residue has settled out. Do not prepare large quantities. Use a freshly prepared solution every few days.

Hydroquinone. 0.5 per cent solution. Dissolve 0.5 grams of hydroquinone in 100 milliliters of water made slightly acid with a drop of concentrated sulphuric acid. Store in the refrigerator.

Sodium succinate. 20 per cent solution. Dissolve 20 grams of anhydrous sodium succinate in 100 milliliters of water. Store in the refrigerator.

Potassium phosphate. Standard solution. Dissolve 0.4394 g. of potassium dihydrogen phosphate in 1000 milliliters of water. Each milliliter contains 0.1 milligram of phosphorus.

Ammonium hydroxide. 1:1 dilution

Boric acid. Saturated solution. Dissolve 50 grams of boric acid in about 800 milliliters of warm water and dilute to 1 liter.



# PROCEDURE:

1. Pipet an aliquot of 1 milliliter of the ash solution, representing 0.005 grams of dry plant material., into a 25 milliliters volumetric flask. The aliquot should not contain more than 0.3 milligrams of phosphorus. The effective range of phosphorus concentrations for this procedure is from 0.002 to 0.300 milligrams of phosphorus in the aliquot. If fluorides are present, add 5 milliliters of the saturated boric acid solution. If the solution is acid, add 5 drops of the brom-phenol blue indicator and neutralize by adding ammonium hydroxide until the color turns blue. If boric acid has been added, 1:10 ammonium hydroxide is satisfactory, but if the original acid extract is used alone, then 1:1 ammonium hydroxide is more convenient.
2. Add the following reagents in the order listed, shaking well after each addition. The maximum lapse of time between each addition which will not affect the color development is 5 minutes, but the blanks must be treated in the same manner.
  - (a) 2.0 milliliters of the ammonium molybdate solution.
  - (b) 2.0 milliliters of the hydroquinone solution.
  - (c) 2.5 milliliters of the sodium succinate solution.
3. Make up to volume with distilled water and allow to stand for 30 minutes. The color is stable between 30 minutes and 4 hours. Determine the optical density of the color in a Coleman Universal Spectrophotometer, using a wave length of 775  $m\mu$  and a PC-5 filter.

## 7. Determination of manganese

Manganese was determined in aliquots of the ash solution by the procedure of Willard and Great-house (1917)

### REAGENTS

Sodium metaperiodate. Fine powder

Phosphoric acid. 85 per cent

Sulfuric acid. Concentrated

Sodium sulfite. Fine powder

Potassium permanganate. Standard solution containing 0.0250 milligrams of manganese per milliliter.

Prepare a 0.10 N standard potassium permanganate solution.

Add 22.8 milliliters of the standard solution to a 250 milliliters Erlenmeyer flask. Add about 50 milliliters of distilled water and 1 milliliter of concentrated sulfuric acid. Heat to boiling and reduce the permanganate by adding sodium sulfite powder. Avoid a large excess of sulfite. Boil off the excess sulfur dioxide and dilute to one liter. Each milliliter of this solution contains 0.0250 milligrams of manganese.

### PROCEDURE

1. Pipet 20 milliliters of the ash solution, representing 0.1000 grams of dry plant material, into a 25 milliliters volumetric flask.
2. Add 1 milliliter of 85 per cent phosphoric acid and about 50 milligrams of sodium periodate. Mix well, and place in water bath at 95° C. Let stand for two hours.

3. Cool, make to volume with distilled water, mix,  
and measure optical density in the Coleman spectro-  
photometer using a PC-4 filter, a wave length of  
530  $m\mu$  and a water blank.
4. Prepare a series of standard solutions containing  
0.0125 to 0.250 milligrams of manganese per 25  
milliliter volumetric flask. Develop color as  
above. Plot optical density against concentration  
of manganese.

### 8. Determination of copper.

Copper was determined polarographically according to the procedure outlined by Reeds and Cunnings (1941).

#### REAGENTS

Nitric acid. Concentrated solution

Sulfuric-perchloric mixture. Mix equal volumes of concentrated sulfuric acid and 72 per cent solution of perchloric acid.

Ammonium hydroxide. Concentrated solution

Ammonium hydroxide solution. a 1:40 solution.

Sodium citrate supporting electrolyte. Dissolve 105.07 grams of citric acid and 20 grams of sodium hydroxide in distilled water and make up to a volume of 2 liters.

Acid fuchsin solution. A 0.05 per cent solution in distilled water.

Nitrogen gas.

#### PROCEDURE

1. Place 0.5 to 2 grams of plant material in a 30 milliliters kjeldahl flask. Add 10 milliliters of concentrated nitric acid, 2 milliliters of the sulfuric-perchloric solution and a drop of kerosene to prevent frothing.
2. Let stand overnight.
3. Heat until the solution is colorless or a pale yellow.
4. Let cool.
5. Add 5 milliliter of water and heat to boiling.
6. Add a slight excess of concentrate ammonium hydroxide

(5 milliliters were used)

7. Boil for a minute. Bumping occurs readily and each sample must be taken separately and shaken all the time while heating to prevent any loss of material from sudden boiling.
8. Filter through a Whatman No 2 filter paper into a 100 milliliters beaker, wash the kjeldahl flask and filter paper with the 1:40 ammonium hydroxide solution.
9. Evaporate the filtrate to dryness at slow heat on a sand bath. Be careful toward the end of the evaporation because the drying salts splatter easily.
10. Take up the residue in 9 milliliters of sodium citrate solution, add 1 milliliter of 0.05 per cent acid fuchsin.
11. Remove the oxygen by bubbling nitrogen 20 minutes.
12. Run the curves with an applied potential from 0.2 volts to - 2.8 volts. The half wave potential for copper in the sodium citrate supporting electrolyte is - 0.15 volts against a saturated calomel electrode.
13. Measure the diffusion current from the polarogram and calculate the corresponding amount of copper.

## 9. Determination of nitrogen

### REAGENTS

Hydrochloric acid. Standard 0.02 N solution

Boric acid. 2 per cent solution. Dissolve 10 grams of acid in 500 milliliters of hot water.

Methyl purple indicator. Commercially prepared solution. (Fleisher chemical company) Add 2 drops to the boric acid solution per 100 milliliters.

Sodium hydroxide. 30 per cent solution. Dissolve 150 grams of sodium hydroxide pellets in 350 milliliters of water.

Ranker's solution. Dissolve 32 grams of salicylic acid in 1 liter of concentrated sulphuric acid.

Sodium thiosulphate. Dissolve 50 grams of sodium thiosulphate in 100 milliliters of water.

Selenium . Powdered metal

Potassium sulphate-Copper sulphate mixture. Pulverize in a mortar 3 parts of copper sulphate with 1 part of potassium sulphate.

### PROCEDURE

1. Weigh from 40 to 50 milligrams of the finely ground dry plant material into a 50 milliliters micro Kjeldahl digestion flask. A long handled micro weighing tube will be found very convenient.
2. Add 2 milliliters of Ranker's solution, mix.
3. Add 5 drops of the thiosulphate solution and gently warm.
4. After several minutes, add about 5 milligrams of the powdered

selenium by means of a micro spatula. Boil until the solution is clear. Usually 40 to 60 minutes are sufficient for the digestion of dry powdered grass material.

5. Transfer the digested solution to the micro still and carefully wash out the flask with 2 rinsings of water.
6. Add 10 milliliters of 30 per cent sodium hydroxide and distill for 10 minutes into 5 milliliters of 2 per cent boric acid.
7. Titrate to a purple color with standard 0.02 N hydrochloric acid.
8. Run a blank determination using all the reagents as in the determination of the unknown.

#### CALCULATIONS

$$\text{per cent N} = \frac{(\text{mls. HCl used} - \text{mls. HCL blank}) \times 0.28}{\text{gs. sample}}$$

#### 10. Determination of total carbohydrates.

The total carbohydrates in the dry plant material was determined by preliminary hydrolysis, and then estimating the reducing sugar by the micro procedure described by Heinze and Murneek (1940).

#### REAGENTS

Hydrochloric acid. Concentrated solution

Lead acetate. Neutral, 25 per cent solution

Potassium oxalate. 25 per cent solution

Potassium hydroxide. 25 per cent solution

Methyl red indicator. 0.1 per cent solution in 60 per cent ethyl alcohol.

Copper reagent "50". Dissolve 25 grams of sodium carbonate and 25 grams of sodium potassium tartrate (Rochelle salt) in about 600 milliliters of water. Then add 75 milliliters of a 10 per cent copper sulphate solution by means of a pipette dipping below the surface of the solution. Add 20 grams of sodium bicarbonate and 1 gram of potassium iodide and mix. Add 200 milliliters of a solution of potassium iodate containing 3.567 grams of iodate per liter. Mix. Bring to volume in a 1 liter volumetric flask. Store in a dark bottle. The iodate content may be varied according to the amount of sugar to be determined. If the reagents are made up as directed, less than 4.40 milligrams of glucose per 10 milliliters of solution may be determined.



Potassium iodide-potassium oxalate. Dissolve 2.50 grams of

potassium iodide and 2.5 grams of potassium oxalate in water and dilute to 100 milliliters. This reagent should be stored in a dark bottle and prepared fresh each week. The development of free iodine on standing will seriously disturb the determination of sugar.

Sodium thiosulphate. Standard 0.0200 N solution. Dissolve

24.82 grams of sodium thiosulphate,  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , in about 800 milliliters of boiled water. Add 10 milliliters of 0.1 N sodium hydroxide. Dilute to 1 liter. This solution is about 0.1. Let stand several days before standardizing. To standardize the solution, accurately weigh from 0.12 to 0.17 grams of potassium iodate into a 250 milliliters Erlenmeyer flask. Dissolve the salt in 50 milliliters of boiled water. Titrate immediately with the thiosulphate to a straw color. Add 3 drops of a starch indicator, or enough to give a blue color, and complete the titration to the disappearance of the starch-iodine blue color. Calculate the normality of the thiosulphate. One milliequivalent of potassium iodate is 0.03567 grams.

$$N \text{ of thiosulphate} = \frac{\text{gs. potassium iodate}}{(\text{mls. thiosulphate}) \times 0.03567}$$

Prepare the standard 0.0200 N thiosulphate by dilution as follows:

$$V_x = \frac{0.0200}{V_1 N_1}$$

where  $V_x$  = final volume of the dilution

$V_1$  = volume of the thiosulphate to be diluted

$N_1$  = normality of the thiosulphate to be diluted.

Sulphuric acid. Approximately 1 N solution. Dilute 28.4 milliliters of concentrated sulphuric acid (sp. gr. 1.835) to 1 liter.

Starch indicator. Add 1 gram of the "soluble" starch to 100 milliliters of a saturated sodium chloride solution.

Agitate, and store in the refrigerator.

#### PROCEDURE:

1. Weigh 1 gram of dry plant material into a 500 milliliters reflux flask, add 200 milliliters of water, and 20 milliliters of 25 per cent hydrochloric acid.
2. Reflux for 2.5 hours. Swirl the flask occasionally to wash down particles adhering to the glass.
3. Add 5 drops of the methyl red indicator, and neutralize with 25 per cent sodium hydroxide. The neutralization must be done carefully.
4. Add 30 milliliters of the 25 per cent neutral lead acetate, mix. Test for complete precipitation by adding a drop of the lead acetate.
5. Add 10 milliliters of 25 per cent potassium oxalate. Mix. Test for complete precipitation of the lead by adding a drop of the oxalate.
6. Filter into a 500 milliliters volumetric flask through Whatman No. 2 filter paper. Bring to volume and mix.
7. The magnitude of the aliquot depends on the concentration of reducing sugar. Ordinarily 3 milliliters will be

satisfactory. Three milliliters correspond to 15 milligrams of the dry plant material.

8. Pipet an aliquot of 3 milliliters of the solution into an 8 x 1 inch test tube. Add 10 milliliters of reagent "50". Mix, cover the tubes with a small beaker and immerse in a boiling water bath for 15 minutes.
9. Cool in running water for 3 minutes, or until the temperature is about 30° C.
10. Add 2 milliliters of the potassium iodide-potassium oxalate solution. Mix.
11. Add 10 milliliters of the 1 N sulphuric acid. Agitate thoroughly. Let stand for 5 minutes.
12. Titrate with 0.0200 N standard thiosulphate until the straw color has almost disappeared. Add 3 drops of the starch indicator, and complete the titration.
13. Prepare a calibration curve by obtaining the titration values for a series of standard glucose solutions containing from 0.1 to 4.4 milligrams of glucose per aliquot.
14. Titrate a blank, substituting 3 milliliters of water for the plant extract.

#### CALCULATIONS:

The number of milliliters of thiosulphate used to titrate the blank, minus the milliliters of the thiosulphate used to titrate the sample is proportional to the sugar present, calculated as glucose.

Read the amount of sugar in the sample from the calibration curve.

$$\text{per cent glucose} = \frac{\text{mgs. found} \times 0.1}{\text{Gs. sample}}$$

# 11. Determination of ascorbic acid.

The method used for the determination of ascorbic acid was the photometric procedure described by Morell (1941) and Bessey (1938)

A solution of 0.5 per cent oxalic acid was used as an extractant which according to Ponting (1943) may be substituted for metaphosphoric acid.

## REAGENTS:

Oxalic acid. 0.5 per cent solution in distilled water.

Dye solution. 34.4 milligrams of sodium 2,6- dichlorobenzenone-indophenol per liter of distilled water.

## PROCEDURE:

1. Macerate a sample of about 5 grams of fresh plant material with 150 milliliters of 0.5 per cent oxalic acid in a Waring Blender for 5 minutes, then make up to 200 milliliters.
2. Filter the macerate with suction through 2 thicknesses of Cenco No 13250 filter paper. Discard the first 10 milliliters of the extract.
3. Set the Coleman Spectrophotometer, using a wave length of 520 and a PC-4 filter, to read 100 per cent transmission on the galvanometer scale, against a blank tube containing only distilled water.

4. Pipet aliquots of 5 milliliters of the extract into matched Coleman photometer tubes, quickly deliver 5 milliliters of the dye into the sample and shake vigorously for about 5 seconds.
5. Take the transmission readings 15 and 30 seconds after the time the dye has been added. These readings are designated as  $G_1$  and  $G_2$ .
6. Reduce the extract with a few crystals of ascorbic acid to give the transmission reading ,  $G_{s_r}$ .
7. Adjust the galvanometer reading to give the value of  $G_{s_r}$  with 5 milliliters of 0.5 per cent oxalic acid plus 5 milliliters of dye and a few crystals of ascorbic acid.
8. Mix 5 milliliters of 0.5 per cent oxalic acid with 5 milliliters of dye to give the transmission readings  $G_{b_1}$  and  $G_{b_2}$ .

CALCULATION:

From the average value of  $G_s$  and  $G_b$ , the amount of ascorbic acid,  $C$ , is obtained as follows:

$$C = K (\log G_s - \log G_b)$$

With the amount of dye used and the dilution of the sample to 200 milliliters, the value of  $K$  was found to be 8 by calculation from the standard curve.

Preparation of the standard curve:

Prepare a fresh solution of ascorbic acid by dissolving 25 milligrams in 250 milliliters of 0.5 per cent oxalic acid. Add 14 aliquots, each differing by 1 milliliter increments (corresponding to 1 to 14 micrograms of ascorbic acid per milliliter) to a series of 14 100 milliliters volumetric flasks and make up to volume with 0.5 per cent oxalic acid.

Use aliquots of 5 milliliters of these standards to obtain the standard curve, following the steps described above.

Plot the values of the expression,  $\log G_s - \log G_b$ , on millimeter graph paper against the known amounts of ascorbic acid in the aliquot.

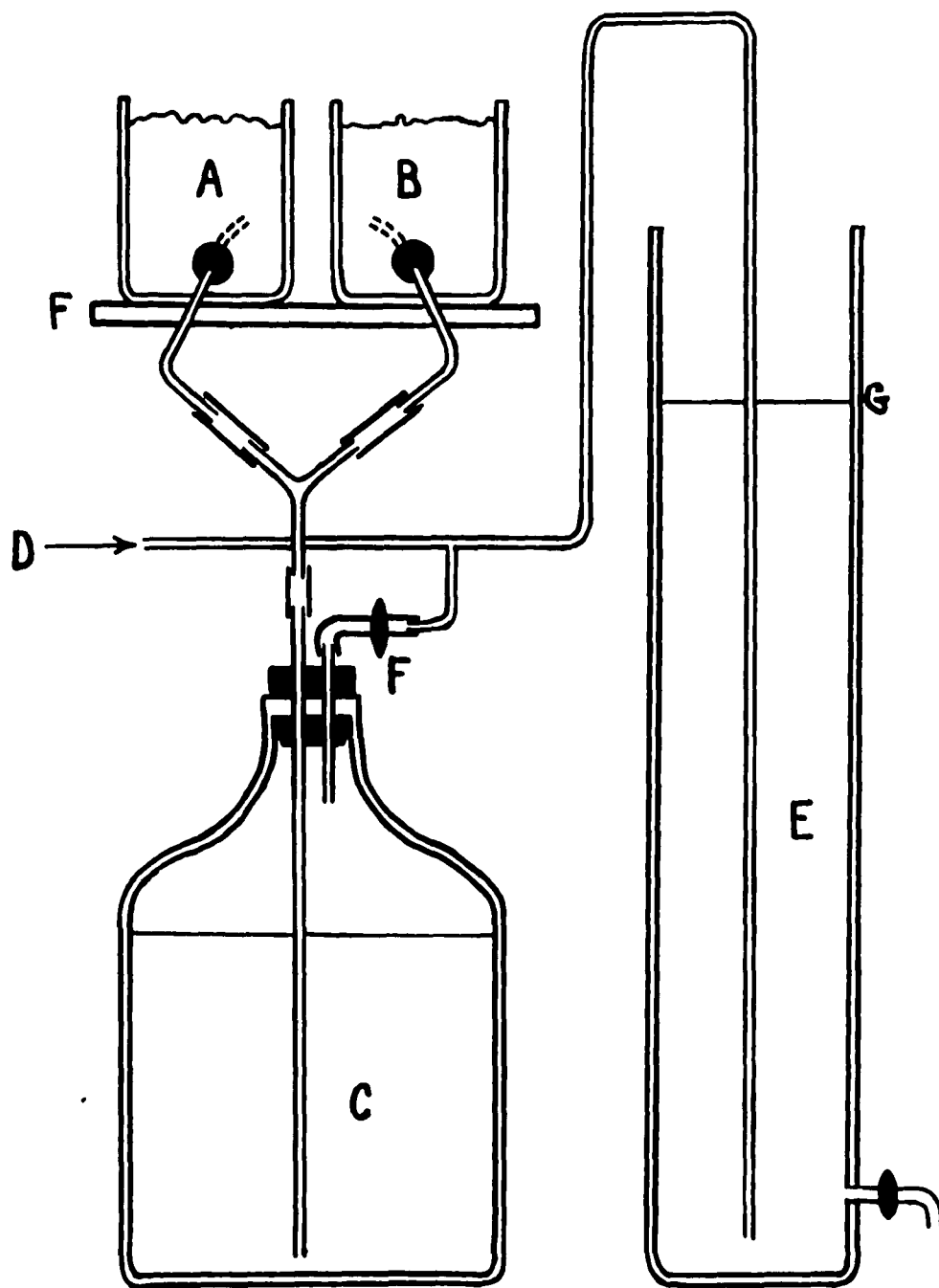


Figure 1. Mechanical arrangement of the cultures.



Experiment 1

Table 1. Experimental results obtained from wheat plants grown on various levels of iron in the nutrient solutions; averages expressed per 10 plants.

| Parts per million<br>of iron in the<br>nutrient solution | Grams of fresh<br>weight | Grams of<br>dry matter | Grams total<br>carbohydrate | Grams total<br>nitrogen | Grams total<br>chlorophyll | Milligrams of<br>iron |
|----------------------------------------------------------|--------------------------|------------------------|-----------------------------|-------------------------|----------------------------|-----------------------|
| 0                                                        | 22.3                     | 3.65                   | 0.57                        | 0.13                    | 0.078                      | 0.307                 |
| 1                                                        | 22.3                     | 3.83                   | 0.63                        | 0.14                    | 0.076                      | 0.283                 |
| 2                                                        | 25.6                     | 4.20                   | 0.59                        | 0.16                    | 0.094                      | 0.437                 |
| 3                                                        | 32.2                     | 5.54                   | 0.77                        | 0.20                    | 0.117                      | 0.543                 |
| 4                                                        | 26.5                     | 4.36                   | 0.62                        | 0.15                    | 0.092                      | 0.340                 |
| 5                                                        | 27.0                     | 4.46                   | 0.62                        | 0.14                    | 0.091                      | 0.357                 |
| 6                                                        | 28.9                     | 5.24                   | 0.74                        | 0.17                    | 0.106                      | 0.419                 |
| 7                                                        | 22.5                     | 4.38                   | 0.60                        | 0.14                    | 0.074                      | 0.429                 |
| 8                                                        | 23.9                     | 4.38                   | 0.63                        | 0.14                    | 0.088                      | 0.342                 |
| 9                                                        | 22.4                     | 5.18                   | 0.73                        | 0.15                    | 0.090                      | 0.404                 |

Experiment 1.

Table 2. Experimental results obtained from wheat plants grown on various levels of iron in the nutrient solutions; averages expressed per 10 plants.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|----------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0                                                        | 3.41                                         | 2.11                                                   | 1.96                                                      | —                                                          |
| 1                                                        | 2.83                                         | 2.27                                                   | 1.87                                                      | 0.31                                                       |
| 2.                                                       | 3.33                                         | 2.21                                                   | 2.17                                                      | 0.27                                                       |
| 3                                                        | 4.24                                         | 2.95                                                   | 2.87                                                      | 0.42                                                       |
| 4                                                        | 3.11                                         | 2.53                                                   | 2.29                                                      | 0.33                                                       |
| 5                                                        | 3.62                                         | 2.40                                                   | 2.35                                                      | 0.34                                                       |
| 6                                                        | 3.62                                         | 3.10                                                   | 2.82                                                      | 0.45                                                       |
| 7                                                        | 2.53                                         | 2.17                                                   | 2.30                                                      | 0.34                                                       |
| 8                                                        | 3.23                                         | 2.52                                                   | 2.23                                                      | 0.36                                                       |
| 9                                                        | 3.00                                         | 2.59                                                   | 2.76                                                      | 0.52                                                       |

Experiment 1.

Table 3. Experimental results obtained from wheat plants grown on various levels of copper in the nutrient solutions; averages expressed per 10 plants.

| Parts per million<br>of copper in the<br>nutrient solution | Grams of fresh<br>matter | Grams of<br>dry matter | Grams total<br>carbohydrate | Grams total<br>nitrogen | Grams total<br>chlorophyll | Milligrams of<br>iron. |
|------------------------------------------------------------|--------------------------|------------------------|-----------------------------|-------------------------|----------------------------|------------------------|
| 0.00                                                       | 19.8                     | 3.38                   | 0.49                        | 0.12                    | 0.070                      | 0.270                  |
| 0.01                                                       | 21.8                     | 3.39                   | 0.47                        | 0.12                    | 0.074                      | 0.264                  |
| 0.02                                                       | 22.3                     | 3.72                   | 0.53                        | 0.12                    | 0.076                      | 0.305                  |
| 0.03                                                       | 22.3                     | 3.62                   | 0.50                        | 0.13                    | 0.074                      | 0.275                  |
| 0.04                                                       | 24.0                     | 4.10                   | 0.57                        | 0.13                    | 0.075                      | 0.320                  |
| 0.05                                                       | 36.8                     | 4.34                   | 0.60                        | 0.15                    | 0.084                      | 0.356                  |
| 0.06                                                       | 30.4                     | 5.07                   | 0.72                        | 0.16                    | 0.092                      | 0.385                  |
| 0.07                                                       | 23.9                     | 3.70                   | 0.52                        | 0.14                    | 0.081                      | 0.274                  |
| 0.08                                                       | 24.7                     | 4.28                   | 0.59                        | 0.14                    | 0.069                      | 0.334                  |
| 0.09                                                       | 18.5                     | 3.05                   | 0.44                        | 0.09                    | 0.053                      | 0.220                  |

Experiment 1

Table 4. Experimental results obtained from wheat plants grown on various levels of copper in the nutrient solutions; averages expressed per 10 plants.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|------------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0.00                                                       | 2.77                                         | 2.00                                                   | 1.82                                                      | 0.27                                                       |
| 0.01                                                       | 2.62                                         | 1.91                                                   | 1.80                                                      | 0.19                                                       |
| 0.02                                                       | 2.80                                         | 2.09                                                   | 2.05                                                      | 0.15                                                       |
| 0.03                                                       | 2.42                                         | 2.24                                                   | 2.00                                                      | 0.15                                                       |
| 0.04                                                       | 3.06                                         | 2.74                                                   | 2.18                                                      | 0.27                                                       |
| 0.05                                                       | 3.61                                         | 2.72                                                   | 2.34                                                      | 0.27                                                       |
| 0.06                                                       | 4.66                                         | 3.66                                                   | 2.85                                                      | 0.32                                                       |
| 0.07                                                       | 3.30                                         | 2.56                                                   | 2.04                                                      | 0.33                                                       |
| 0.08                                                       | 3.00                                         | 2.96                                                   | 2.22                                                      | 0.42                                                       |
| 0.09                                                       | 2.51                                         | 2.06                                                   | 1.64                                                      | 0.44                                                       |

Experiment 1

Table 5. Experimental results obtained from wheat plants grown on various levels of iron in the nutrient solutions; averages expressed per 100 grams of fresh material.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>iron | Grams of<br>total carbohydrate | Grams of<br>total nitrogen | Milligrams of<br>chlorophyll | Grams of<br>dry matter |
|----------------------------------------------------------|-----------------------|--------------------------------|----------------------------|------------------------------|------------------------|
| 0                                                        | 1.37                  | 2.5                            | 0.59                       | 349                          | 16.3                   |
| 1                                                        | 1.27                  | 2.8                            | 0.63                       | 343                          | 17.2                   |
| 2                                                        | 1.70                  | 2.3                            | 0.63                       | 368                          | 16.4                   |
| 3                                                        | 1.65                  | 2.4                            | 0.61                       | 364                          | 17.2                   |
| 4                                                        | 1.28                  | 2.3                            | 0.58                       | 346                          | 16.5                   |
| 5                                                        | 1.32                  | 2.3                            | 0.52                       | 336                          | 16.5                   |
| 6                                                        | 1.45                  | 2.6                            | 0.60                       | 367                          | 18.1                   |
| 7                                                        | 1.91                  | 2.7                            | 0.63                       | 330                          | 19.5                   |
| 8                                                        | 1.43                  | 2.6                            | 0.57                       | 368                          | 18.3                   |
| 9                                                        | 1.66                  | 3.0                            | 0.60                       | 368                          | 21.3                   |

Experiment 1

Table 6. Experimental results obtained from wheat plants on various concentrations of iron in the nutrient solutions; averages expressed per 100 grams of fresh material (continued).

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|----------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0                                                        | 15.24                                        | 9.4                                                    | 8.8                                                       | ---                                                        |
| 1                                                        | 12.70                                        | 10.2                                                   | 8.4                                                       | 1.4                                                        |
| 2                                                        | 13.02                                        | 8.6                                                    | 8.5                                                       | 1.1                                                        |
| 3                                                        | 13.18                                        | 9.2                                                    | 8.9                                                       | 1.3                                                        |
| 4                                                        | 11.74                                        | 9.5                                                    | 8.7                                                       | 1.2                                                        |
| 5                                                        | 13.39                                        | 8.9                                                    | 8.7                                                       | 1.3                                                        |
| 6                                                        | 12.58                                        | 10.7                                                   | 9.8                                                       | 1.5                                                        |
| 7                                                        | 11.25                                        | 9.7                                                    | 10.3                                                      | 1.5                                                        |
| 8                                                        | 13.49                                        | 10.5                                                   | 9.3                                                       | 1.5                                                        |
| 9                                                        | 12.34                                        | 10.6                                                   | 11.3                                                      | 2.1                                                        |

Experiment 1

Table 7. Experimental results obtained from wheat plants grown on nutrient solutions supplied with various amounts of copper; averages expressed per 100 grams of fresh material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>iron | Grams of<br>total carbohydrate | Grams of<br>total nitrogen | Milligrams of<br>chlorophyll | Grams of dry<br>matter. |
|------------------------------------------------------------|-----------------------|--------------------------------|----------------------------|------------------------------|-------------------------|
| 0.00                                                       | 1.37                  | 2.5                            | 0.6                        | 354                          | 17.1                    |
| 0.01                                                       | 1.21                  | 2.2                            | 0.6                        | 339                          | 15.6                    |
| 0.02                                                       | 1.37                  | 2.4                            | 0.5                        | 340                          | 16.7                    |
| 0.03                                                       | 1.24                  | 2.3                            | 0.6                        | 332                          | 16.3                    |
| 0.04                                                       | 1.34                  | 2.4                            | 0.5                        | 311                          | 17.1                    |
| 0.05                                                       | 1.32                  | 2.2                            | 0.5                        | 315                          | 16.2                    |
| 0.06                                                       | 1.27                  | 2.4                            | 0.5                        | 304                          | 16.7                    |
| 0.07                                                       | 1.14                  | 2.2                            | 0.6                        | 338                          | 15.5                    |
| 0.08                                                       | 1.35                  | 2.4                            | 0.5                        | 280                          | 17.3                    |
| 0.09                                                       | 1.18                  | 2.4                            | 0.5                        | 285                          | 16.5                    |

Experiment 1

Table 8. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 100 grams of fresh material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|------------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0.00                                                       | 14.63                                        | 10.1                                                   | 9.2                                                       | 1.4                                                        |
| 0.01                                                       | 12.01                                        | 8.7                                                    | 8.3                                                       | 0.9                                                        |
| 0.02                                                       | 12.56                                        | 9.4                                                    | 9.2                                                       | 0.7                                                        |
| 0.03                                                       | 10.87                                        | 10.1                                                   | 9.0                                                       | 0.7                                                        |
| 0.04                                                       | 12.77                                        | 11.4                                                   | 9.1                                                       | 1.1                                                        |
| 0.05                                                       | 13.45                                        | 10.1                                                   | 8.7                                                       | 1.0                                                        |
| 0.06                                                       | 15.33                                        | 12.0                                                   | 9.4                                                       | 1.1                                                        |
| 0.07                                                       | 13.84                                        | 10.7                                                   | 8.6                                                       | 1.4                                                        |
| 0.08                                                       | 12.12                                        | 12.0                                                   | 8.9                                                       | 1.7                                                        |
| 0.09                                                       | 13.55                                        | 11.1                                                   | 8.8                                                       | 1.6                                                        |



Experiment 1.

Table 9. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of dry material.

| Parts per million<br>of iron in the<br>solution. | Milligrams of<br>iron. | Grams of total<br>carbohydrate | Grams of total<br>nitrogen | Grams of<br>chlorophyll | Grams of fresh<br>material |
|--------------------------------------------------|------------------------|--------------------------------|----------------------------|-------------------------|----------------------------|
| 0                                                | 8.4                    | 15.6                           | 3.64                       | 2.14                    | 612                        |
| 1                                                | 7.4                    | 16.4                           | 3.68                       | 1.99                    | 582                        |
| 2                                                | 10.4                   | 14.3                           | 3.35                       | 2.24                    | 610                        |
| 3                                                | 9.6                    | 13.9                           | 3.55                       | 2.12                    | 581                        |
| 4                                                | 7.8                    | 14.1                           | 3.53                       | 2.10                    | 608                        |
| 5                                                | 8.0                    | 14.0                           | 3.18                       | 2.04                    | 606                        |
| 6                                                | 8.0                    | 14.2                           | 3.30                       | 2.03                    | 551                        |
| 7                                                | 9.8                    | 13.7                           | 3.22                       | 1.69                    | 513                        |
| 8                                                | 7.8                    | 14.4                           | 3.11                       | 2.01                    | 546                        |
| 9                                                | 7.8                    | 14.0                           | 2.80                       | 1.73                    | 470                        |

Experiment 1.

Table 10. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of dry material. (continued)

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|----------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0                                                        | 93.3                                         | 57.8                                                   | 53.8                                                      | ---                                                        |
| 1                                                        | 74.0                                         | 59.2                                                   | 48.8                                                      | 8.1                                                        |
| 2                                                        | 79.4                                         | 52.6                                                   | 51.6                                                      | 6.5                                                        |
| 3                                                        | 76.6                                         | 53.2                                                   | 51.8                                                      | 7.6                                                        |
| 4                                                        | 71.3                                         | 58.0                                                   | 52.6                                                      | 7.5                                                        |
| 5                                                        | 81.1                                         | 53.8                                                   | 52.6                                                      | 7.7                                                        |
| 6                                                        | 69.3                                         | 59.2                                                   | 53.8                                                      | 8.5                                                        |
| 7                                                        | 57.7                                         | 49.6                                                   | 52.6                                                      | 7.8                                                        |
| 8                                                        | 73.7                                         | 57.6                                                   | 50.8                                                      | 8.1                                                        |
| 9                                                        | 58.0                                         | 50.0                                                   | 53.2                                                      | 10.0                                                       |

Experiment 1.

Table 11. Experimental results obtained from wheat plants grown on nutrient solutions varying in the concentrations of copper; averages expressed per 100 grams of dry material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>iron | Grams of total<br>carbohydrate | Grams of total<br>nitrogen | Grams of<br>chlorophyll | Grams of fresh<br>material |
|------------------------------------------------------------|-----------------------|--------------------------------|----------------------------|-------------------------|----------------------------|
| 0.00                                                       | 8.0                   | 14.6                           | 3.54                       | 2.07                    | 585                        |
| 0.01                                                       | 7.8                   | 14.0                           | 3.55                       | 2.17                    | 643                        |
| 0.02                                                       | 8.2                   | 14.2                           | 3.26                       | 2.04                    | 600                        |
| 0.03                                                       | 7.6                   | 13.9                           | 3.71                       | 2.04                    | 615                        |
| 0.04                                                       | 7.6                   | 14.0                           | 3.17                       | 1.82                    | 584                        |
| 0.05                                                       | 8.2                   | 13.9                           | 3.36                       | 1.94                    | 619                        |
| 0.06                                                       | 7.6                   | 14.3                           | 3.05                       | 1.82                    | 600                        |
| 0.07                                                       | 7.4                   | 14.1                           | 3.84                       | 2.18                    | 644                        |
| 0.08                                                       | 7.8                   | 13.9                           | 3.13                       | 1.62                    | 579                        |
| 0.09                                                       | 7.2                   | 14.5                           | 2.87                       | 1.73                    | 608                        |

Experiment 1.

Table 12. Experimental results obtained from wheat plants grown on nutrient solutions varying in the concentrations of copper; averages expressed per 100 grams of dry material

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>31 days after<br>drying | Milligrams of<br>ascorbic acid<br>105 days after<br>drying |
|------------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0.00                                                       | 82.0                                         | 59.2                                                   | 53.8                                                      | 8.2                                                        |
| 0.01                                                       | 77.2                                         | 56.2                                                   | 53.2                                                      | 5.6                                                        |
| 0.02                                                       | 75.3                                         | 56.2                                                   | 55.2                                                      | 4.1                                                        |
| 0.03                                                       | 66.9                                         | 62.0                                                   | 55.2                                                      | 4.1                                                        |
| 0.04                                                       | 74.6                                         | 66.8                                                   | 53.2                                                      | 6.5                                                        |
| 0.05                                                       | 83.3                                         | 62.6                                                   | 53.8                                                      | 6.3                                                        |
| 0.06                                                       | 92.0                                         | 72.2                                                   | 56.2                                                      | 6.4                                                        |
| 0.07                                                       | 89.2                                         | 69.2                                                   | 55.2                                                      | 8.8                                                        |
| 0.08                                                       | 70.1                                         | 69.2                                                   | 51.8                                                      | 10.0                                                       |
| 0.09                                                       | 82.3                                         | 67.6                                                   | 53.8                                                      | 9.4                                                        |

Experiment 1.

Table 13. Percentages of the original amounts of ascorbic acid found in wheat plants grown on various levels of iron, at each determinations after the drying of the plants.

| Parts per million<br>of iron in the<br>nutrient solution | Percentage of<br>ascorbic acid<br>at harvest | Percentage of<br>ascorbic acid<br>just after<br>drying | Percentage of<br>ascorbic acid<br>31 days after<br>drying | Percentage of<br>ascorbic acid<br>105 days after<br>drying |
|----------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0                                                        | 100                                          | 61.7                                                   | 57.7                                                      | ---                                                        |
| 1                                                        | 100                                          | 80.3                                                   | 66.1                                                      | 11.0                                                       |
| 2                                                        | 100                                          | 66.1                                                   | 65.3                                                      | 8.4                                                        |
| 3                                                        | 100                                          | 69.8                                                   | 67.5                                                      | 9.9                                                        |
| 4                                                        | 100                                          | 80.9                                                   | 74.1                                                      | 10.2                                                       |
| 5                                                        | 100                                          | 66.5                                                   | 65.0                                                      | 9.7                                                        |
| 6                                                        | 100                                          | 85.1                                                   | 77.9                                                      | 11.9                                                       |
| 7                                                        | 100                                          | 86.2                                                   | 91.5                                                      | 13.3                                                       |
| 8                                                        | 100                                          | 77.8                                                   | 68.9                                                      | 11.1                                                       |
| 9                                                        | 100                                          | 85.9                                                   | 91.6                                                      | 17.0                                                       |

Experiment 1.

Table 14. Percentages of the original amounts of ascorbic acid found in wheat plants grown on various levels of copper, at each determinations after the drying of the plants.

| Parts per million<br>of copper in the<br>nutrient solution | Percentage of<br>ascorbic acid<br>at harvest | Percentage of<br>ascorbic acid<br>just after<br>drying | Percentage of<br>ascorbic acid<br>31 days after<br>drying | Percentage of<br>ascorbic acid<br>105 days after<br>drying |
|------------------------------------------------------------|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------|
| 0.00                                                       | 100                                          | 72.0                                                   | 65.6                                                      | 10.0                                                       |
| 0.01                                                       | 100                                          | 72.4                                                   | 68.1                                                      | 7.2                                                        |
| 0.02                                                       | 100                                          | 74.9                                                   | 73.3                                                      | 5.4                                                        |
| 0.03                                                       | 100                                          | 92.9                                                   | 82.7                                                      | 6.2                                                        |
| 0.04                                                       | 100                                          | 89.3                                                   | 71.3                                                      | 8.6                                                        |
| 0.05                                                       | 100                                          | 75.1                                                   | 64.7                                                      | 7.4                                                        |
| 0.06                                                       | 100                                          | 78.3                                                   | 61.3                                                      | 7.2                                                        |
| 0.07                                                       | 100                                          | 77.3                                                   | 62.1                                                      | 10.1                                                       |
| 0.08                                                       | 100                                          | 99.0                                                   | 73.4                                                      | 14.1                                                       |
| 0.09                                                       | 100                                          | 81.9                                                   | 64.9                                                      | 11.8                                                       |

Figure 2. Average fresh weight, expressed in grams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 3. Average dry weight, expressed in grams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 4. Average amounts of iron, expressed in milligrams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 5. Average amounts of ascorbic acid at harvest, expressed in milligrams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 6. Average amounts of ascorbic acid just after drying, expressed in milligrams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 7. Average amounts of ascorbic acid 31 days after drying, expressed in milligrams per ten wheat plants, plotted against the concentration of iron in the nutrient solution expressed in parts per million.

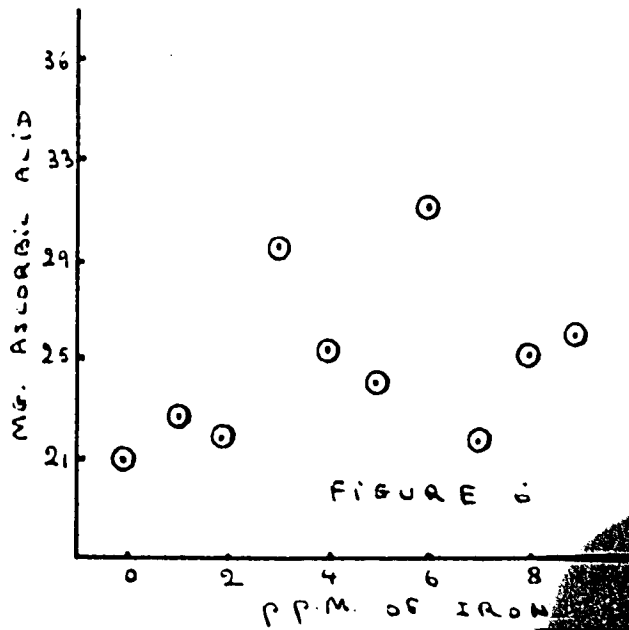
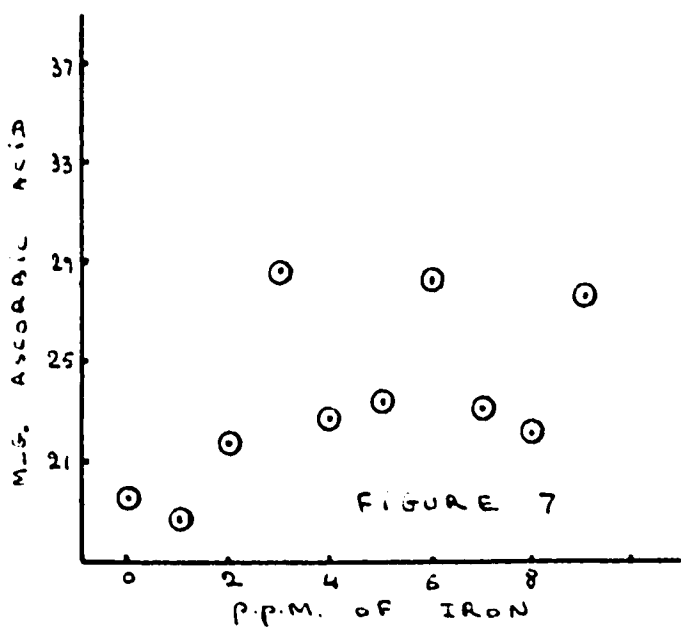
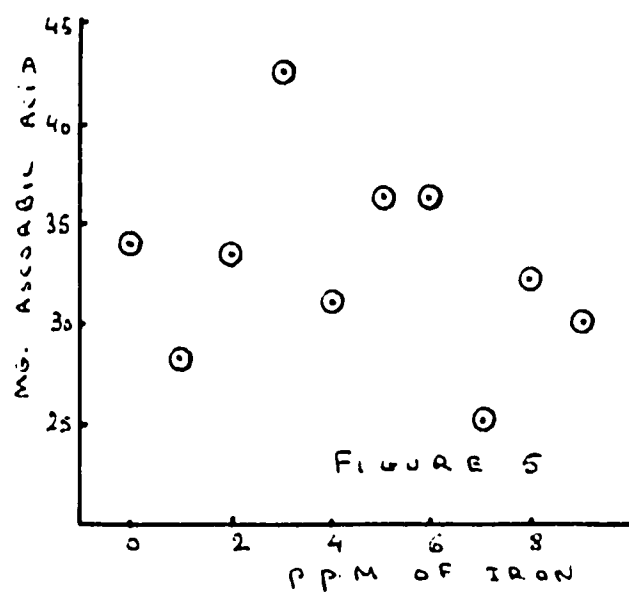
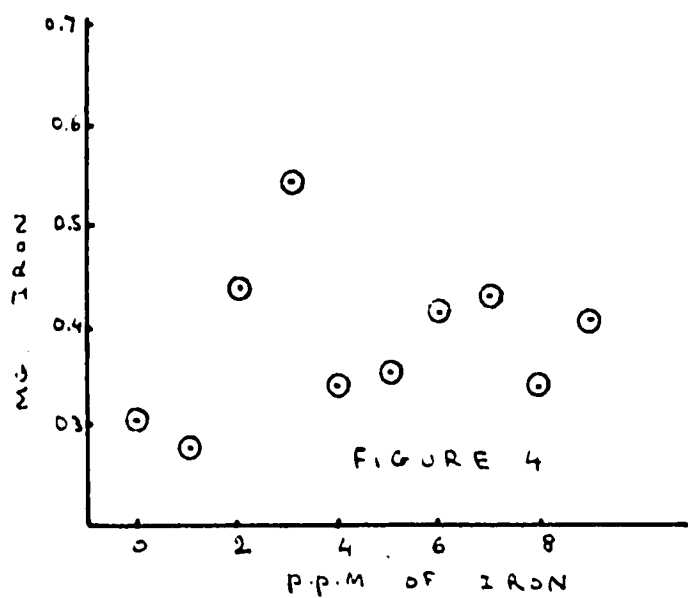
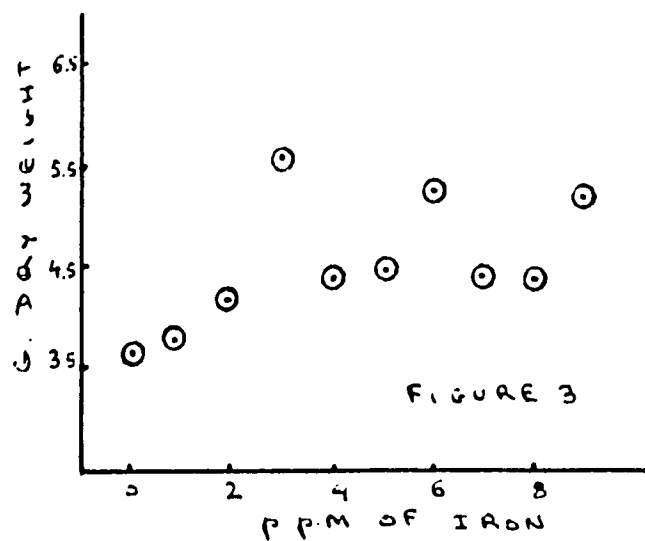
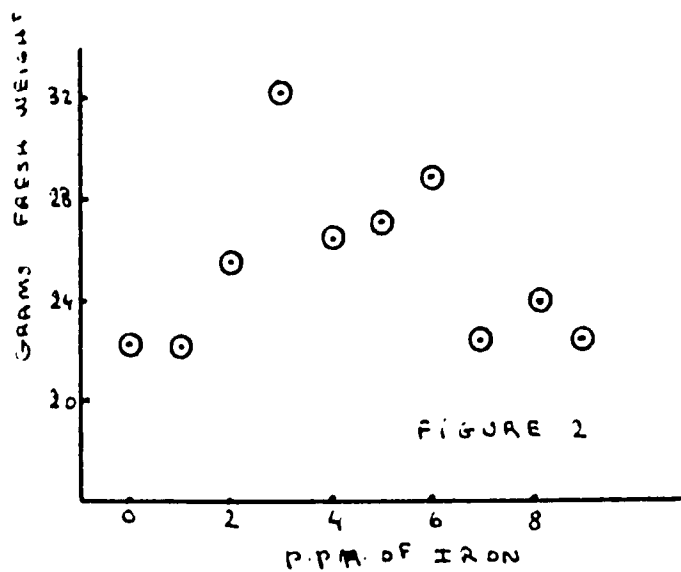




Figure 8. Average amounts of ascorbic acid 105 days after drying, expressed in milligrams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 9. Average amounts of total carbohydrate, expressed in grams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 10. Average amounts of total nitrogen, expressed in grams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million ,

Figure 11. Average amounts of chlorophyll, expressed in grams per ten wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 12. Average amounts of nitrogen, expressed in grams per ten wheat plants, plotted against the amount of iron, expressed in milligrams per ten wheat plants.

Figure 13. Average amounts of chlorophyll, expressed in grams per ten wheat plants, plotted against the amounts of iron, expressed in milligrams per ten wheat plants.

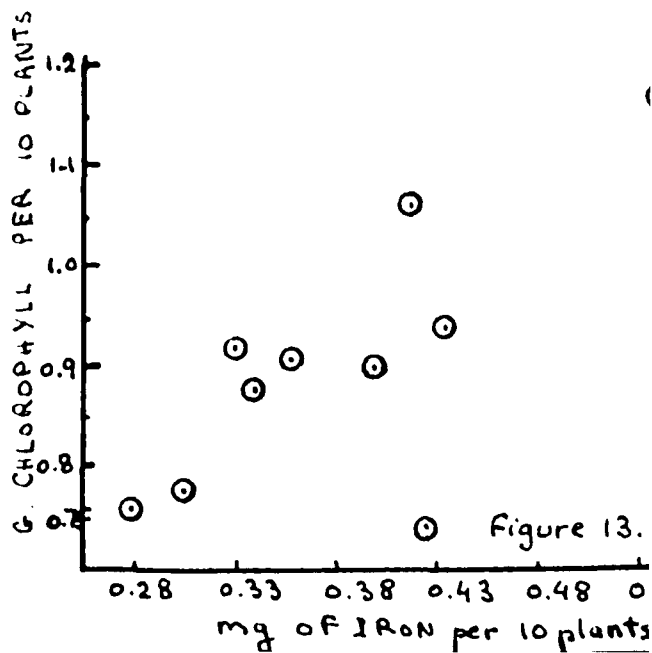
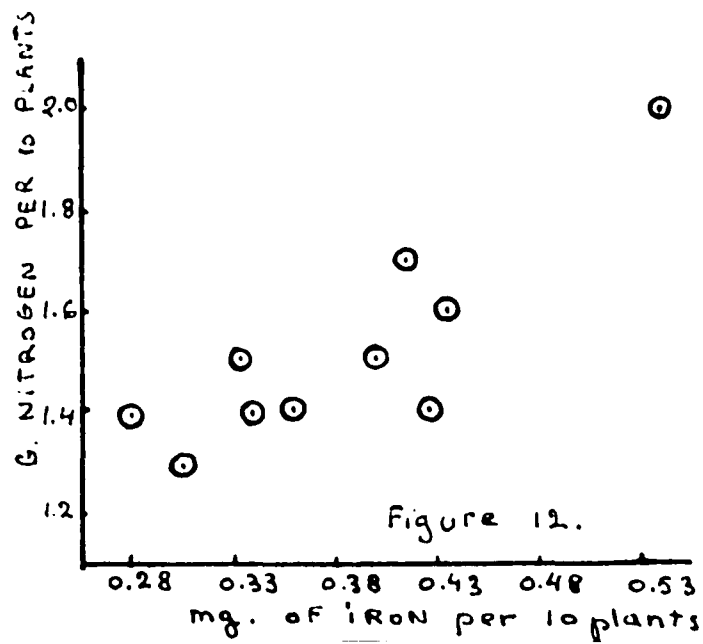
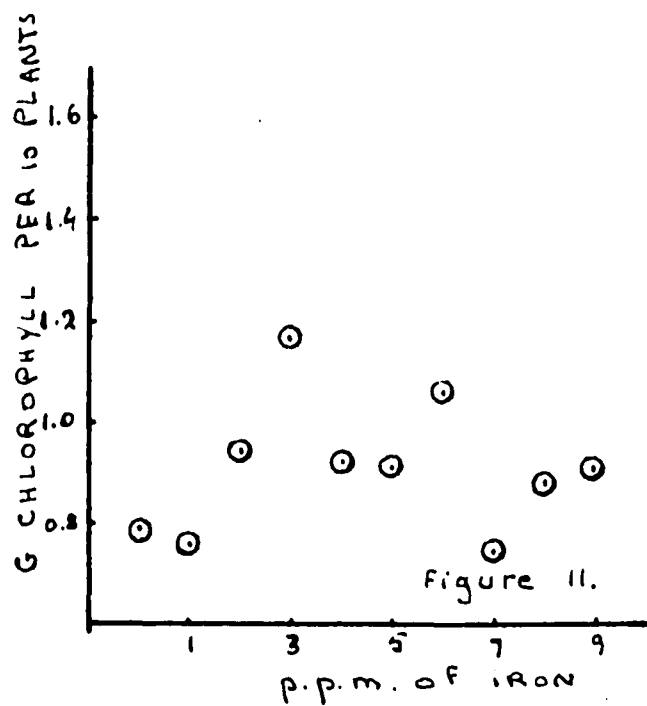
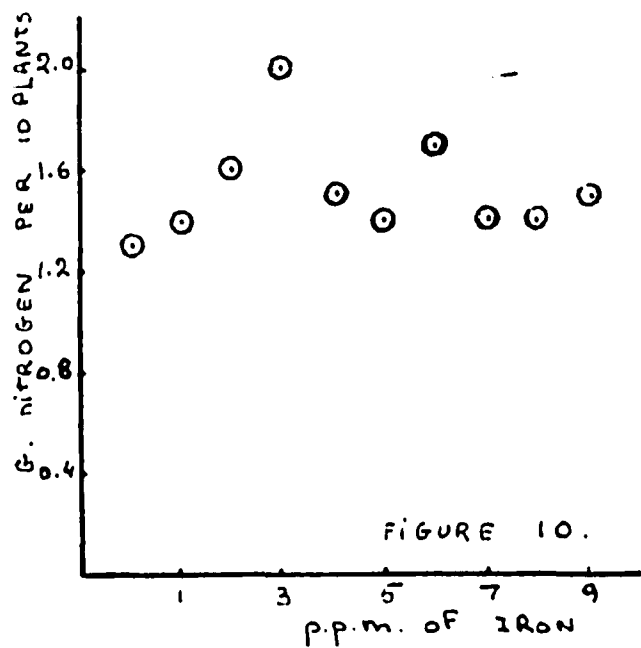
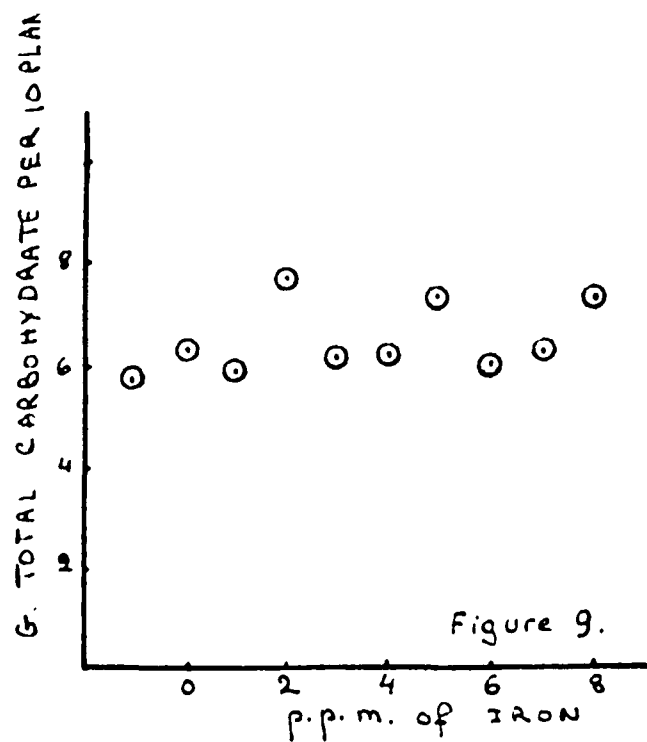
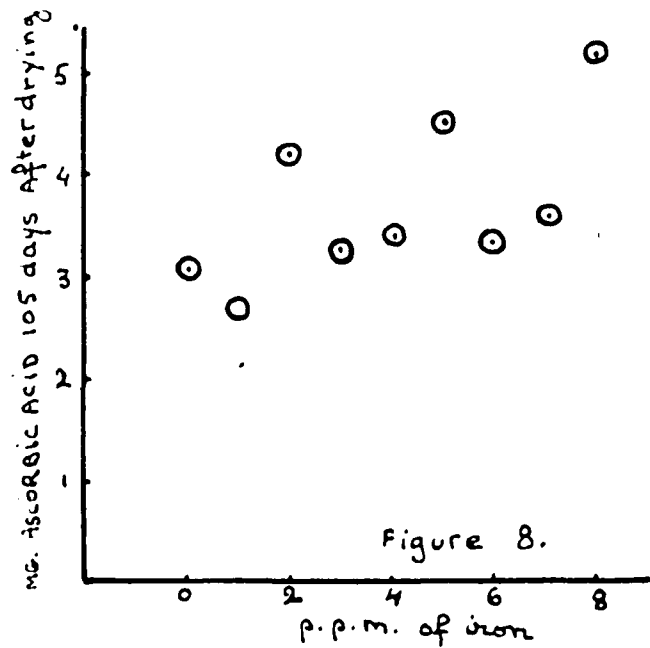
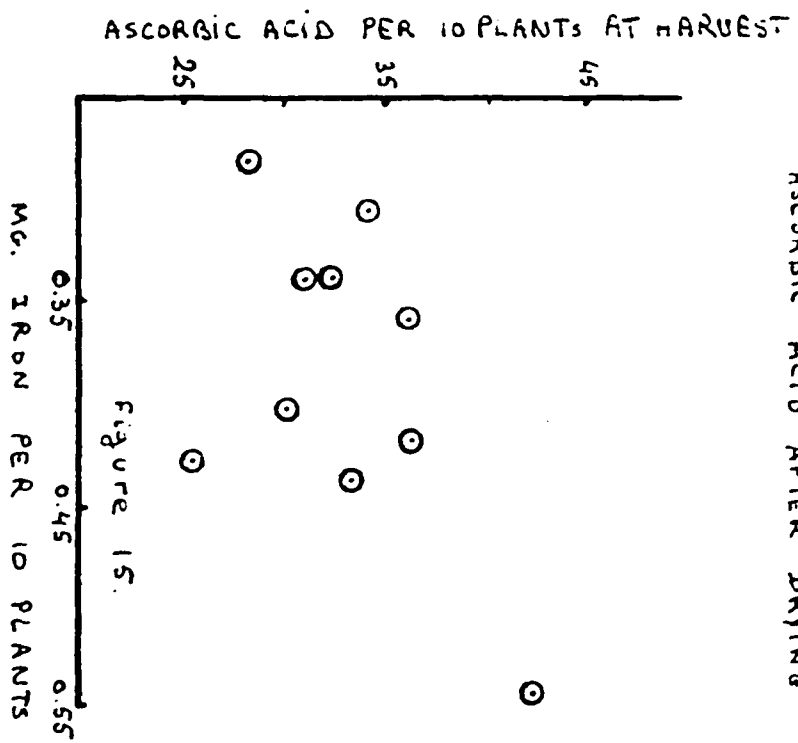
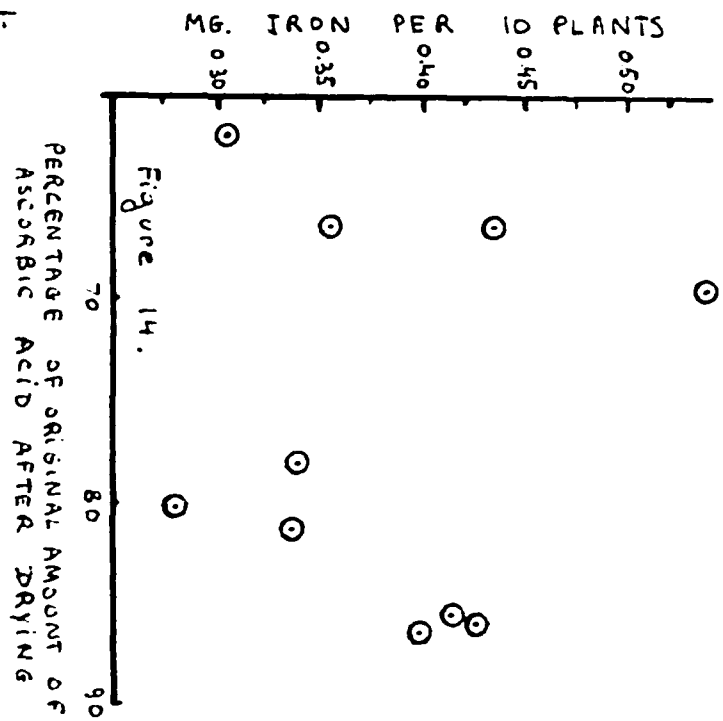


Figure 14. Average amounts of iron, expressed in milligrams per ten wheat plants grown on various concentrations of iron, plotted against the percentage of the original amount of ascorbic acid just after drying.

Figure 15. Average amounts of ascorbic acid at harvest, expressed in milligrams per ten wheat plants grown on various concentrations of iron in the nutrient solution, plotted against the amounts of iron, expressed in milligrams per ten plants.



- Figure 16. Average amounts of iron, expressed in milligrams per 100,grams of fresh material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 17. Average amounts of ascorbic acid just after drying expressed in milligrams per 100 grams of fresh material from wheat plants , plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 18. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 19. Average amounts of ascorbic acid 31 days after drying, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 20. Average amounts of ascorbic acid 105 days after drying, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 21. Average amounts of carbohydrate, expressed in grams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

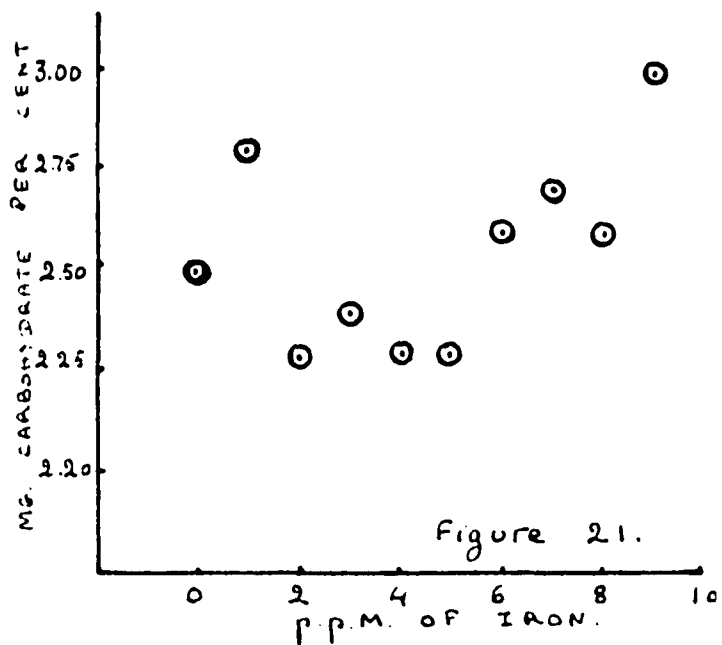
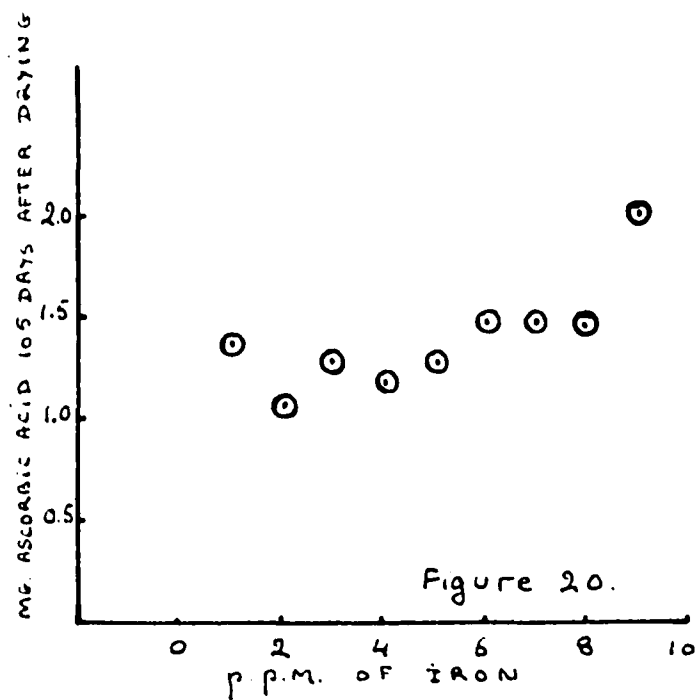
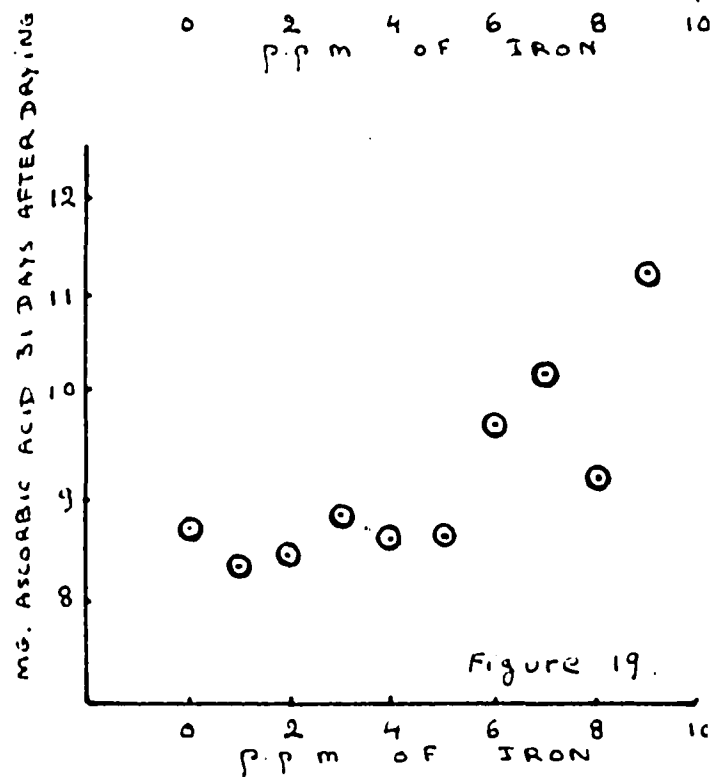
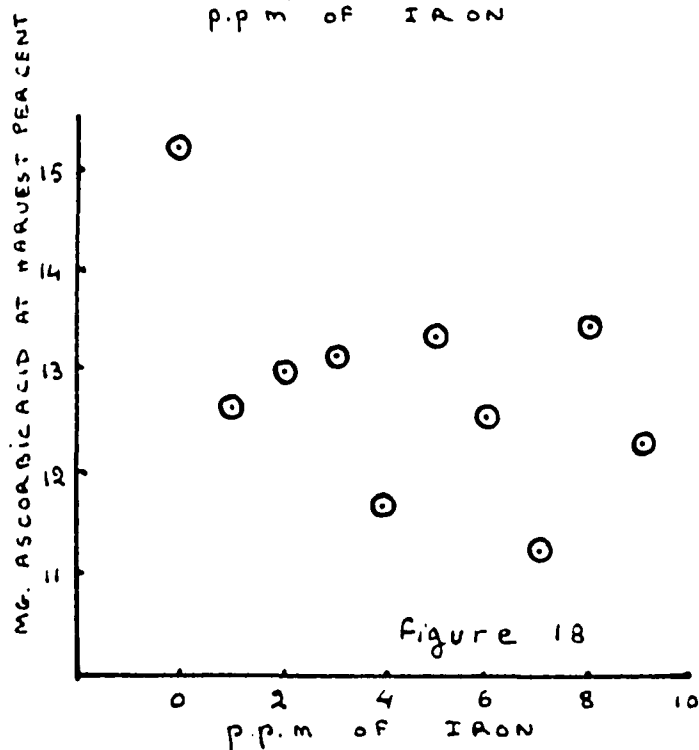
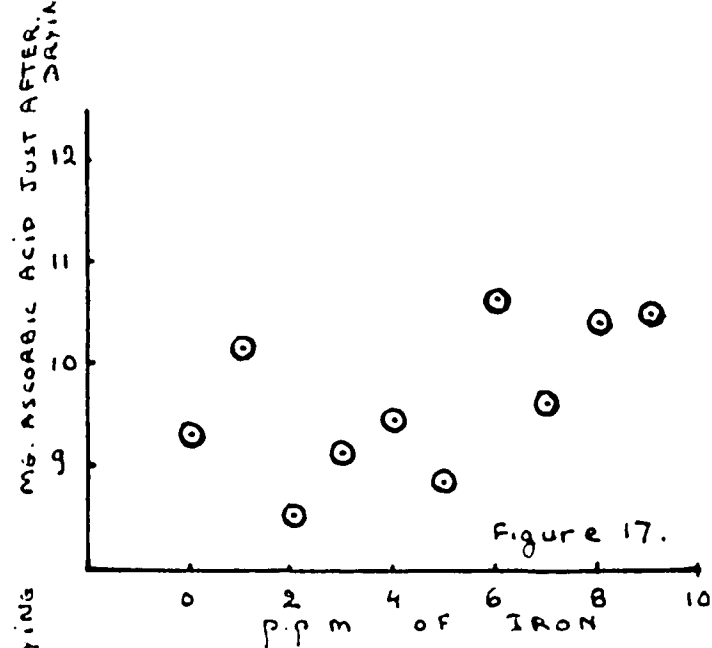
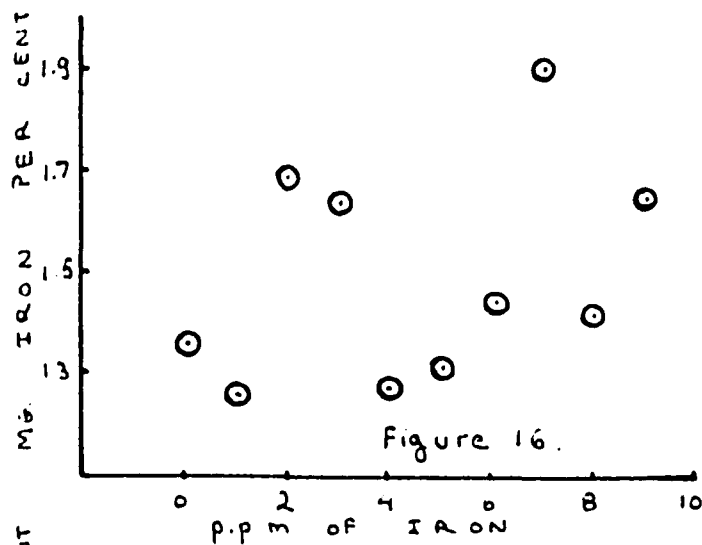


Figure 22. Average amounts of total nitrogen, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 23. Average amounts of chlorophyll, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 24. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 25. Average amounts of total nitrogen, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 26. Average amounts of dry matter, expressed in grams per 100 grams of fresh material from wheat plants grown on various concentrations of iron in the nutrient solution, plotted against the amount of ascorbic acid 31 days after drying, expressed in milligrams per 100 grams of fresh material.

Figure 27. Average amounts of dry matter, expressed in grams per 100 grams of fresh material from wheat plants grown on nutrient solution varyin in concentration of iron, plotted against the percentage of the original amount of ascorbic acid found in the fresh matter just after drying.

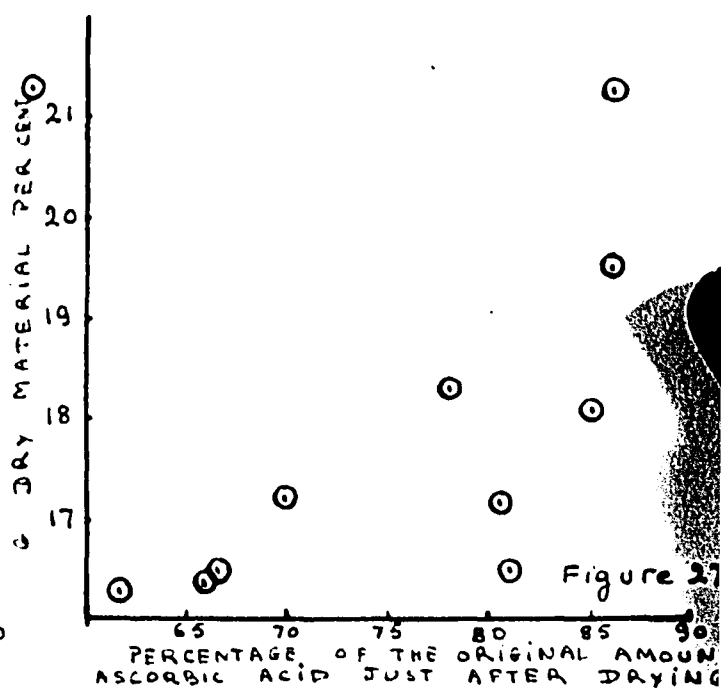
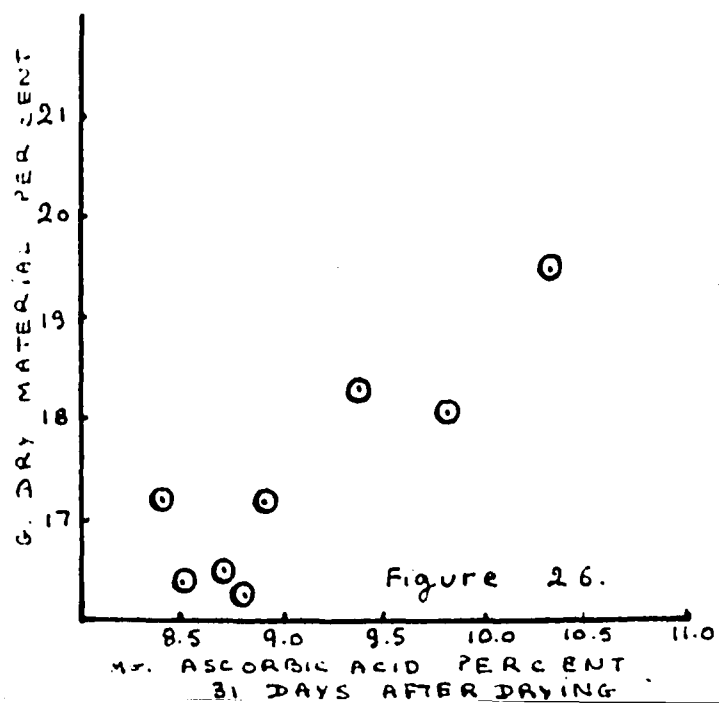
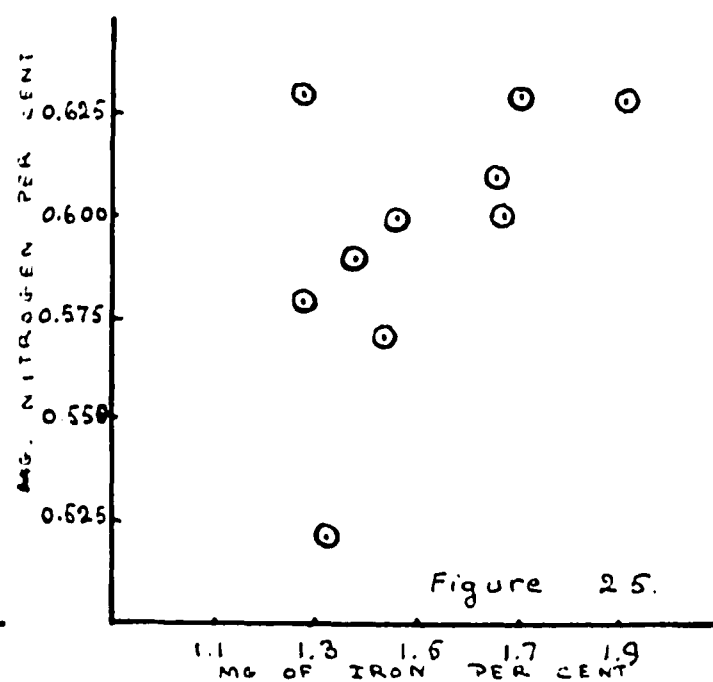
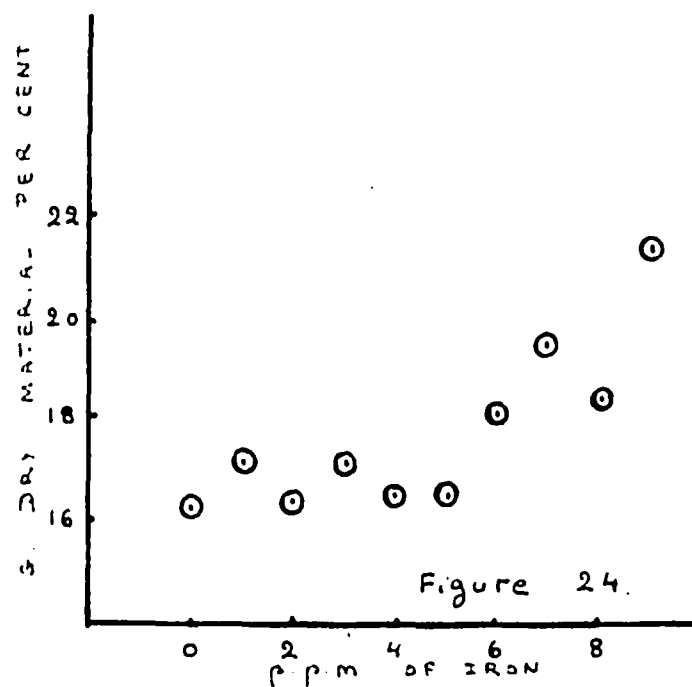
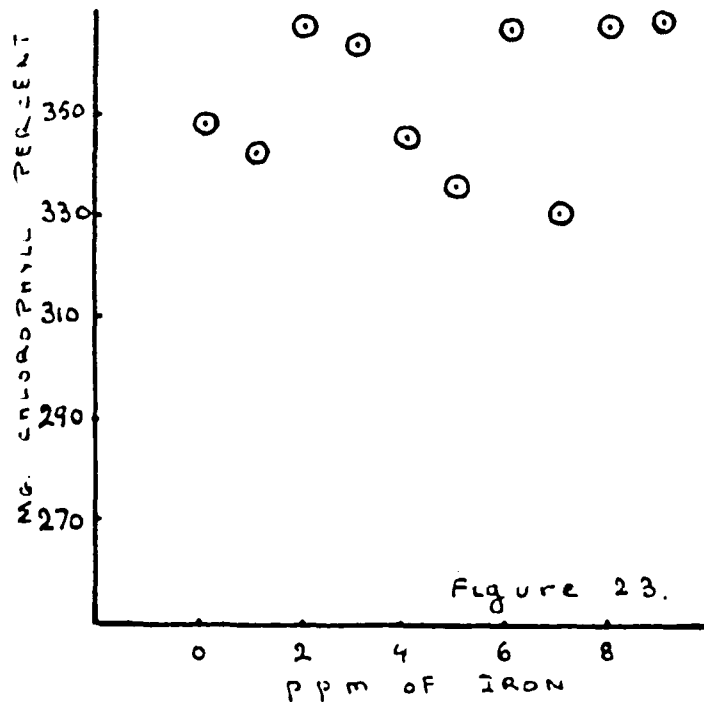
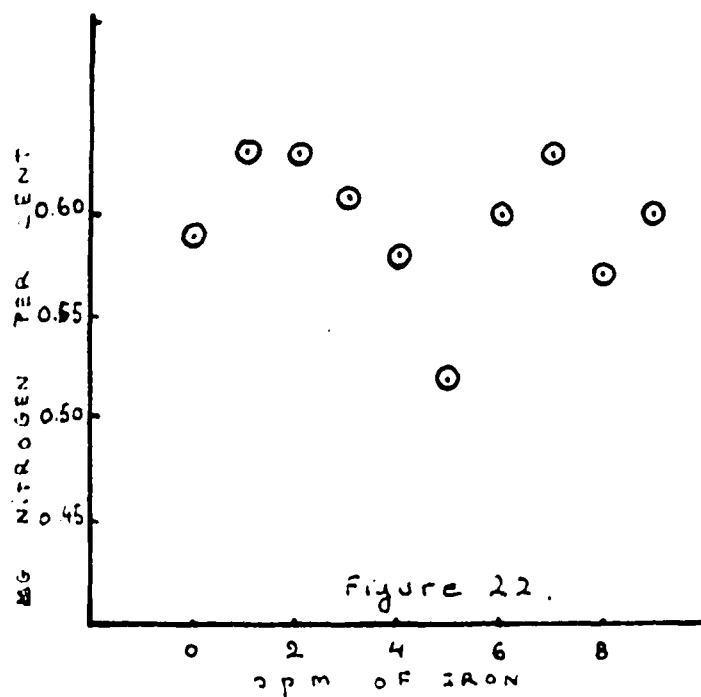




Figure 28. Average amounts of total nitrogen, expressed in grams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 29. Average amounts of chlorophyll, expressed in grams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 30. Average amounts of fresh material, expressed in grams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

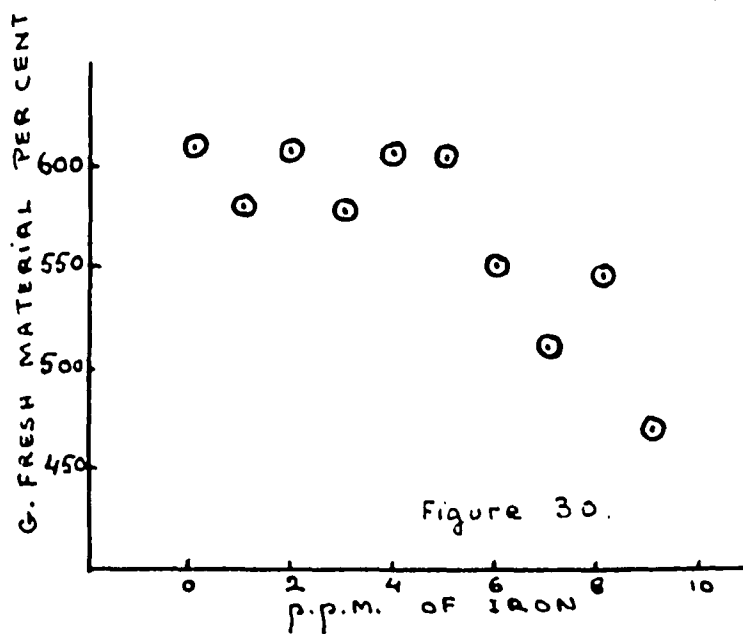
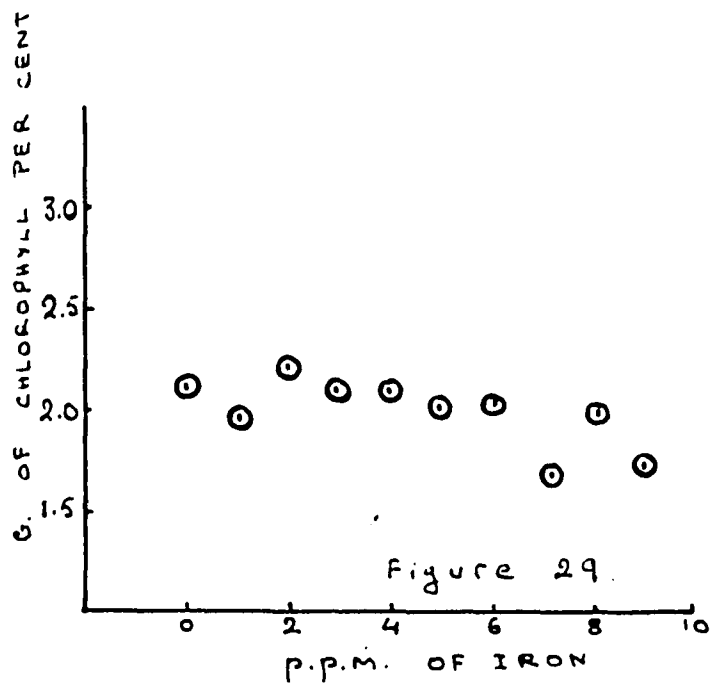
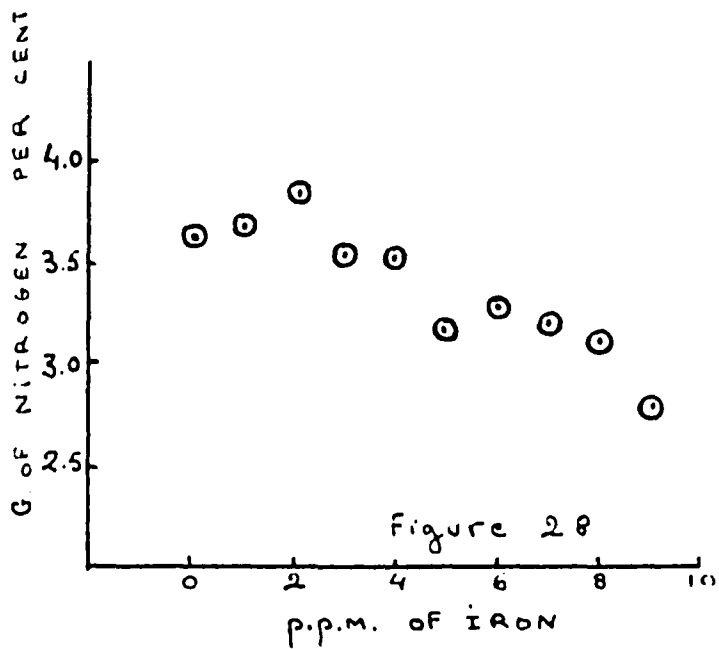


Figure 31. Average amounts of iron, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 32. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 33. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 34. Average amounts of ascorbic acid 31 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 35. Average amounts of ascorbic acid 10r days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 36. Average amounts of carbohydrate, expressed in grams per 100 gramms of dry material from wheat plants, plotted against the concentration of iron in the nutrient solution expressed in parts per million.

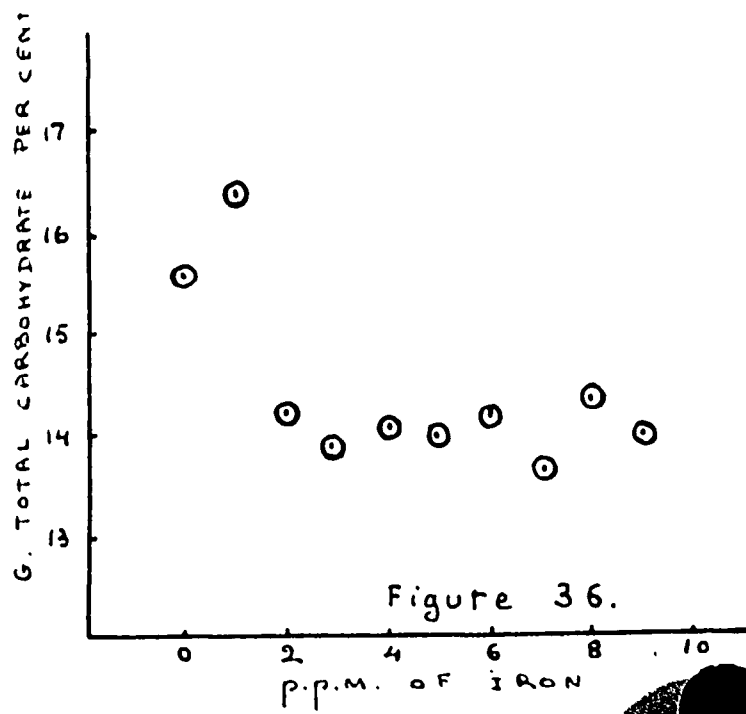
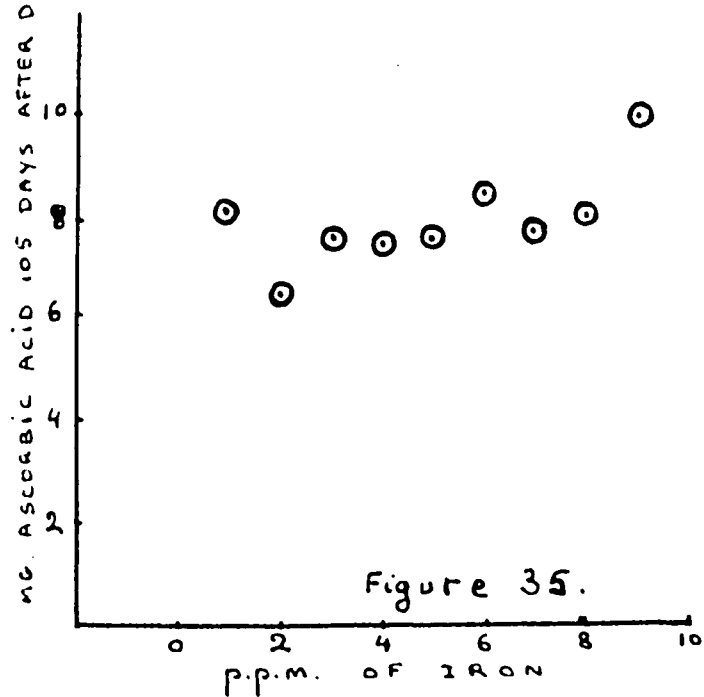
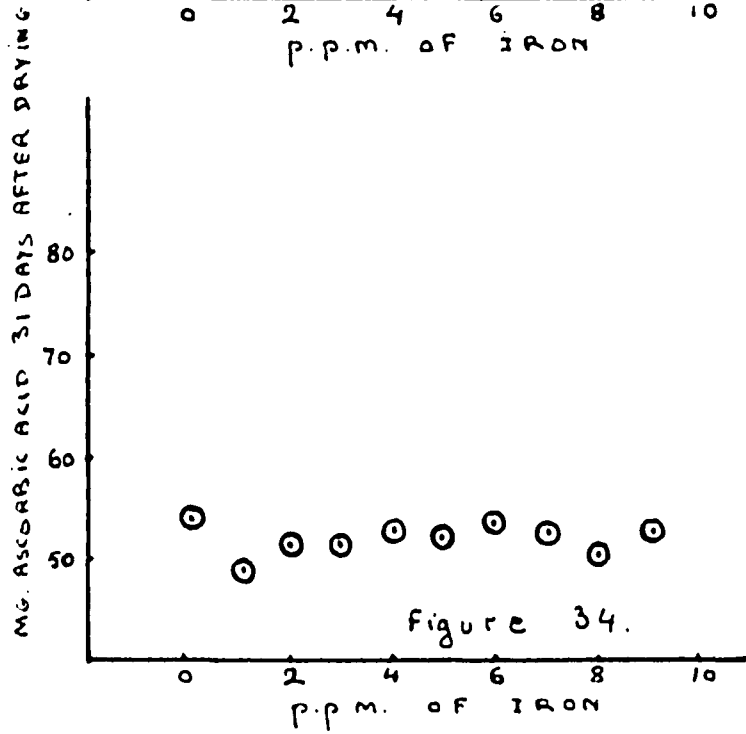
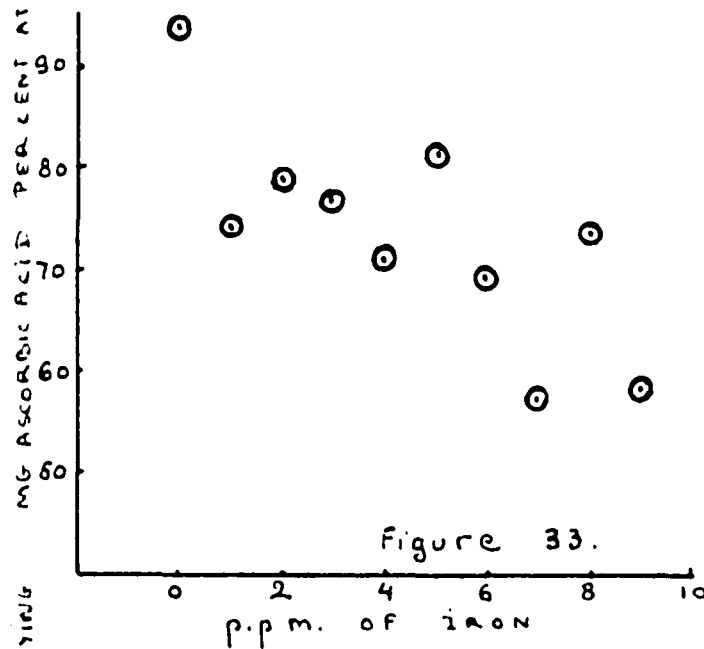
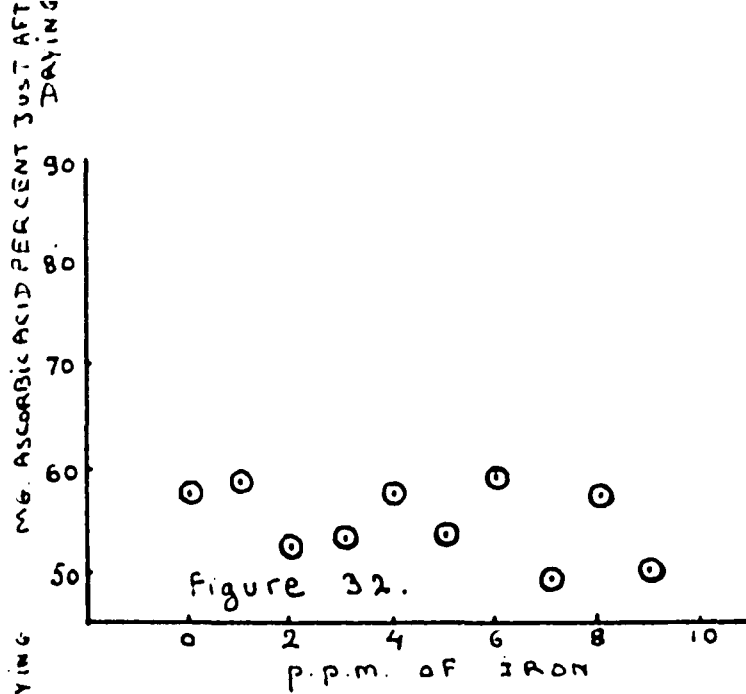
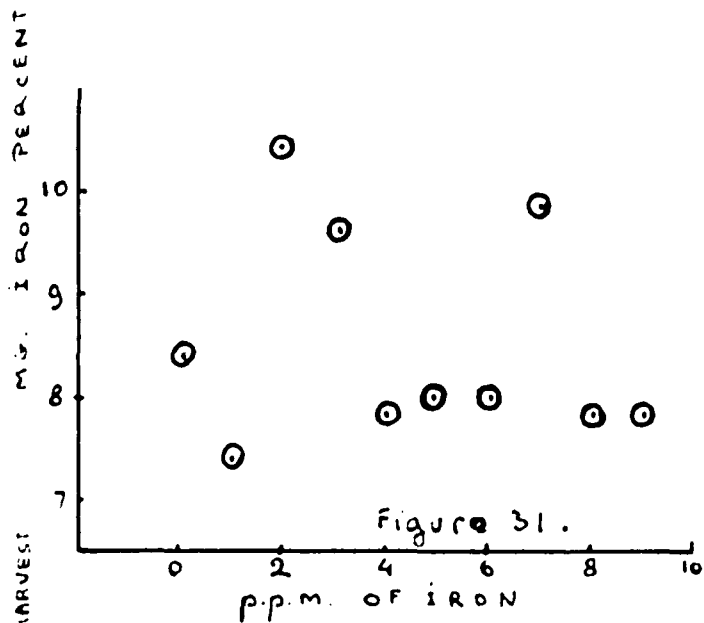
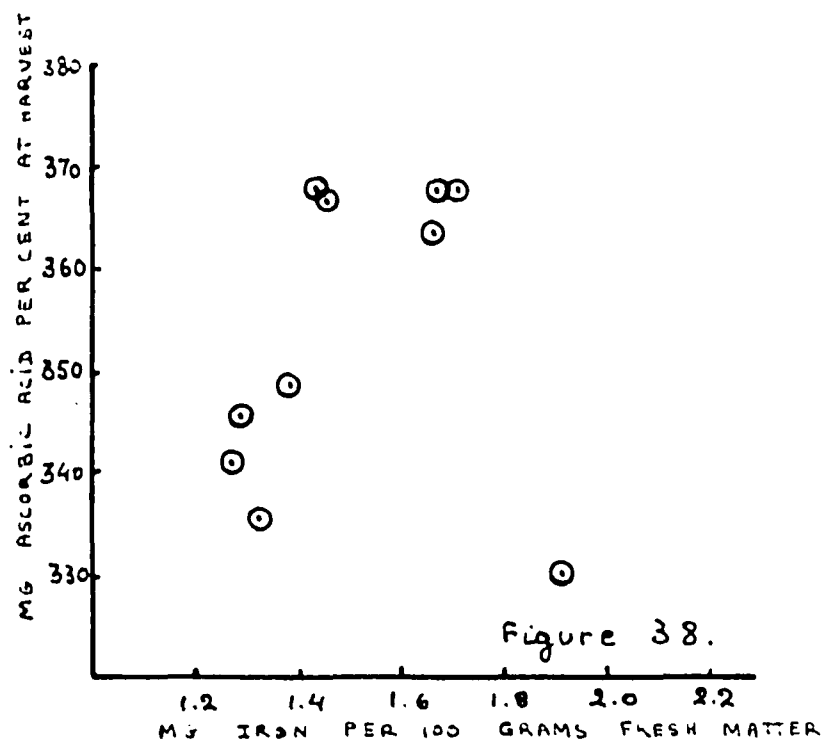
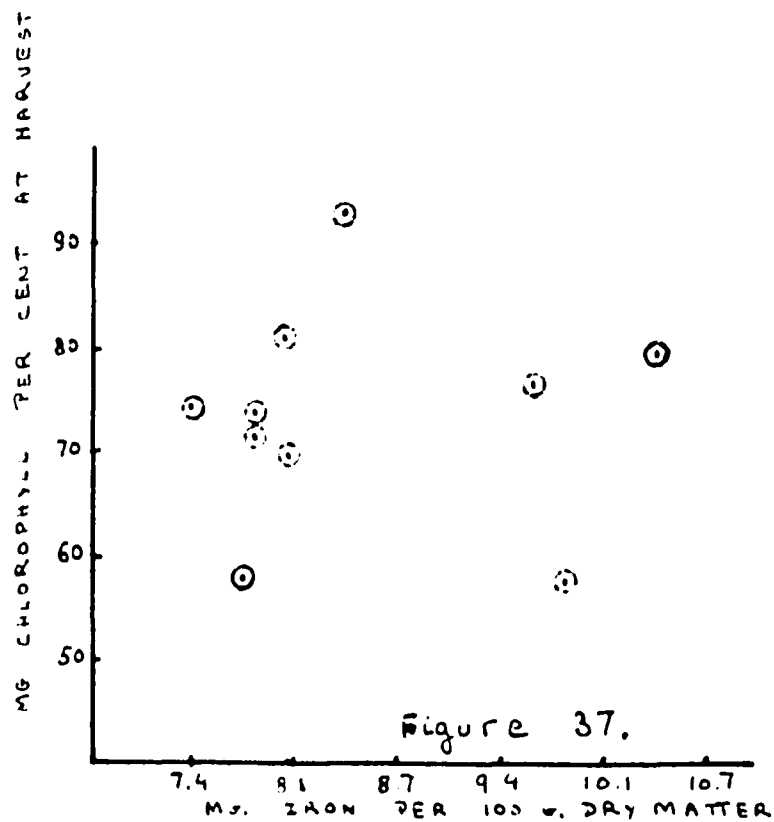


Figure 37. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter.

Figure 38. Average amounts of chlorophyll, expressed in grams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various concentrations of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh matter.



- Figure 39. Average amounts of ascorbic acid 105 days after drying, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.
- Figure 40. Average amounts of total carbohydrate, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.
- Figure 41. Average amounts of total nitrogen, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.
- Figure 42. Average amounts of chlorophyll, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.
- Figure 43. Average total carbohydrate, expressed in grams per 10 plants, grown on nutrient solution supplied with various concentrations of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.
- Figure 44. Average dry weight, expressed in grams per 10 plants, grown on nutrient solutions supplied with various concentrations of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.

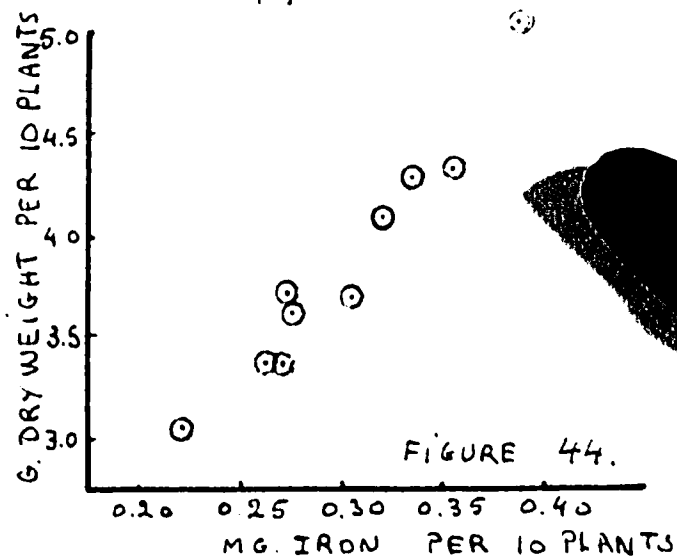
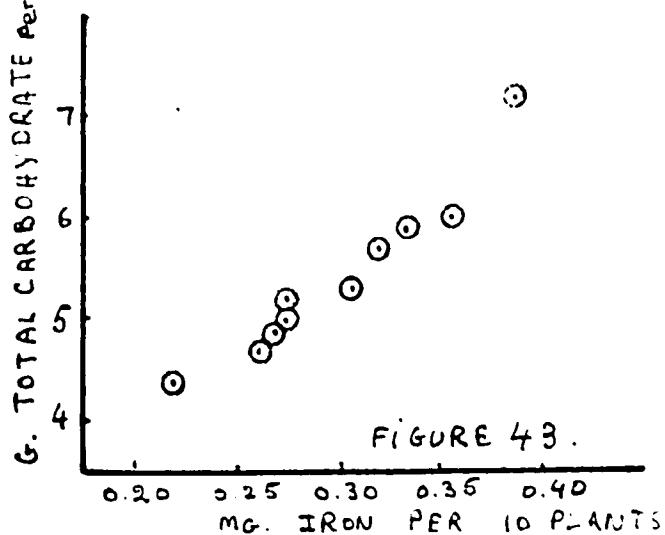
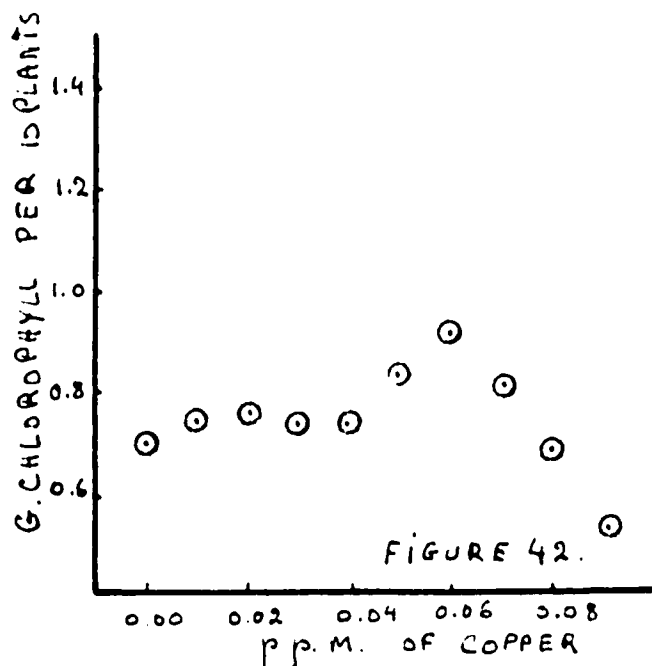
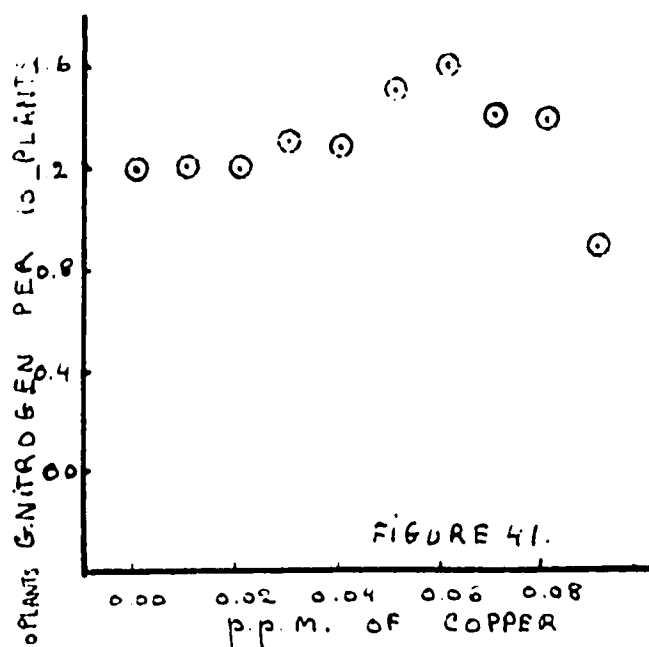
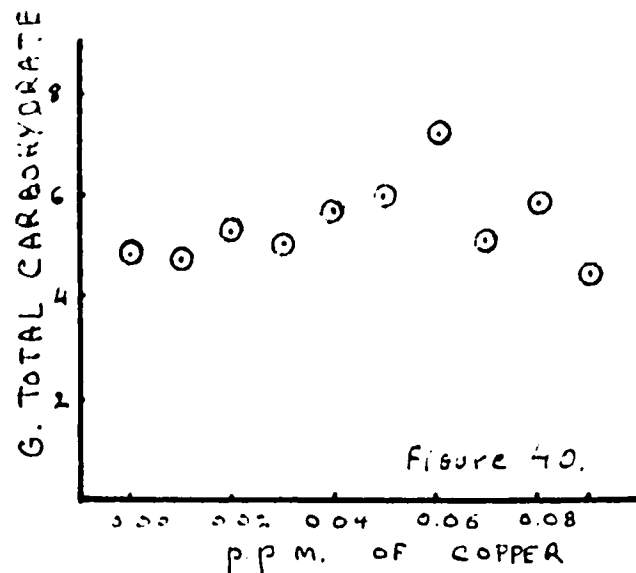
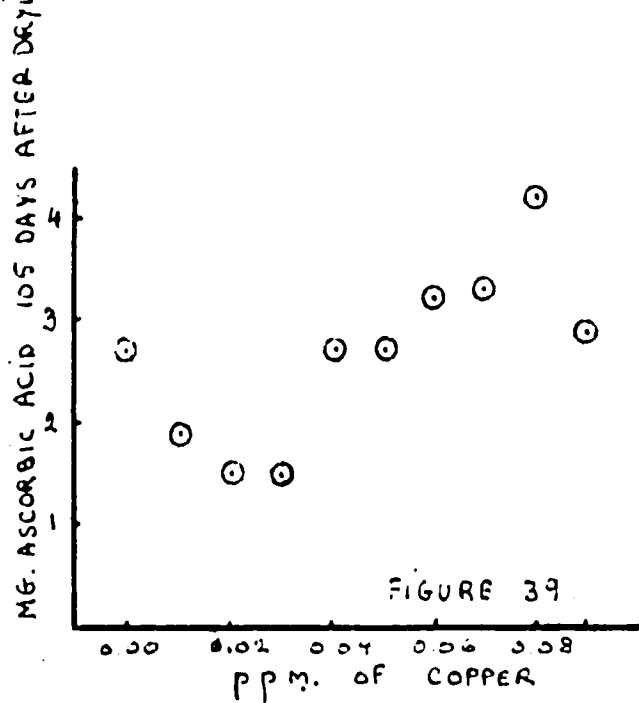




Figure 45. Average fresh weight, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 46. Average amounts of dry material, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 47. Average amounts of iron, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 48. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 49. Average amounts of ascorbic acid just after drying, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 50. Average amounts of ascorbic acid 31 days after drying, expressed in milligrams per 10 wheat plants, plotted against the concentrations of copper in the nutrient solution, expressed in parts per million.

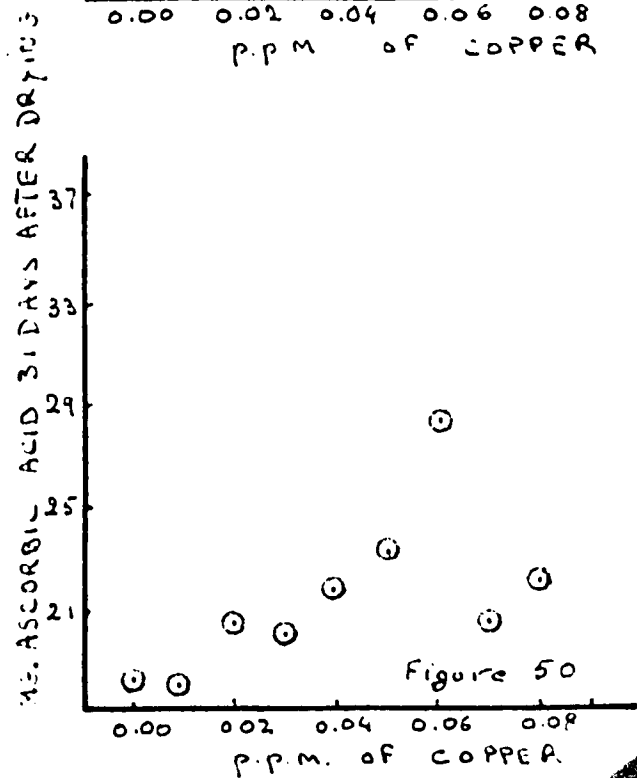
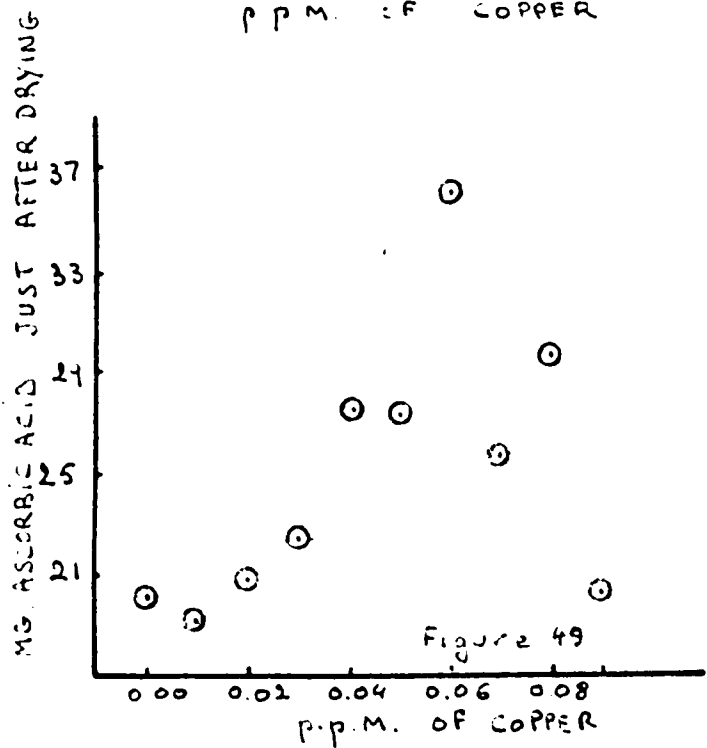
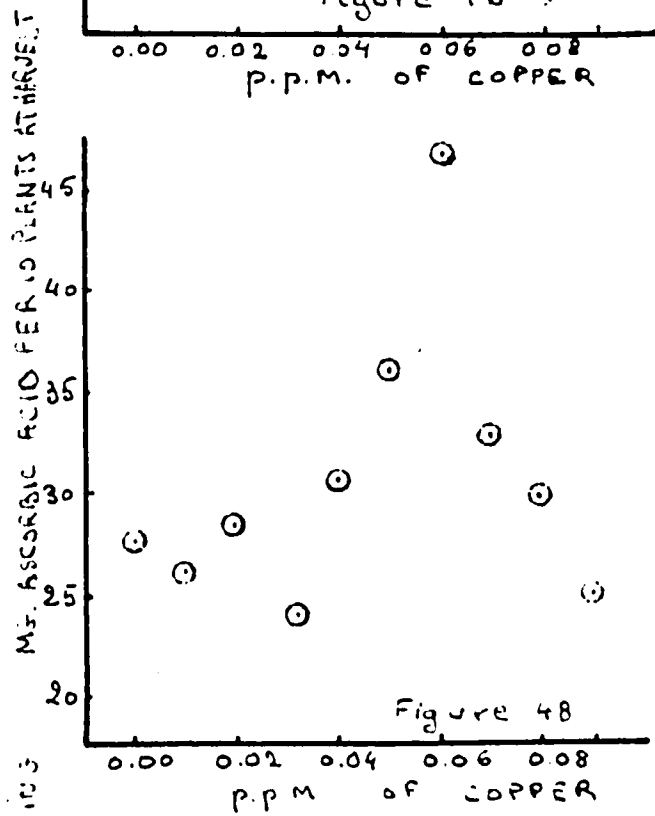
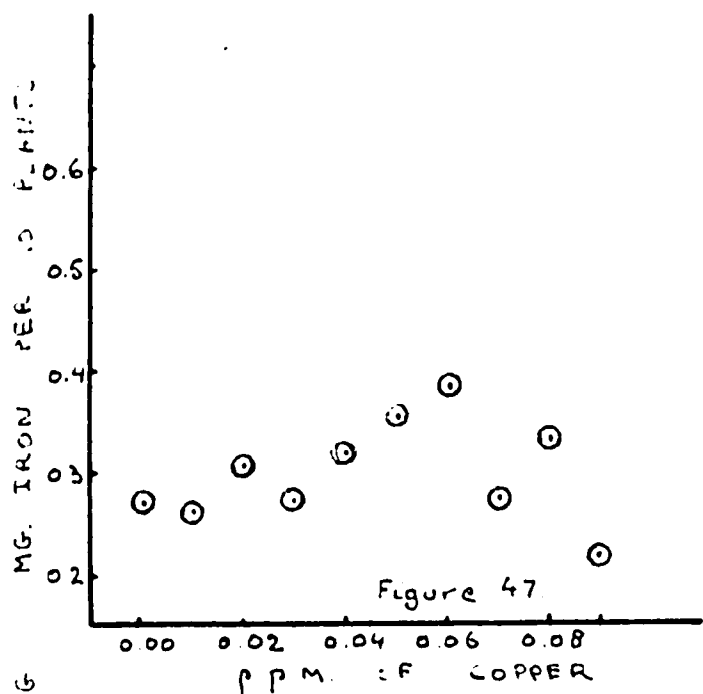
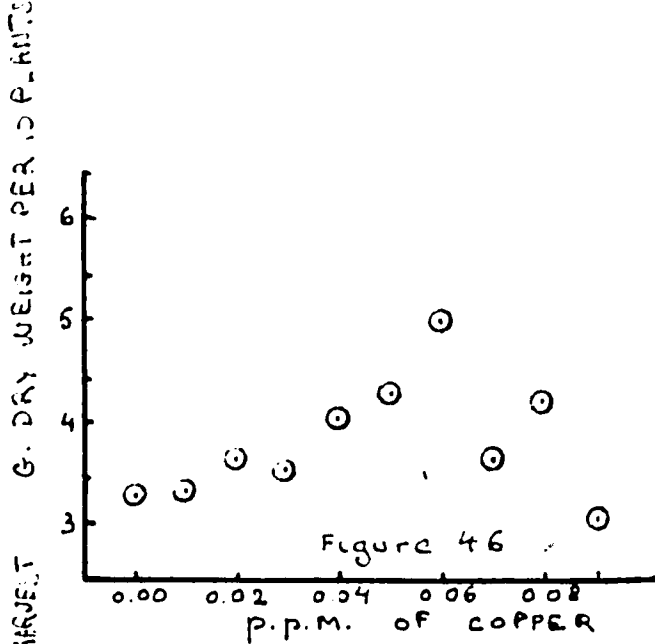
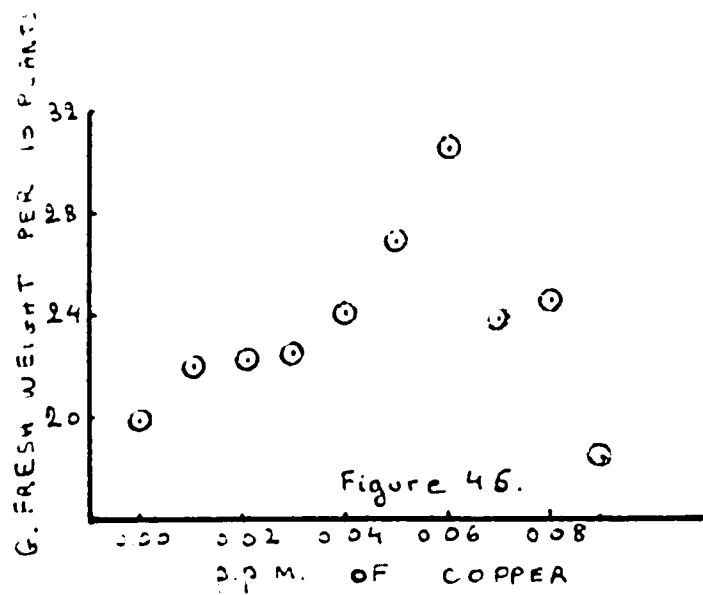


Figure 51. Average amounts of iron, expressed in milligrams per 100 grams of fresh material from wheat plants , plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 52. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 53. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 54. Average amounts of ascorbic acid 31 days after harvest, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 55. Average amounts of ascorbic acid 105 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 56. Amounts of carbohydrate, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

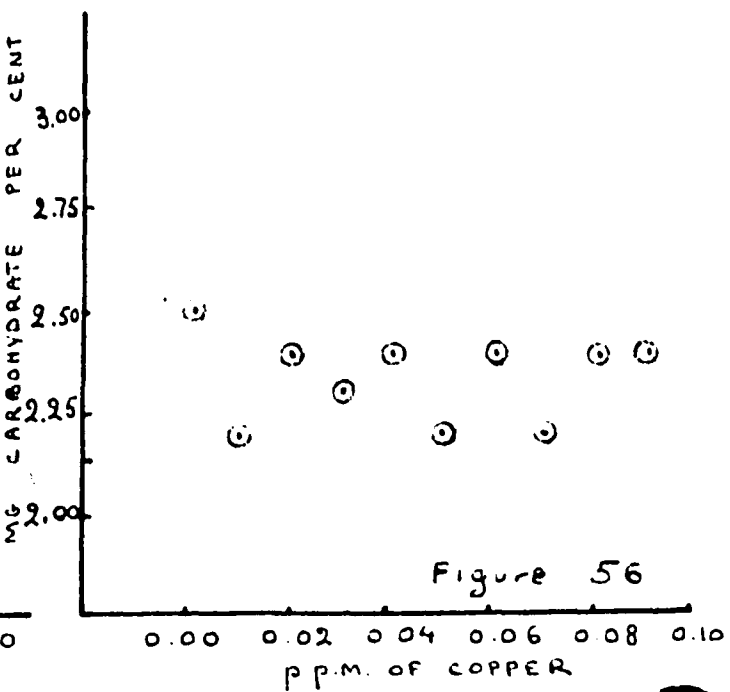
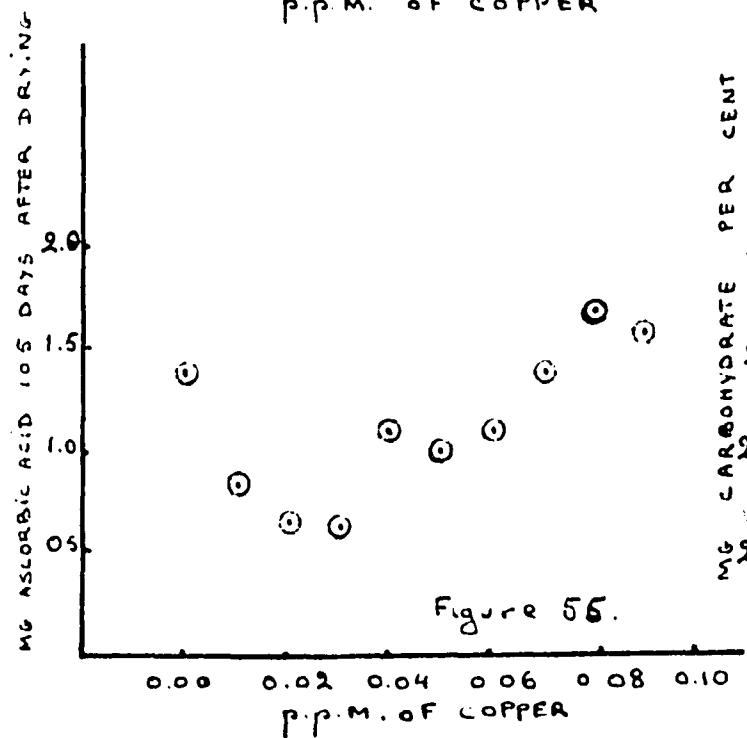
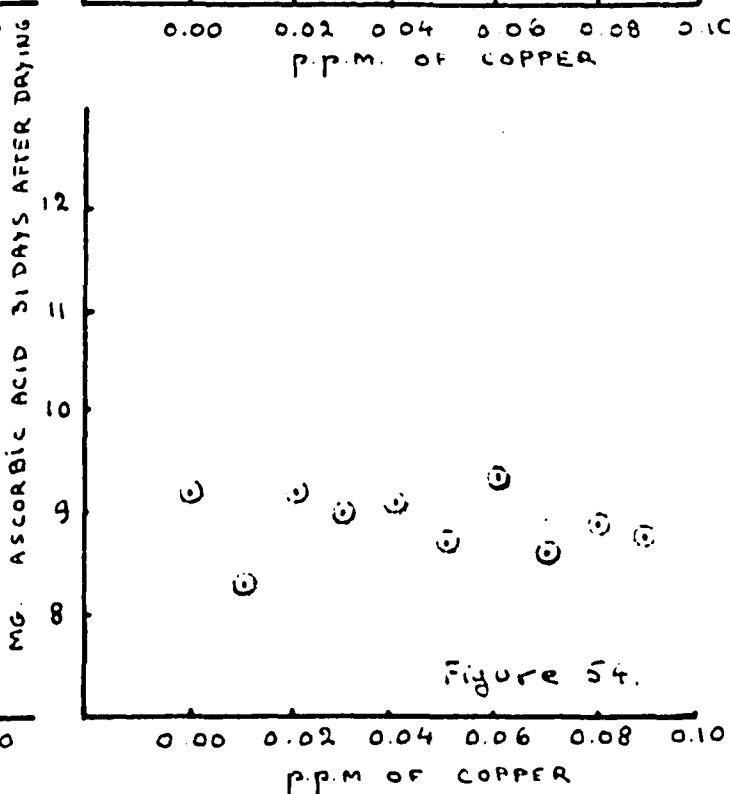
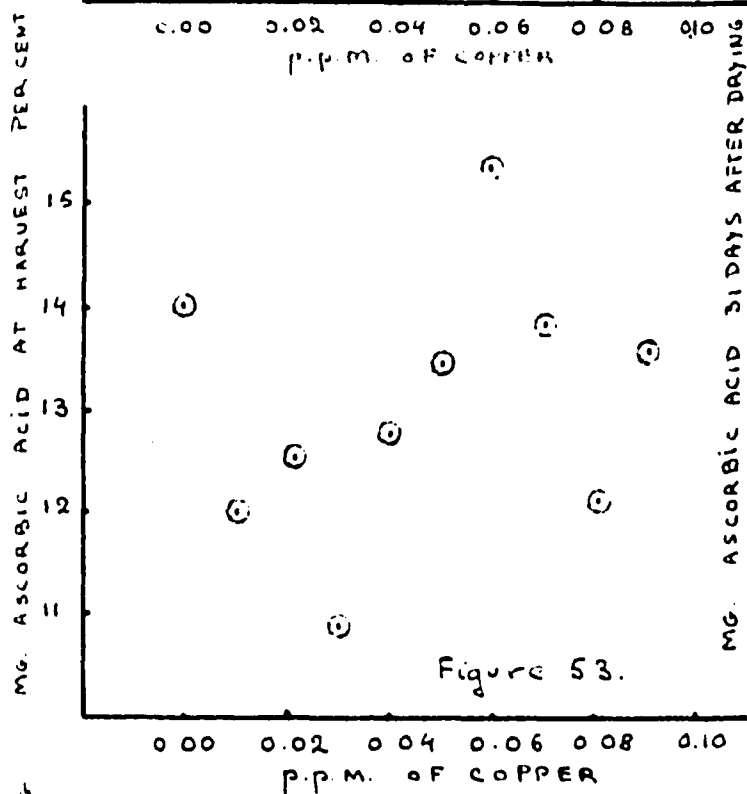
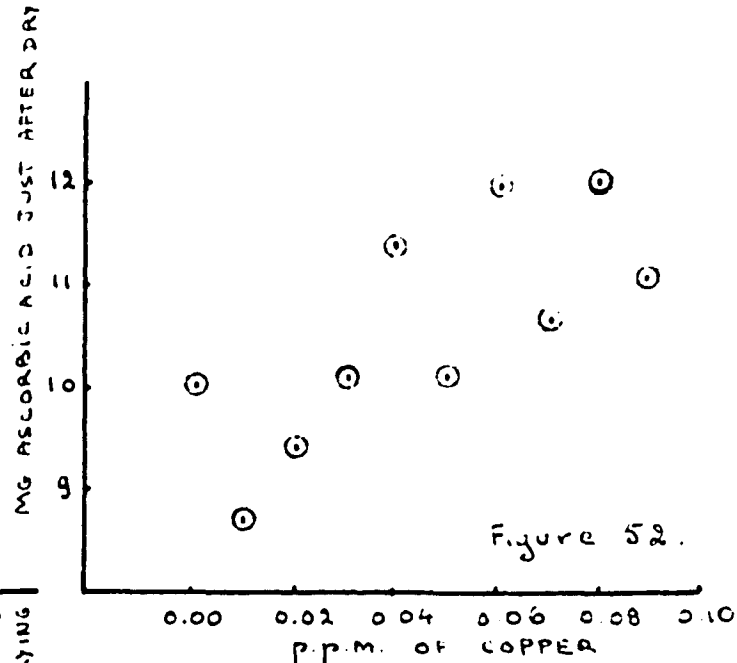
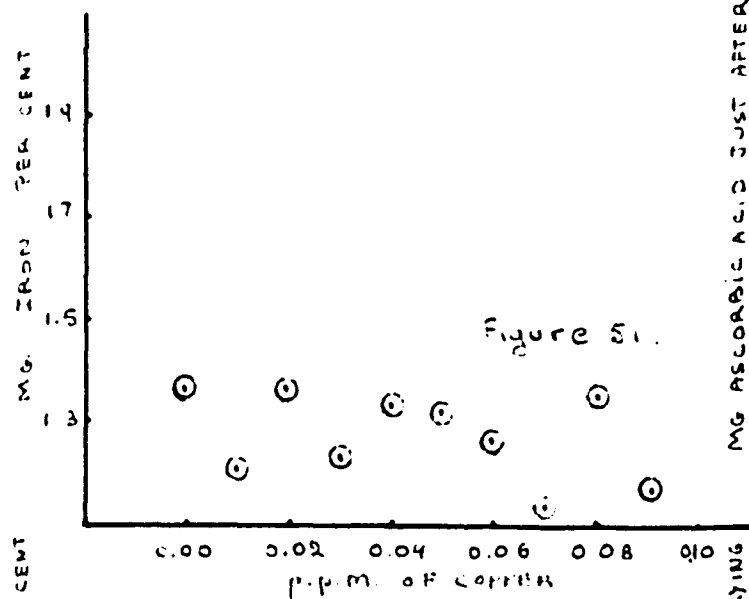


Figure 57. Average amounts of total nitrogen, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

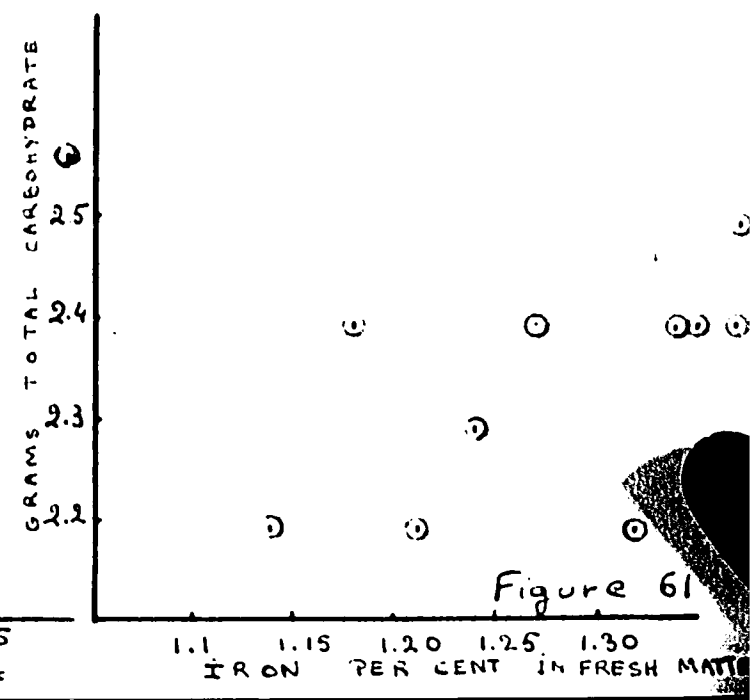
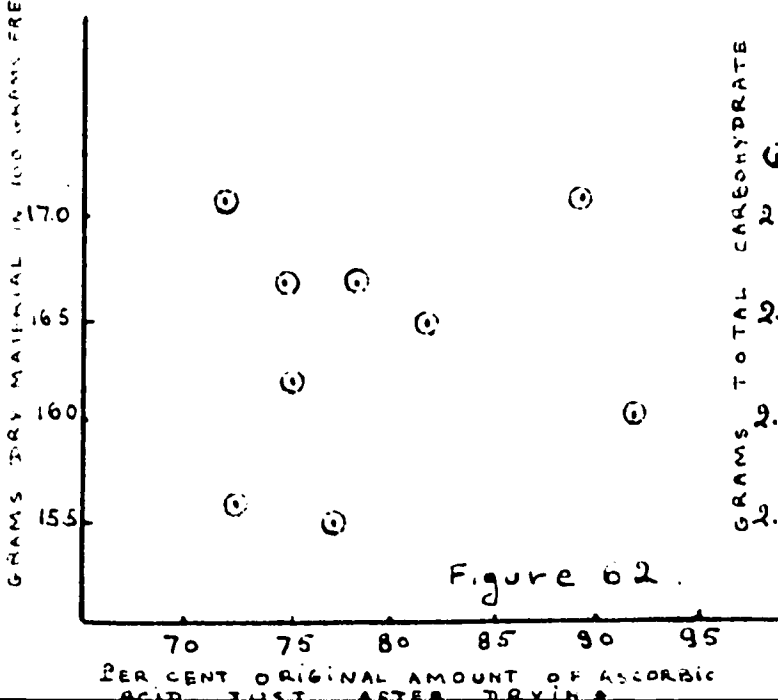
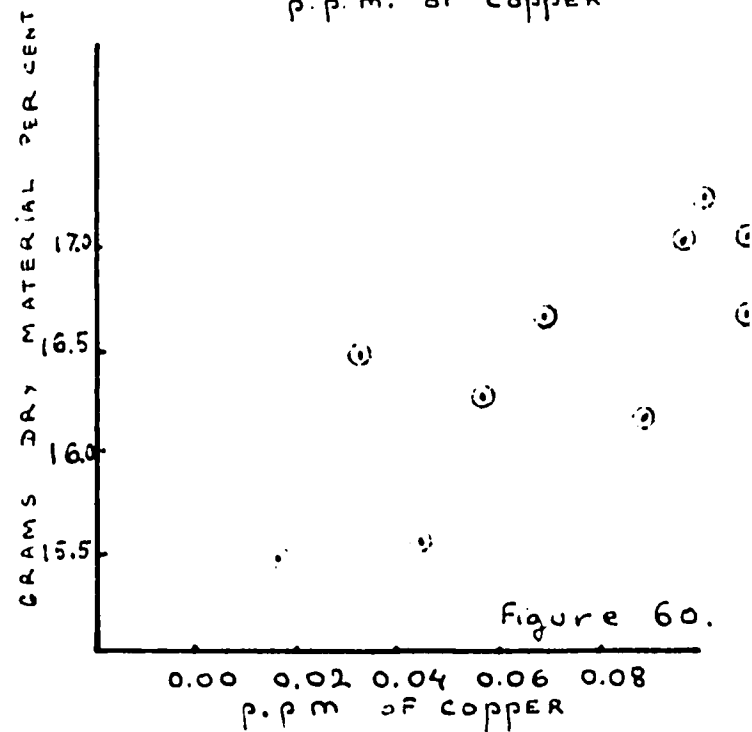
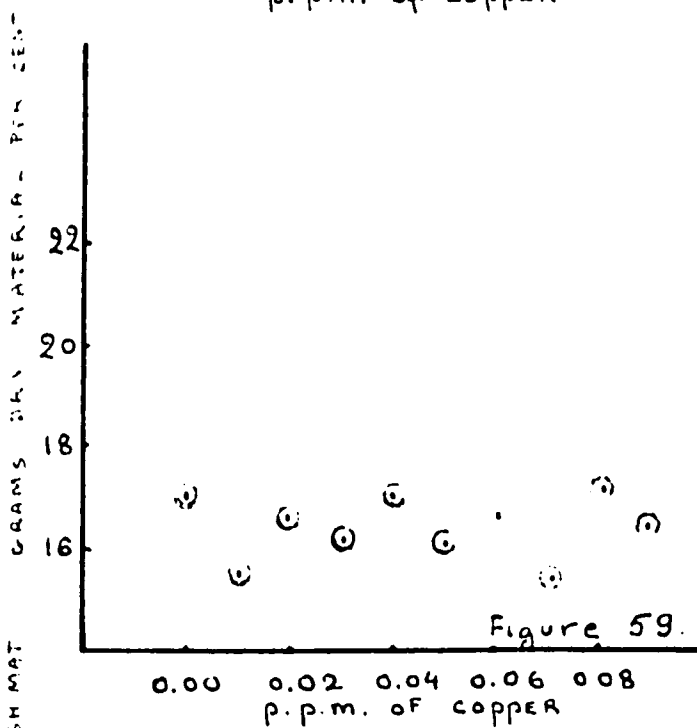
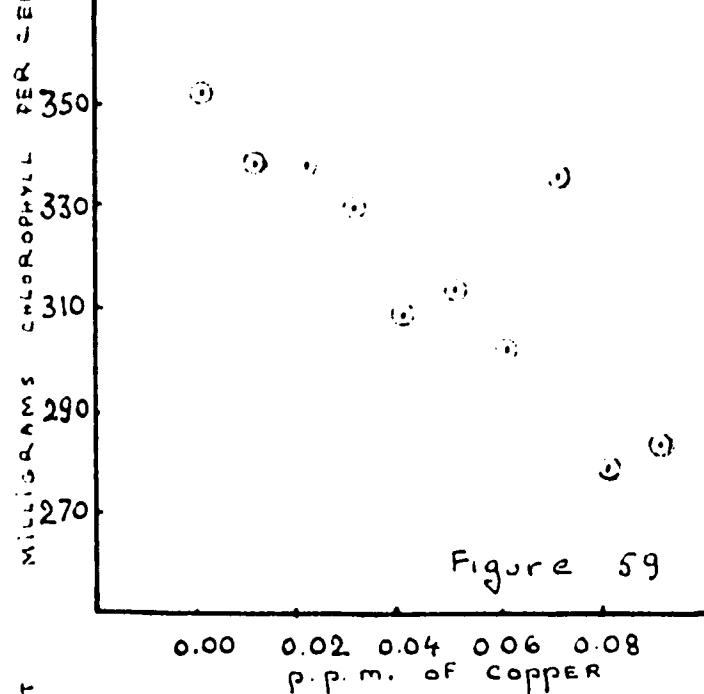
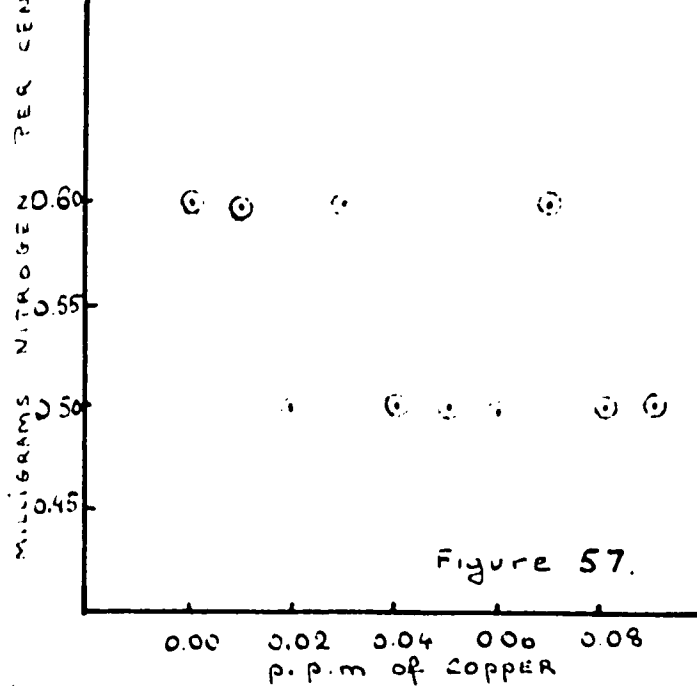
Figure 58. Average amounts of chlorophyll, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 59. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

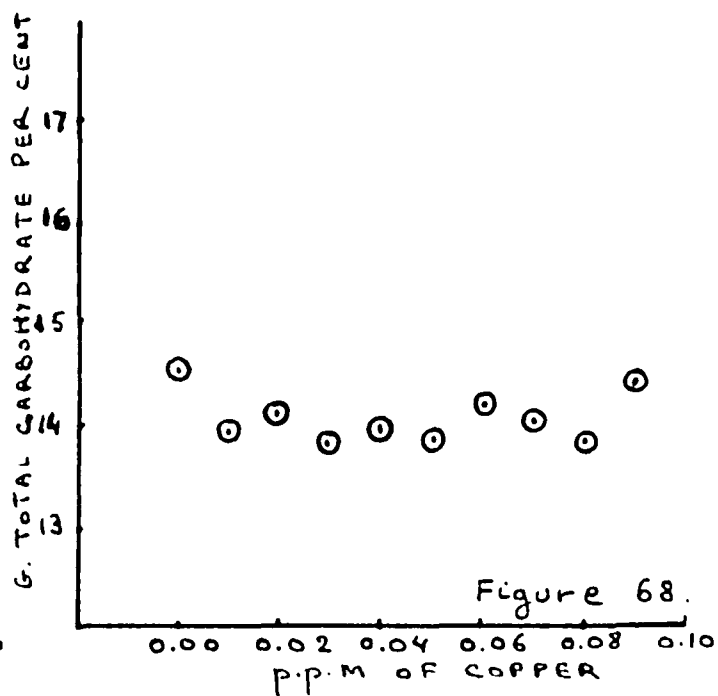
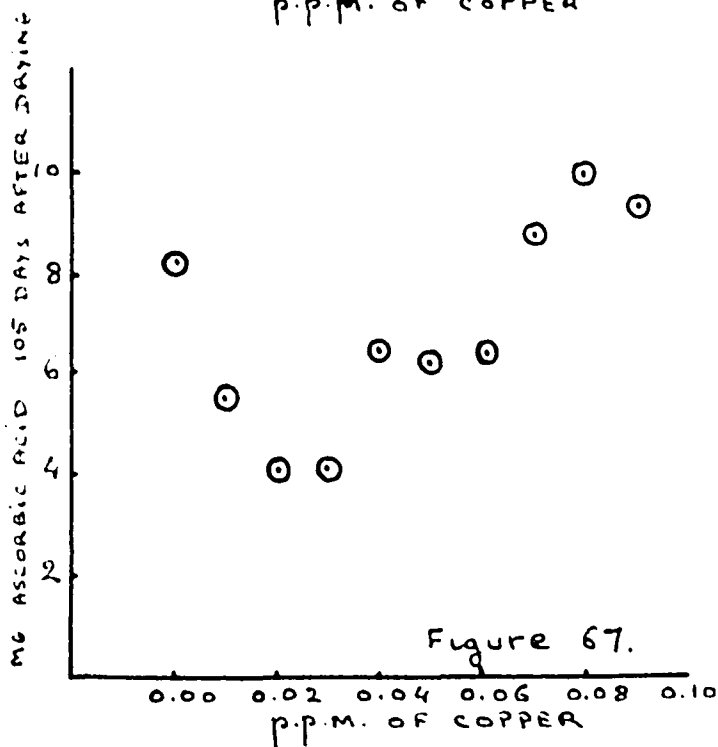
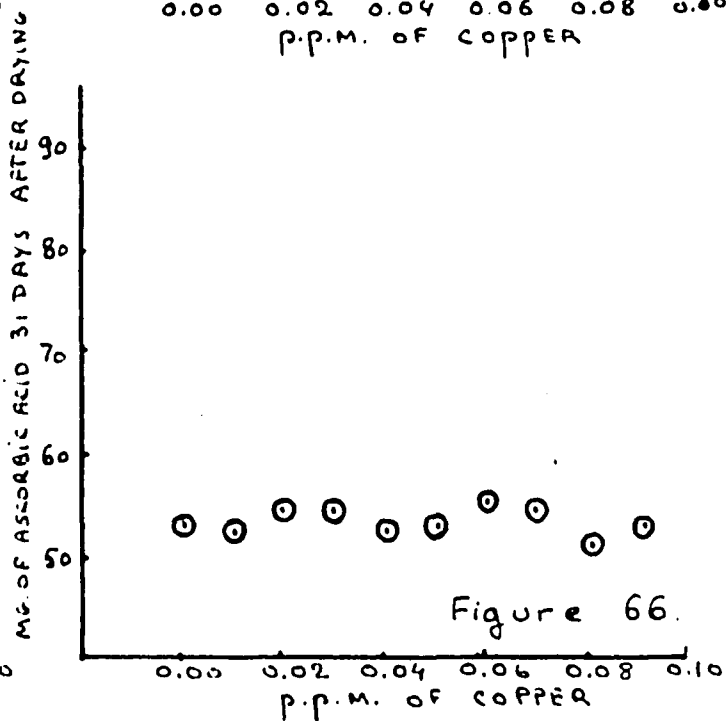
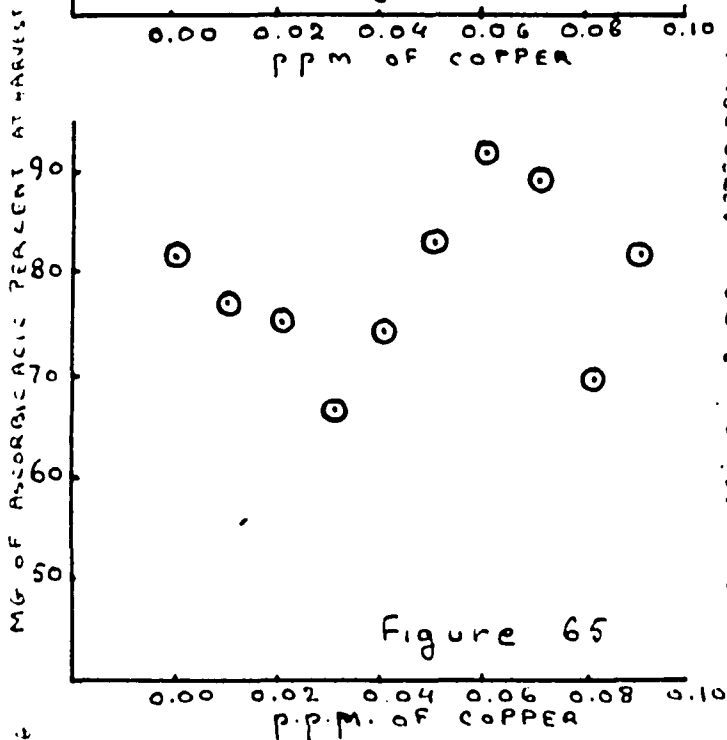
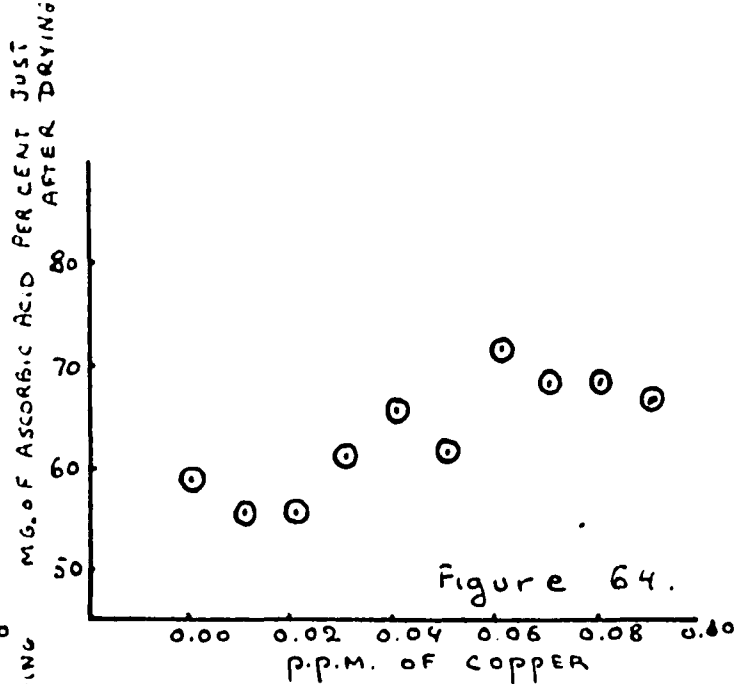
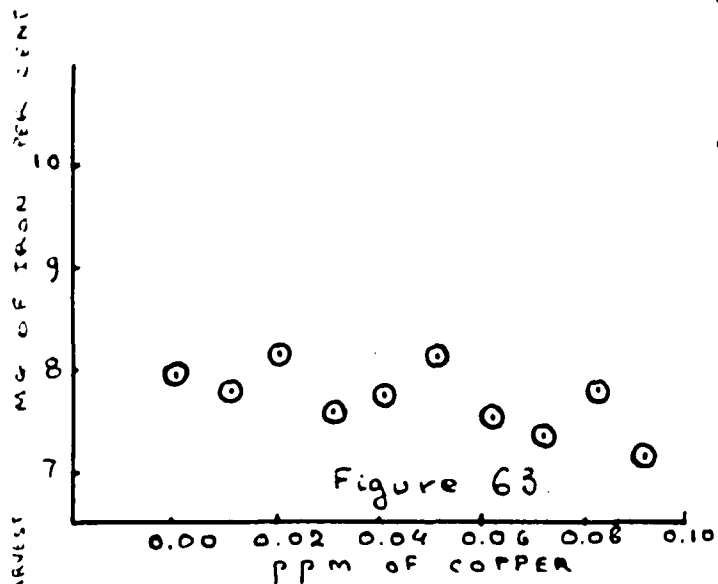
Figure 60. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various concentrations of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh material.

Figure 61. Average amounts of total carbohydrate, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various concentrations of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh material.

Figure 62. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants grown on nutrient solution supplied with various concentrations of copper, plotted against the percentage of the original amount of ascorbic acid just after drying.



- Figure 63. Average amounts of iron, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 64. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 65. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 66. Average amounts of ascorbic acid 31 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 67. Average amounts of ascorbic acid 105 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 68. Average amounts of total carbohydrate, expressed in grams per 100 grams of dry material from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.





- Figure 69. Average amounts of nitrogen, expressed in grams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution expressed in parts per million.
- Figure 70. Average amounts of chlorophyll, expressed in grams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 71. Average amounts of fresh material, expressed in grams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 72. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of dry material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of the corresponding plants material.

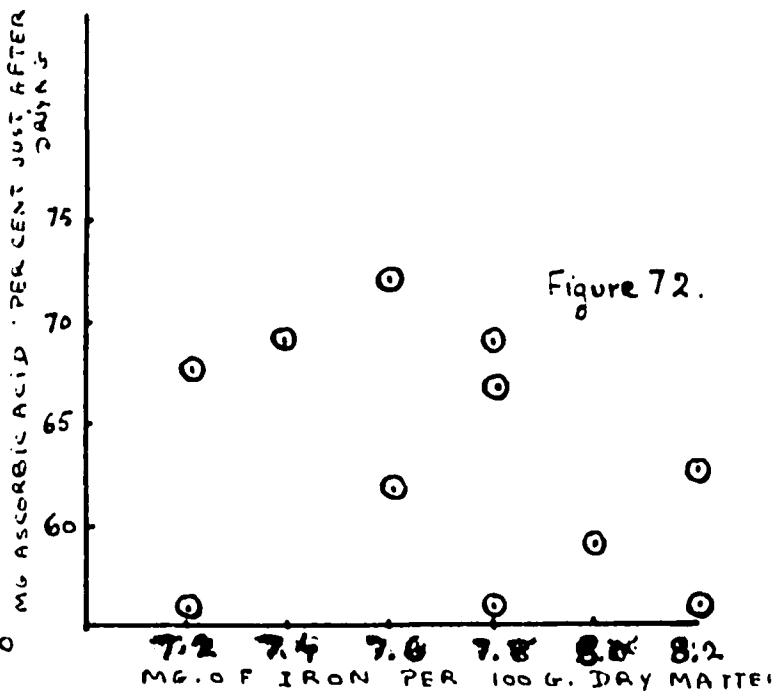
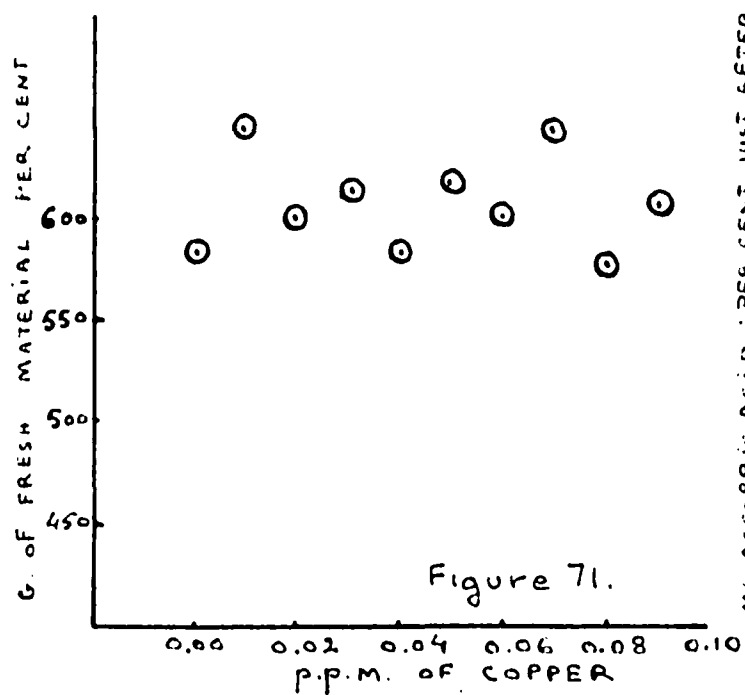
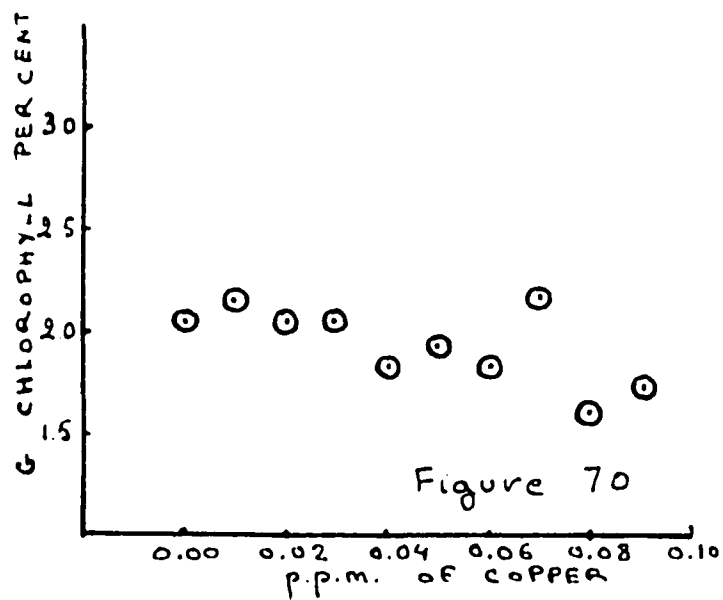
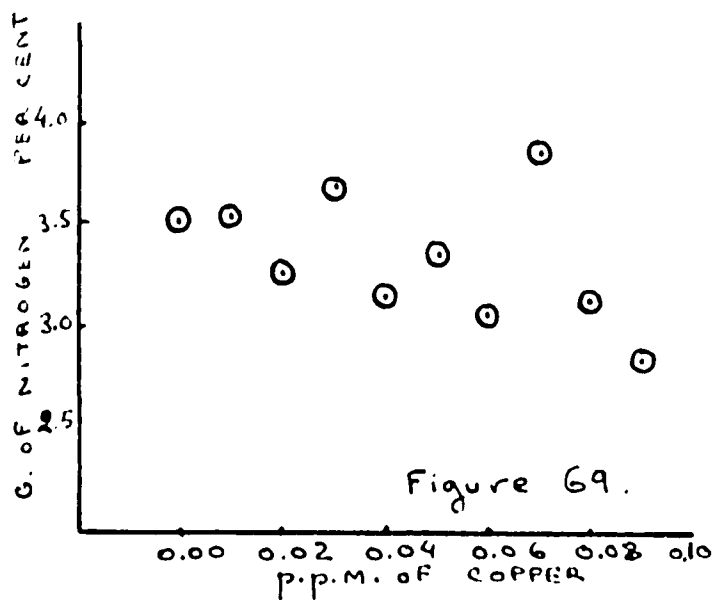


Figure 73. Percentages of the original amounts of ascorbic acid, just after drying, 31 and 105 days after drying, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 74. Percentages of the original amounts of ascorbic acid just after drying, 31 and 105 days after drying, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

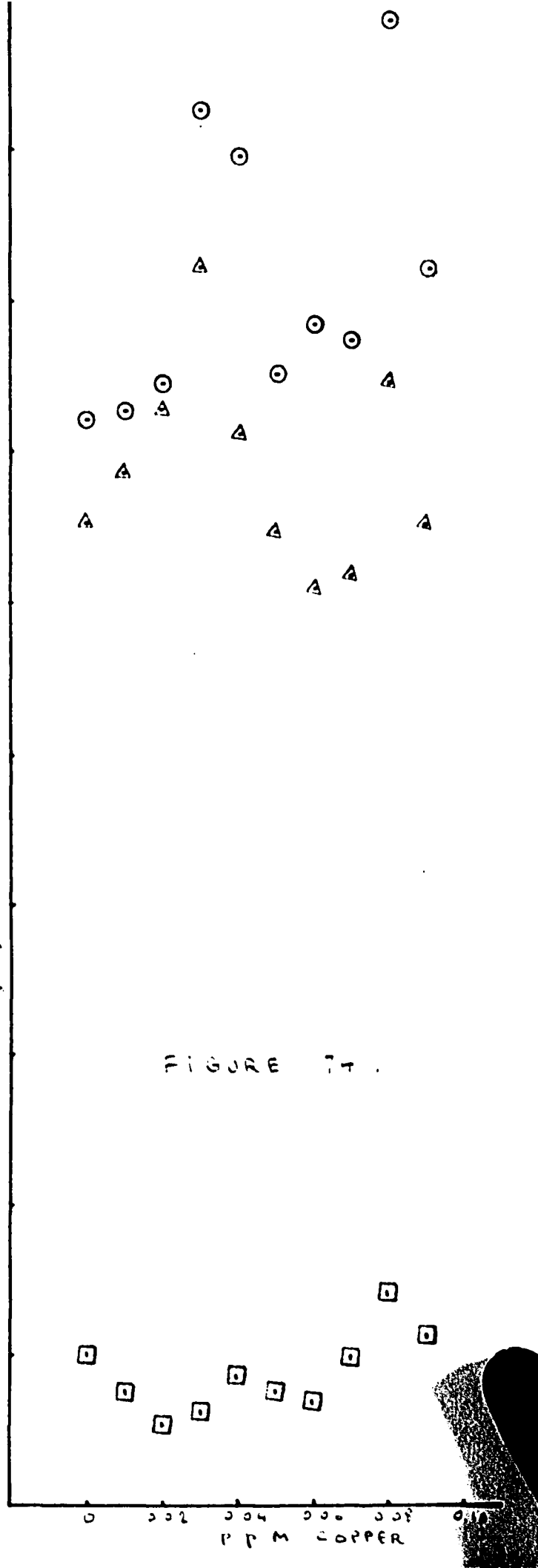
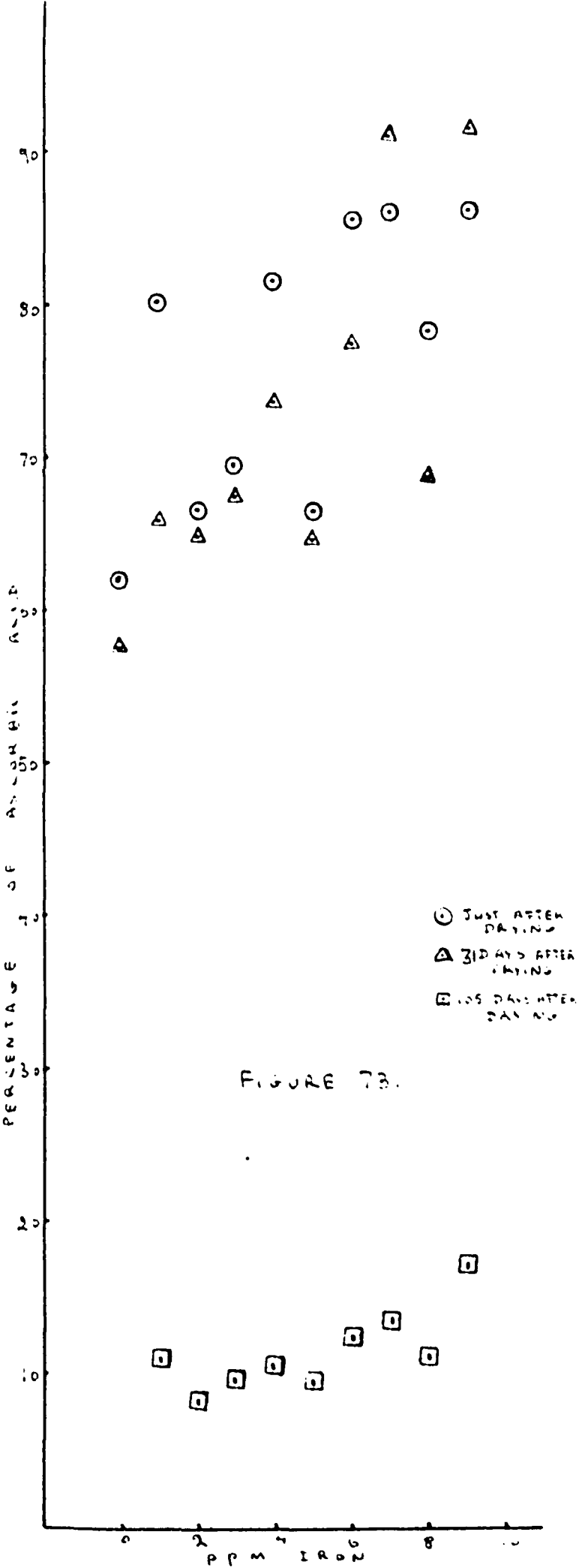
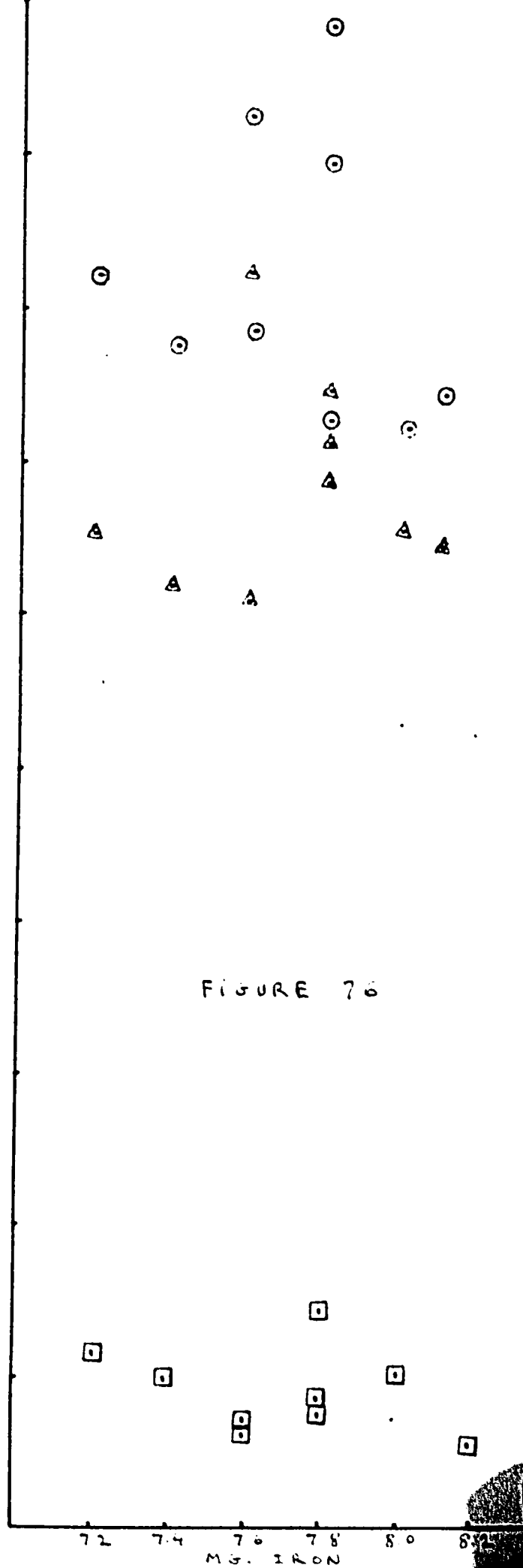
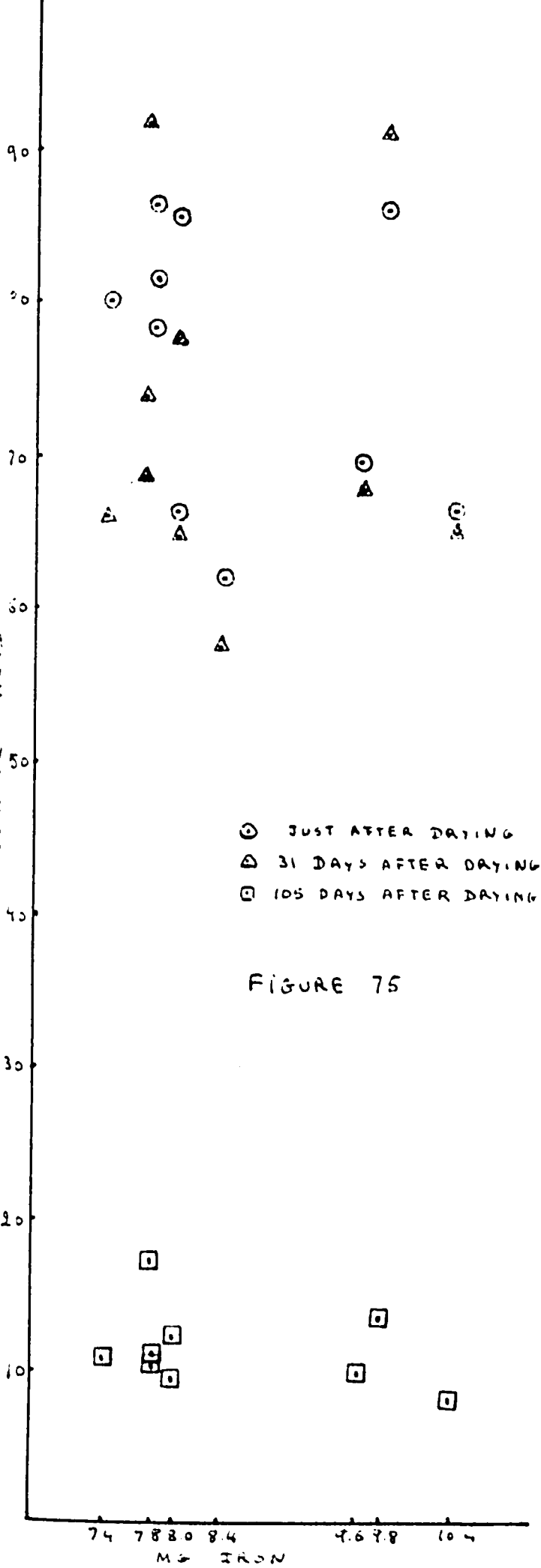


Figure 75. Percentages of the original amounts of ascorbic acid, just after drying, 31 and 105 days after drying, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter.

Figure 76. Percentages of the original amounts of ascorbic acid just after drying, 31 and 105 days after drying, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter.



Experiment 2.

Table 15. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 10 plants.

| Parts per million<br>of iron in the<br>nutrient solution | Fresh weight<br>in grams | Grams of<br>dry matter | Milligrams<br>of iron | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>7 days after<br>drying | Milligrams of<br>ascorbic acid<br>14 days after<br>drying |
|----------------------------------------------------------|--------------------------|------------------------|-----------------------|----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|
| 0                                                        | 6.84                     | 0.84                   | 0.076                 | 1.97                                         | 1.30                                                   | 0.95                                                     | 0.89                                                      |
| 2                                                        | 6.41                     | 0.93                   | 0.142                 | 3.12                                         | 1.10                                                   | 0.31                                                     | 0.29                                                      |
| 4                                                        | 7.28                     | 0.95                   | 0.114                 | 2.94                                         | 1.20                                                   | 0.69                                                     | 0.45                                                      |
| 6                                                        | 7.79                     | 1.02                   | 0.184                 | 3.24                                         | 1.34                                                   | 0.67                                                     | 0.62                                                      |
| 8                                                        | 7.70                     | 1.00                   | 0.208                 | 2.82                                         | 1.22                                                   | 0.82                                                     | 0.79                                                      |
| 10                                                       | 7.40                     | 0.98                   | 0.201                 | 3.05                                         | 1.31                                                   | 1.10                                                     | 0.77                                                      |
| 12                                                       | 7.72                     | 1.00                   | 0.243                 | 3.25                                         | 1.34                                                   | 0.80                                                     | 0.82                                                      |
| 14                                                       | 7.33                     | 0.94                   | 0.216                 | 2.69                                         | 1.42                                                   | 1.08                                                     | 0.62                                                      |
| 16                                                       | 7.40                     | 0.90                   | 0.189                 | 2.57                                         | 1.35                                                   | 0.36                                                     | 0.83                                                      |
| 18                                                       | 9.20                     | 0.98                   | 0.255                 | 2.58                                         | 1.11                                                   | 0.95                                                     | 0.72                                                      |

Experiment 2.

Table 16. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 10 plants.

| Parts per million<br>of copper in the<br>nutrient solution | Fresh weight<br>in grams | Grams of<br>dry matter | Milligrams<br>of iron | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>7 days after<br>drying | Milligrams of<br>ascorbic acid<br>14 days after<br>drying |
|------------------------------------------------------------|--------------------------|------------------------|-----------------------|----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|
| 0.00                                                       | 7.36                     | 0.91                   | 0.214                 | 3.23                                         | 1.16                                                   | 0.75                                                     | 0.82                                                      |
| 0.02                                                       | 6.70                     | 0.89                   | 0.150                 | 2.47                                         | 1.20                                                   | 0.44                                                     | 0.22                                                      |
| 0.04                                                       | 7.02                     | 0.96                   | 0.168                 | 2.43                                         | 1.34                                                   | 0.73                                                     | 0.79                                                      |
| 0.06                                                       | 7.13                     | 1.00                   | 0.188                 | 2.89                                         | 1.09                                                   | 0.29                                                     | 0.16                                                      |
| 0.08                                                       | 7.66                     | 0.91                   | 0.094                 | 2.76                                         | 1.60                                                   | 1.15                                                     | 0.93                                                      |
| 0.10                                                       | 6.94                     | 0.88                   | 0.119                 | 2.32                                         | 1.38                                                   | 0.86                                                     | 0.65                                                      |
| 0.12                                                       | 7.73                     | 0.93                   | 0.163                 | 2.95                                         | 1.64                                                   | 0.93                                                     | 0.98                                                      |
| 0.14                                                       | 7.78                     | 1.02                   | 0.209                 | 3.09                                         | 1.41                                                   | 0.99                                                     | 1.03                                                      |
| 0.16                                                       | 7.35                     | 0.98                   | 0.176                 | 2.55                                         | 1.09                                                   | 0.19                                                     | 0.20                                                      |
| 0.18                                                       | 8.11                     | 0.96                   | 0.137                 | 2.90                                         | 1.56                                                   | 0.95                                                     | 0.87                                                      |



Experiment 2

Table 17. Experimental results from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of fresh material.

| Parts per million of iron in the nutrient solution |    | Milligrams of iron | Milligrams of ascorbic acid at harvest | Milligrams of ascorbic acid just after drying | Milligrams of ascorbic acid 7 days after drying | Milligrams of ascorbic acid 14 days after drying | Grams of dry matter |
|----------------------------------------------------|----|--------------------|----------------------------------------|-----------------------------------------------|-------------------------------------------------|--------------------------------------------------|---------------------|
| 7                                                  | 0  | 1.09               | 28.6                                   | 18.9                                          | 13.8                                            | 12.9                                             | 12.2                |
|                                                    | 2  | 2.22               | 48.7                                   | 17.2                                          | 4.8                                             | 4.5                                              | 14.5                |
|                                                    | 4  | 1.57               | 40.6                                   | 16.5                                          | 9.6                                             | 6.2                                              | 13.1                |
|                                                    | 6  | 2.36               | 41.7                                   | 17.2                                          | 8.6                                             | 7.8                                              | 13.1                |
|                                                    | 8  | 2.70               | 36.7                                   | 15.8                                          | 10.7                                            | 10.3                                             | 13.0                |
|                                                    | 10 | 2.70               | 41.1                                   | 17.6                                          | 14.8                                            | 10.4                                             | 13.2                |
|                                                    | 12 | 3.16               | 42.2                                   | 17.4                                          | 10.3                                            | 10.6                                             | 13.0                |
|                                                    | 14 | 2.94               | 36.6                                   | 19.4                                          | 14.7                                            | 8.4                                              | 12.8                |
|                                                    | 16 | 2.56               | 34.9                                   | 18.2                                          | 11.7                                            | 11.3                                             | 12.2                |
|                                                    | 18 | 3.33               | 33.6                                   | 14.6                                          | 12.4                                            | 9.4                                              | 12.8                |

Experiment 2.

Table 18. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 100 grams of fresh material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>iron | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>7 days after<br>drying | Milligrams of<br>ascorbic acid<br>14 days after<br>drying | Grams of<br>dry matter |
|------------------------------------------------------------|-----------------------|----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|------------------------|
| 0.00                                                       | 2.39                  | 43.7                                         | 15.7                                                   | 10.1                                                     | 11.1                                                      | 12.3                   |
| 0.02                                                       | 2.23                  | 36.8                                         | 17.9                                                   | 6.6                                                      | 3.3                                                       | 13.3                   |
| 0.04                                                       | 2.38                  | 34.4                                         | 18.9                                                   | 10.4                                                     | 11.2                                                      | 13.6                   |
| 0.06                                                       | 2.63                  | 40.4                                         | 15.2                                                   | 4.1                                                      | 2.2                                                       | 14.0                   |
| 0.08                                                       | 1.23                  | 36.1                                         | 20.9                                                   | 15.0                                                     | 12.2                                                      | 11.9                   |
| 0.10                                                       | 1.70                  | 33.3                                         | 19.7                                                   | 12.4                                                     | 9.3                                                       | 12.6                   |
| 0.12                                                       | 2.12                  | 38.4                                         | 21.3                                                   | 12.1                                                     | 12.8                                                      | 12.1                   |
| 0.14                                                       | 2.69                  | 39.7                                         | 18.1                                                   | 12.7                                                     | 13.2                                                      | 13.1                   |
| 0.16                                                       | 2.39                  | 34.6                                         | 14.9                                                   | 2.6                                                      | 2.8                                                       | 13.3                   |
| 0.18                                                       | 1.69                  | 35.8                                         | 19.1                                                   | 11.7                                                     | 10.6                                                      | 11.8                   |

Table 19. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of dry plant material.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>iron | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>7 days after<br>drying | Milligrams of<br>ascorbic acid<br>14 days after<br>drying | Grams of<br>fresh matter |
|----------------------------------------------------------|-----------------------|----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|--------------------------|
| 0                                                        | 9.0                   | 235                                          | 155.1                                                  | 113.0                                                    | 105.6                                                     | 820                      |
| 2                                                        | 15.3                  | 336                                          | 118.6                                                  | 33.4                                                     | 30.7                                                      | 690                      |
| 4                                                        | 12.0                  | 310                                          | 126.1                                                  | 72.9                                                     | 47.2                                                      | 763                      |
| 6                                                        | 18.0                  | 318                                          | 131.6                                                  | 65.3                                                     | 60.9                                                      | 763                      |
| 8                                                        | 20.9                  | 282                                          | 121.8                                                  | 82.2                                                     | 78.9                                                      | 769                      |
| 10                                                       | 20.5                  | 311                                          | 133.5                                                  | 112.4                                                    | 78.9                                                      | 758                      |
| 12                                                       | 24.3                  | 325                                          | 133.9                                                  | 79.6                                                     | 81.7                                                      | 769                      |
| 14                                                       | 23.0                  | 286                                          | 151.3                                                  | 115.1                                                    | 66.0                                                      | 781                      |
| 16                                                       | 21.0                  | 286                                          | 149.5                                                  | 95.6                                                     | 92.5                                                      | 820                      |
| 18                                                       | 26.0                  | 263                                          | 112.9                                                  | 96.7                                                     | 73.6                                                      | 781                      |

Experiment 2

Table 20. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 100 grams of dry material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of,<br>iron | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>ascorbic acid<br>just after<br>drying | Milligrams of<br>ascorbic acid<br>7 days after<br>drying | Milligrams of<br>ascorbic acid<br>14 days after<br>drying | Grams of<br>fresh matter |
|------------------------------------------------------------|------------------------|----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|--------------------------|
| 0.00                                                       | 23.5                   | 355                                          | 127.3                                                  | 82.4                                                     | 90.1                                                      | 813                      |
| 0.02                                                       | 16.3                   | 277                                          | 134.8                                                  | 49.9                                                     | 24.3                                                      | 752                      |
| 0.04                                                       | 17.5                   | 253                                          | 139.2                                                  | 76.3                                                     | 82.5                                                      | 735                      |
| 0.06                                                       | 18.3                   | 289                                          | 108.6                                                  | 29.3                                                     | 15.8                                                      | 714                      |
| 0.08                                                       | 10.3                   | 303                                          | 175.7                                                  | 126.1                                                    | 102.7                                                     | 840                      |
| 0.10                                                       | 13.5                   | 264                                          | 157.0                                                  | 98.2                                                     | 73.9                                                      | 794                      |
| 0.12                                                       | 17.5                   | 317                                          | 176.1                                                  | 99.8                                                     | 105.9                                                     | 826                      |
| 0.14                                                       | 20.5                   | 303                                          | 138.2                                                  | 97.1                                                     | 100.9                                                     | 763                      |
| 0.16                                                       | 18.0                   | 260                                          | 111.7                                                  | 19.6                                                     | 20.8                                                      | 752                      |
| 0.18                                                       | 14.3                   | 303                                          | 162.1                                                  | 99.1                                                     | 90.2                                                      | 847                      |

Experiment 2.

Table 21. Percentages of the original amounts of ascorbic acid found in wheat plants grown on nutrient solutions varying in concentrations of iron, at each determination after the drying of the plants.

| Parts per million<br>of iron in the<br>nutrient solution | Percentages of<br>ascorbic acid<br>at harvest | Percentages of<br>ascorbic acid<br>just after<br>drying | Percentages of<br>ascorbic acid<br>7 days after<br>drying | Percentages of<br>ascorbic acid<br>14 days after<br>drying. |
|----------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------|
| 0                                                        | 100                                           | 66.1                                                    | 48.1                                                      | 45.0                                                        |
| 2                                                        | 100                                           | 35.2                                                    | 9.9                                                       | 9.1                                                         |
| 4                                                        | 100                                           | 40.6                                                    | 23.5                                                      | 15.2                                                        |
| 6                                                        | 100                                           | 41.3                                                    | 20.5                                                      | 19.2                                                        |
| 8                                                        | 100                                           | 43.1                                                    | 29.1                                                      | 28.0                                                        |
| 10                                                       | 100                                           | 42.9                                                    | 36.1                                                      | 25.4                                                        |
| 12                                                       | 100                                           | 41.2                                                    | 24.5                                                      | 25.1                                                        |
| 14                                                       | 100                                           | 52.9                                                    | 40.2                                                      | 23.1                                                        |
| 16                                                       | 100                                           | 52.2                                                    | 33.4                                                      | 32.2                                                        |
| 18                                                       | 100                                           | 42.9                                                    | 36.8                                                      | 28.0                                                        |

Experiment 2.

Table 22. Percentages of the original amounts of ascorbic acid found in wheat plants grown on nutrient solutions varying in concentrations of copper, at each determination after the drying of the plants.

| Parts per million<br>of copper in the<br>nutrient solution | Percentages of<br>ascorbic acid<br>at harvest | Percentages of<br>ascorbic acid<br>just after<br>drying | Percentages of<br>ascorbic acid<br>7 days after<br>drying | Percentages of<br>ascorbic acid<br>14 days after<br>drying. |
|------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------|
| 0.00                                                       | 100                                           | 35.8                                                    | 23.2                                                      | 25.4                                                        |
| 0.02                                                       | 100                                           | 48.6                                                    | 18.0                                                      | 9.0                                                         |
| 0.04                                                       | 100                                           | 55.0                                                    | 30.2                                                      | 32.6                                                        |
| 0.06                                                       | 100                                           | 37.5                                                    | 10.1                                                      | 5.5                                                         |
| 0.08                                                       | 100                                           | 58.0                                                    | 41.6                                                      | 33.9                                                        |
| 0.10                                                       | 100                                           | 59.5                                                    | 37.2                                                      | 28.0                                                        |
| 0.12                                                       | 100                                           | 55.6                                                    | 31.5                                                      | 33.4                                                        |
| 0.14                                                       | 100                                           | 45.6                                                    | 32.1                                                      | 33.3                                                        |
| 0.16                                                       | 100                                           | 43.0                                                    | 7.5                                                       | 8.0                                                         |
| 0.18                                                       | 100                                           | 53.5                                                    | 32.7                                                      | 29.8                                                        |

Figure 77. Average amounts of iron, expressed in milligrams per 10 plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 78. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 79. Average amounts of ascorbic acid just after drying, expressed in milligrams per 10 wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 80. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 10 wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 81. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 10 wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million .

Figure 82. Average amount of fresh weight, expressed in milligrams per 10 wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

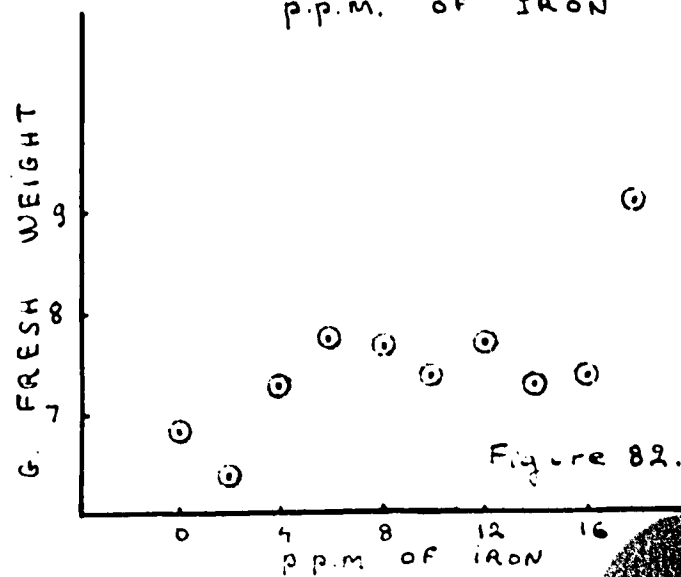
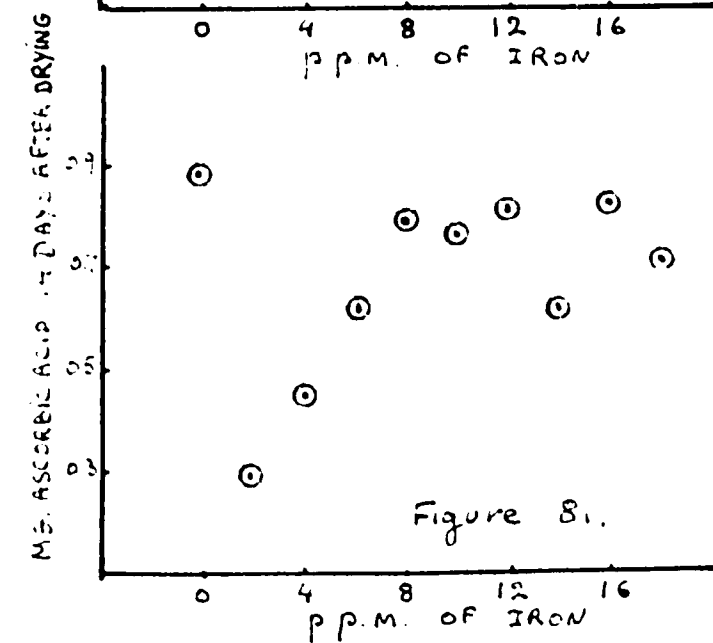
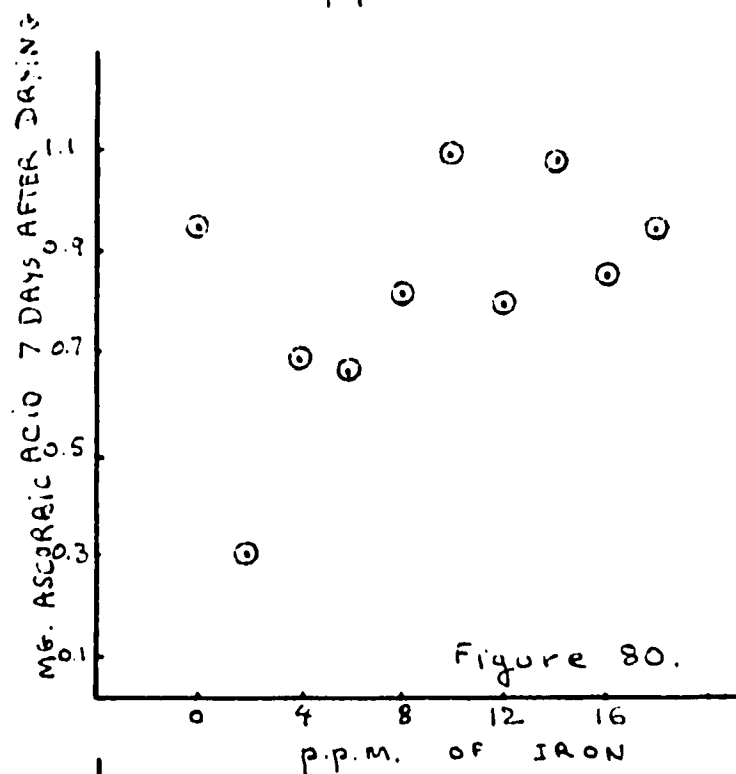
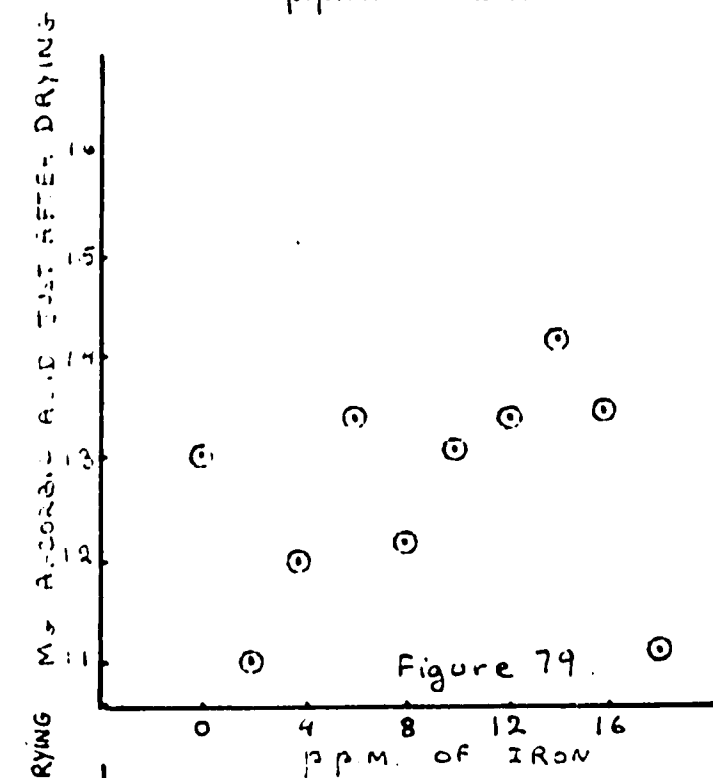
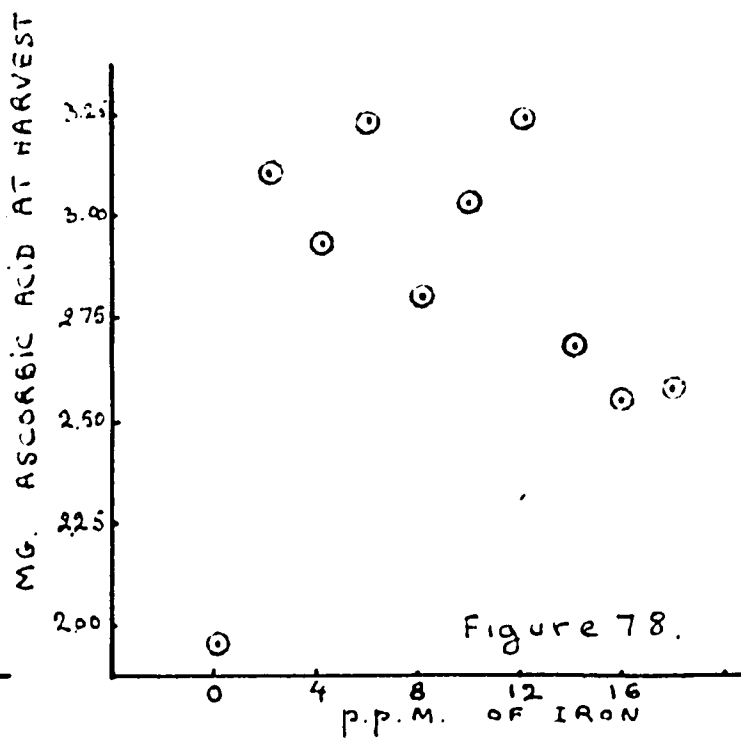
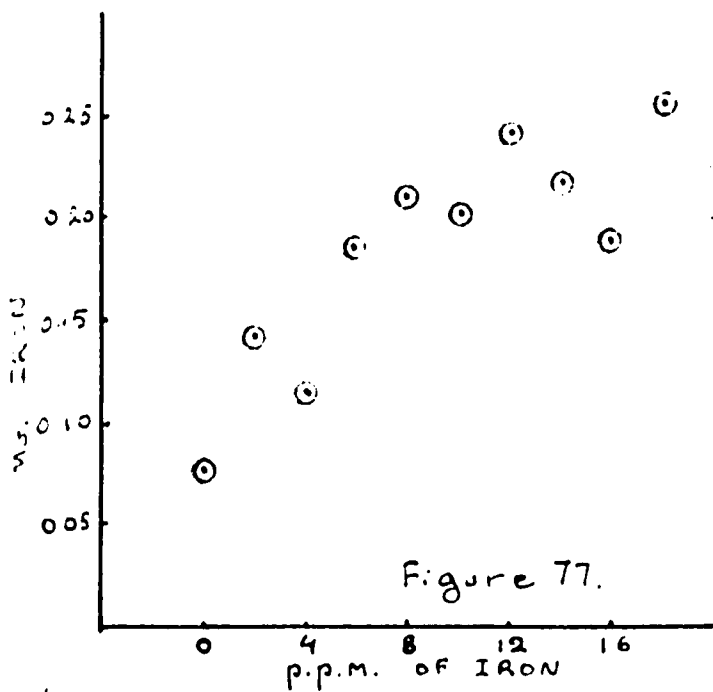




Figure 83. Average amounts of dry weight, expressed in grams per 10 wheat plants , plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 84. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the amount of iron, expressed in milligrams per 10 wheat plants

Figure 85. Average percentages of the original amount of ascorbic acid just after drying in wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amount of iron , expressed in milligrams per 10 wheat plants.

Figure 86. Average percentages of the original amount of ascorbic acid 7 days after drying, in wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 10 wheat plants.

Figure 87. Average percentage of the original amount of ascorbic acid 14 days after drying, in wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 10 wheat plants.

Figure 88. Average amounts of dry weight, expressed in grams per 10 wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 10 wheat plants.

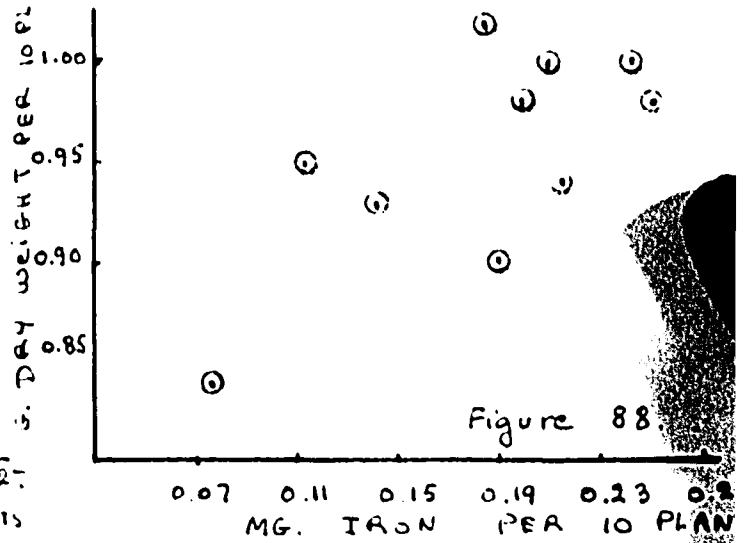
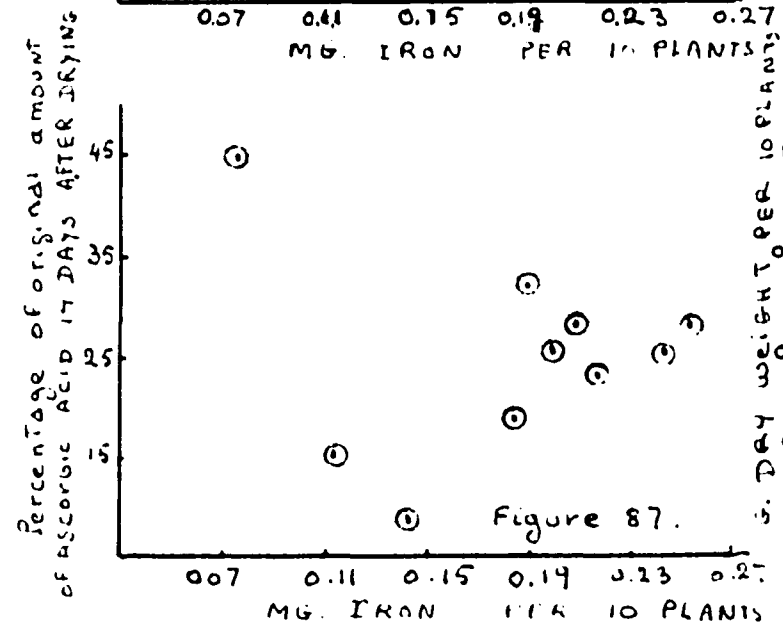
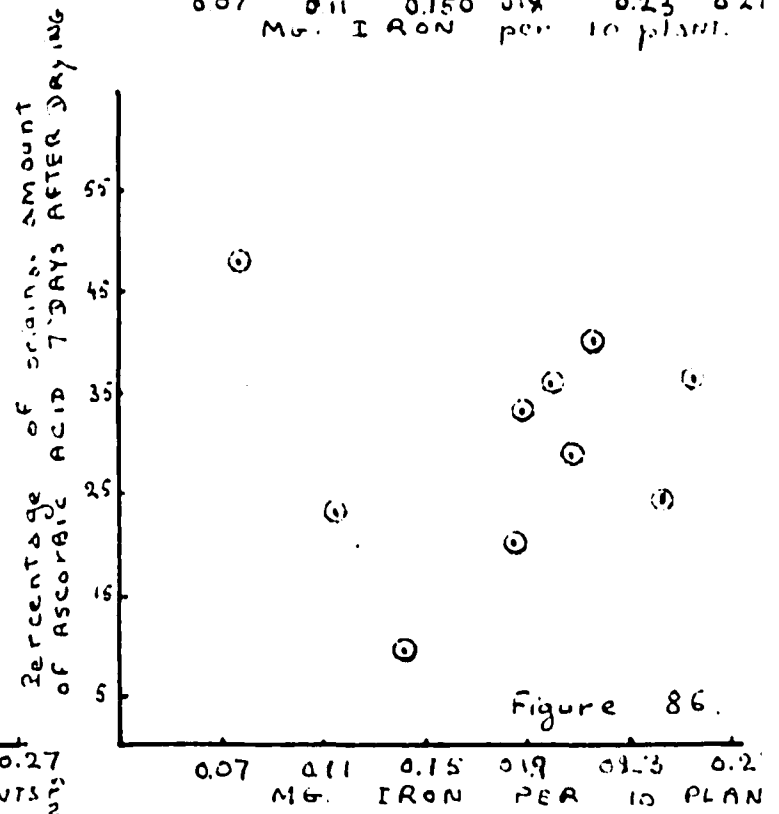
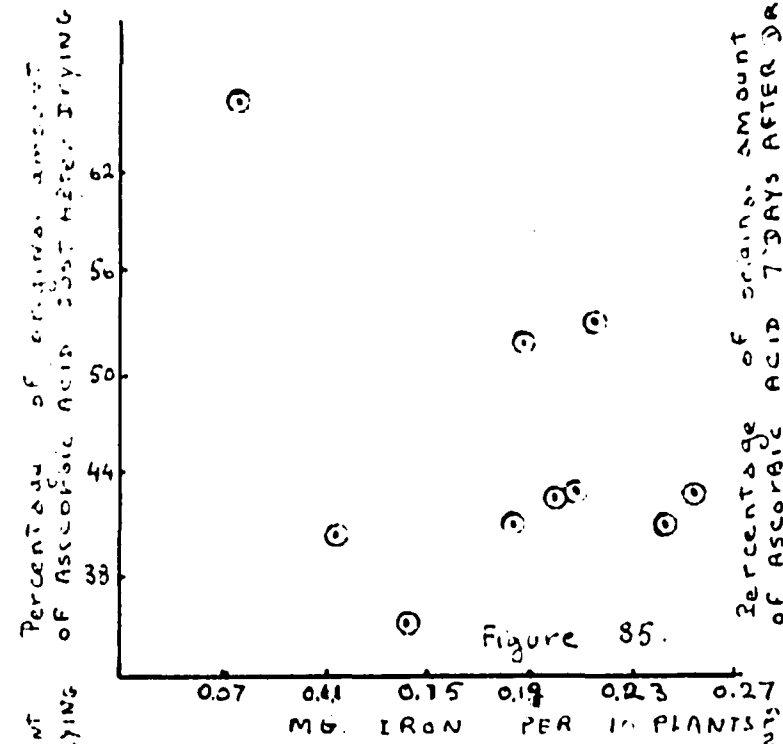
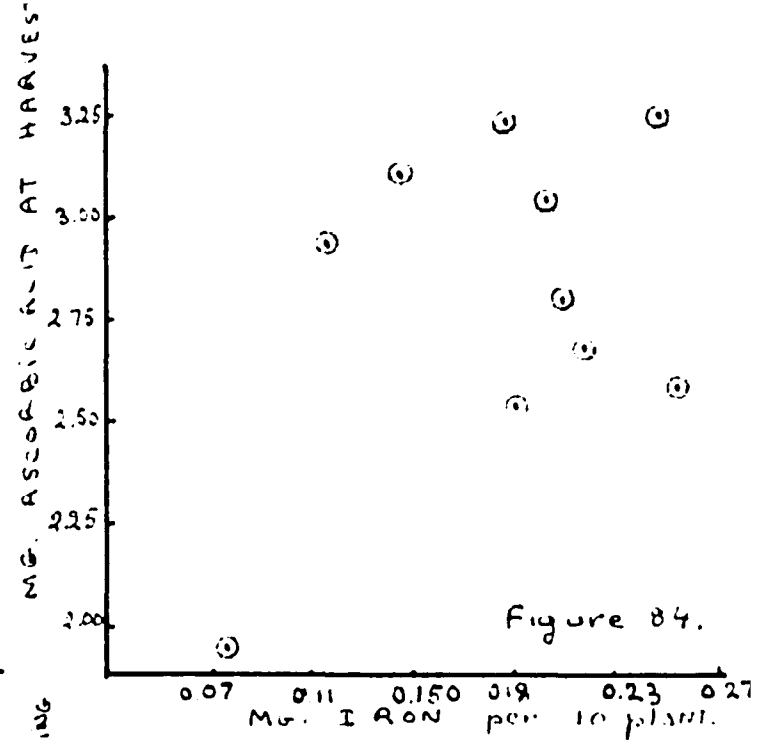
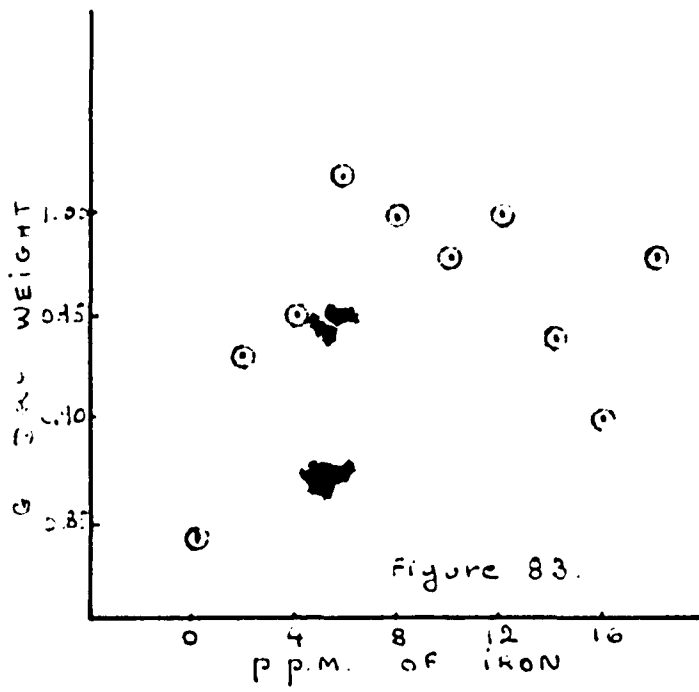


Figure 89. Average amounts of iron, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 90. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 91. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 92. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 93. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 94. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

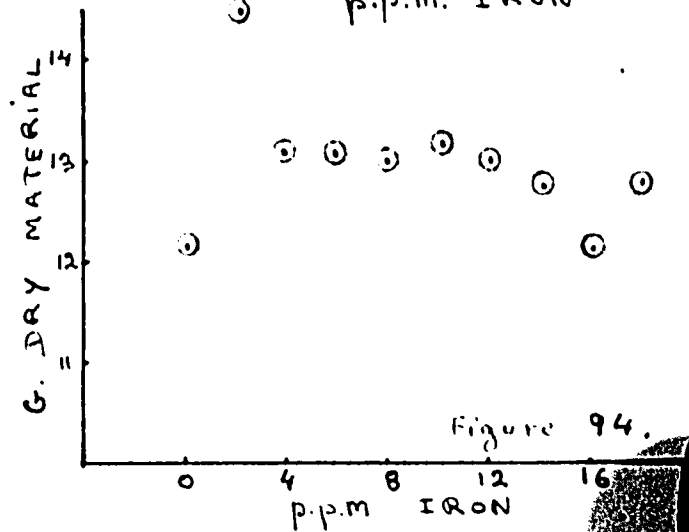
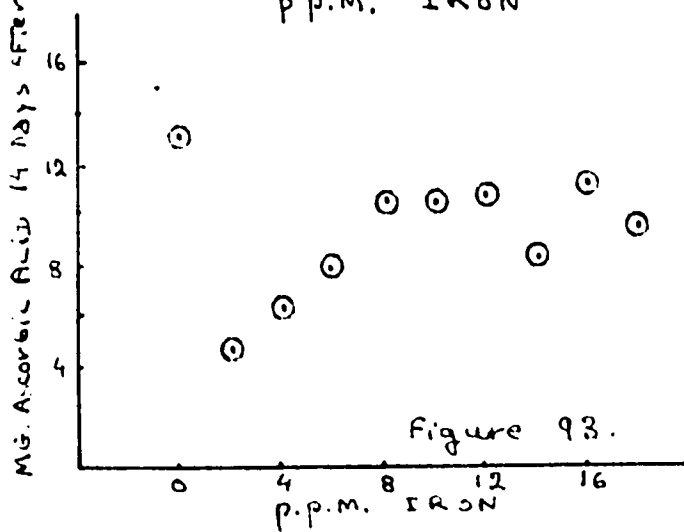
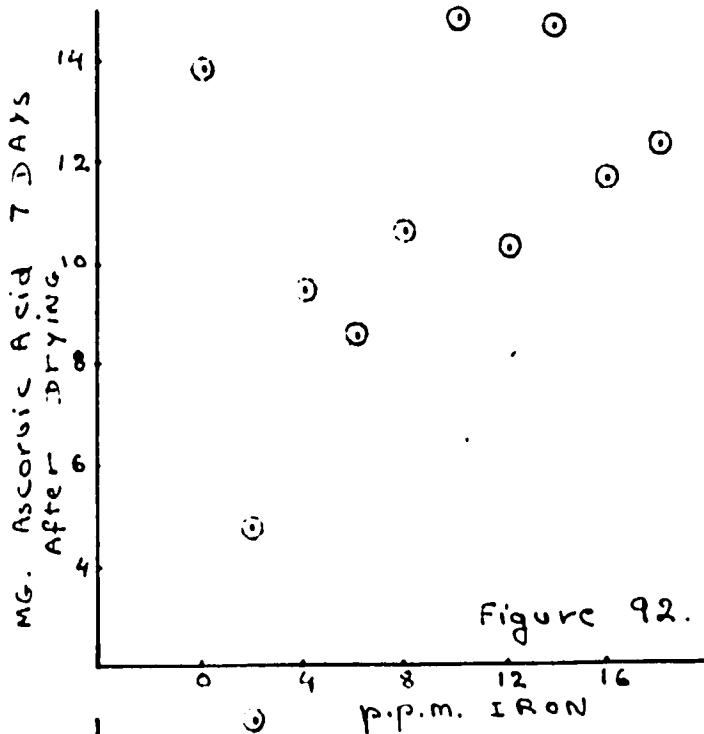
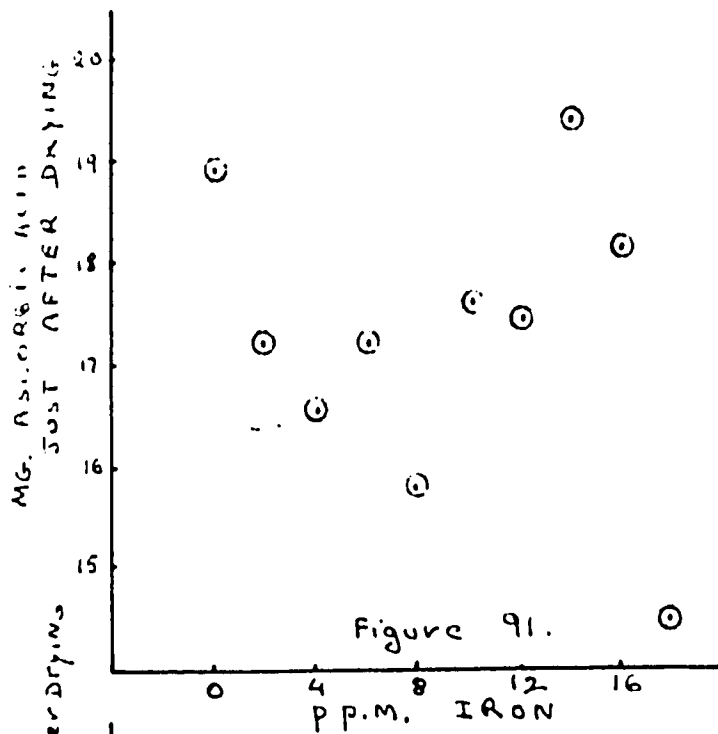
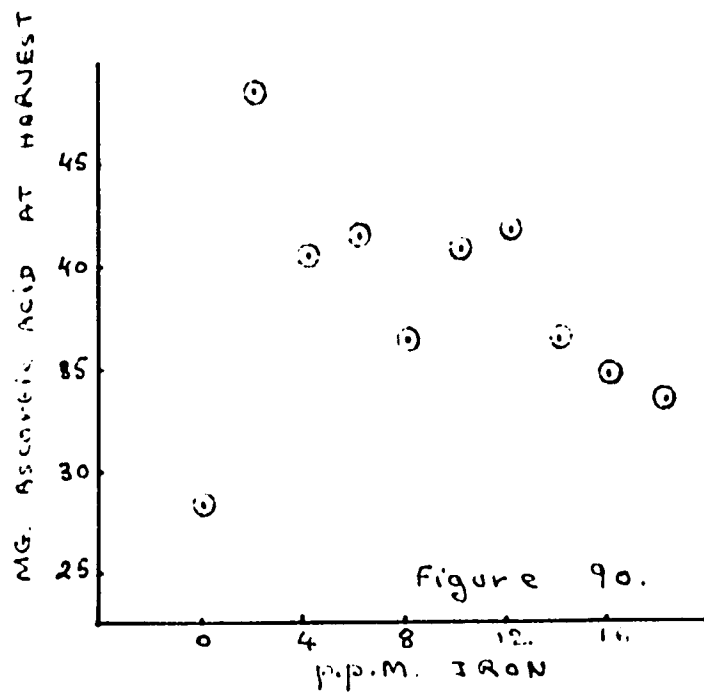
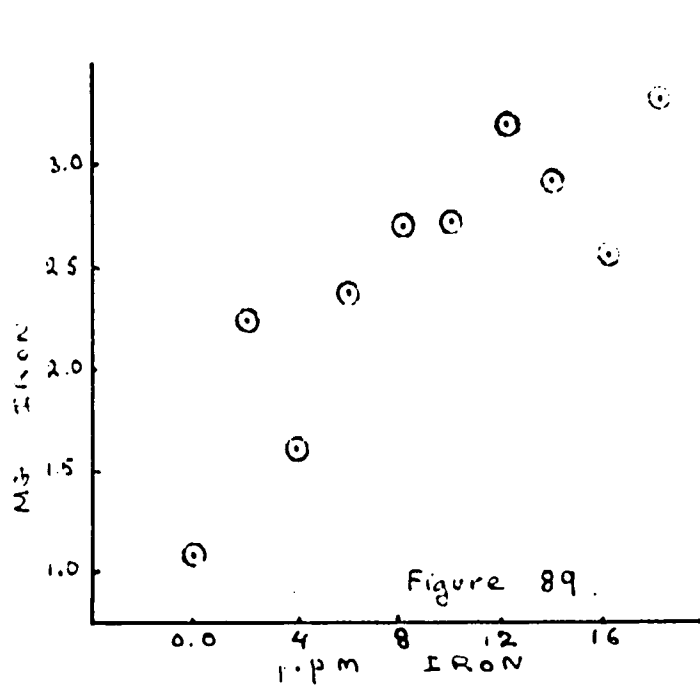


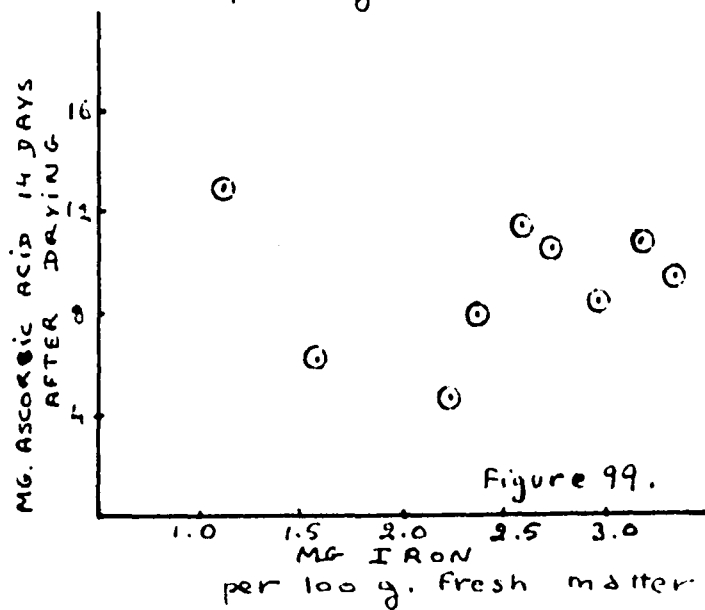
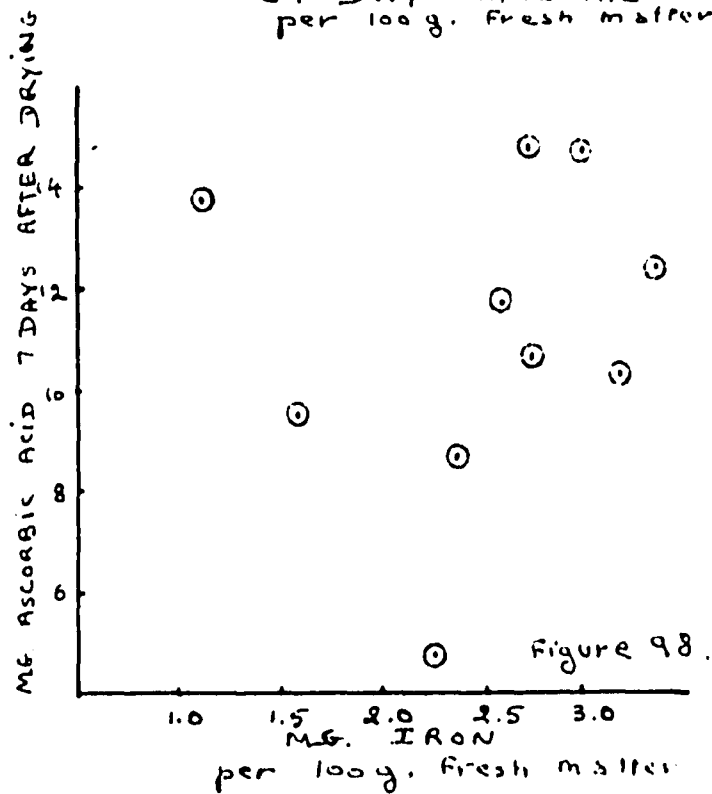
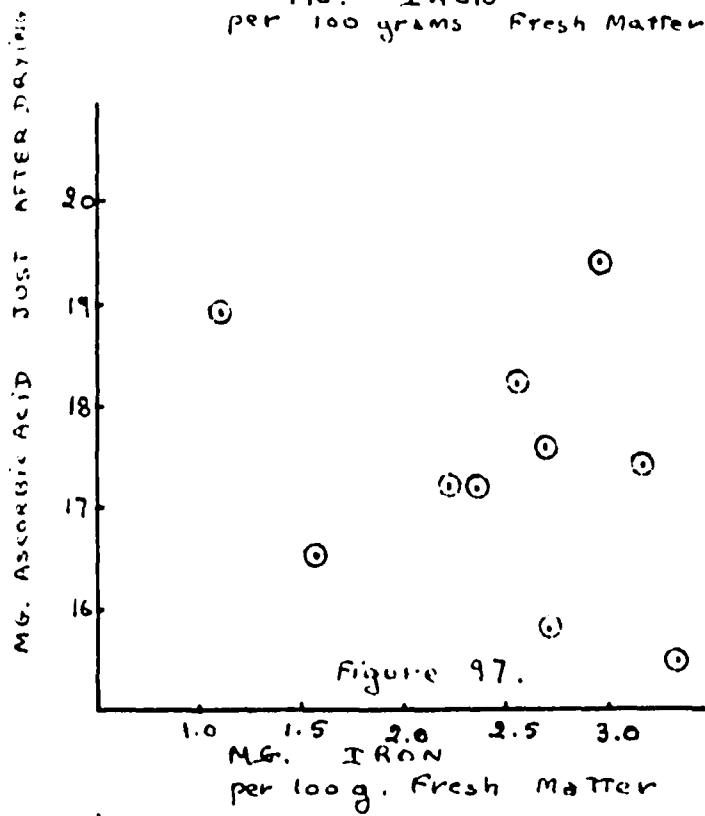
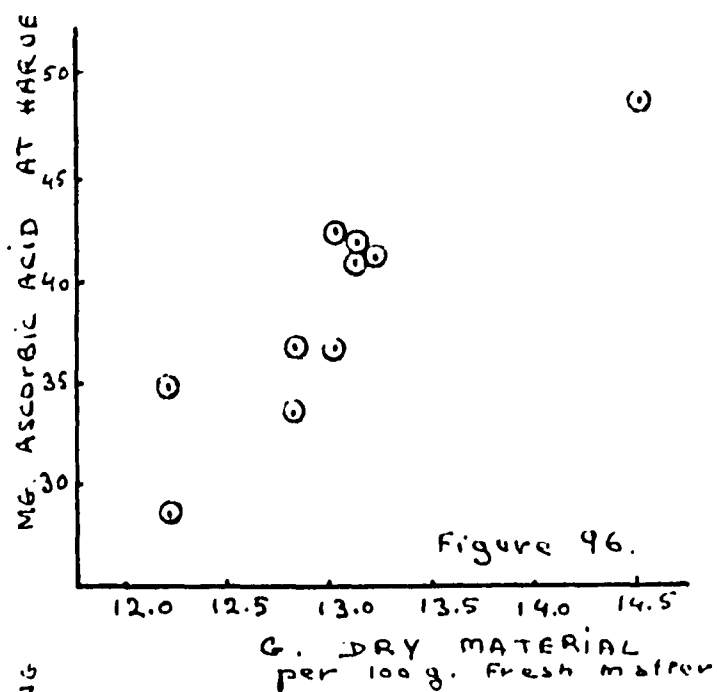
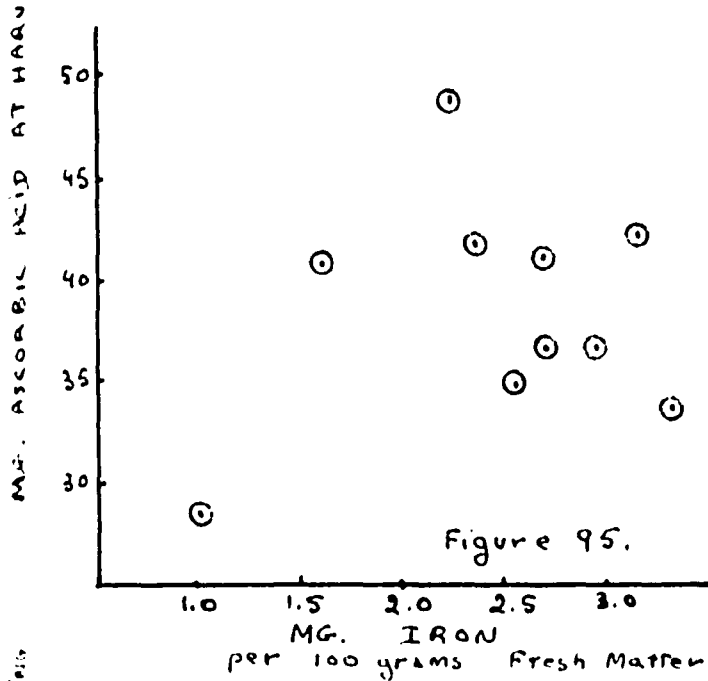
Figure 95. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh material.

Figure 96. Average amount of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solution supplied with various amounts of iron in the nutrient solution, plotted against the amount of dry matter, expressed in grams per 100 grams of fresh matter.

Figure 97. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh material.

Figure 98. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh material.

Figure 99. Average amounts of ascorbic acid 14 days after drying expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh material



- Figure 100. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh material.
- Figure 101. Average amounts of dry material, expressed in grams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron expressed in milligrams per 100 grams of fresh material.
- Figure 102. Average amounts of ascorbic acid , expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh matter.
- Figure 103. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh matter.
- Figure 104. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of fresh matter,

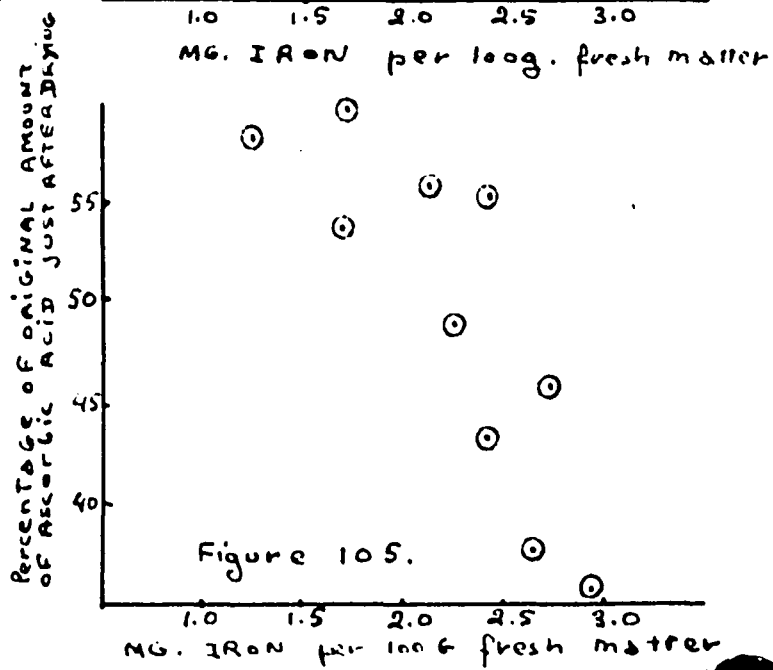
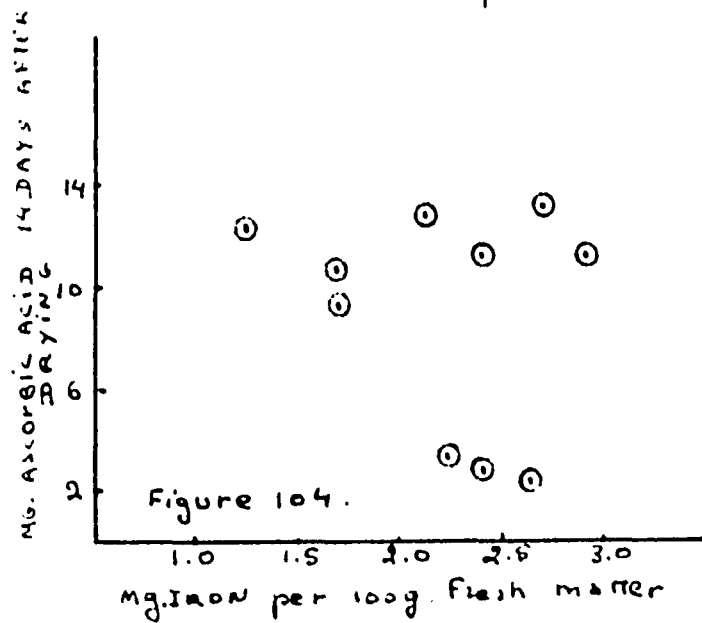
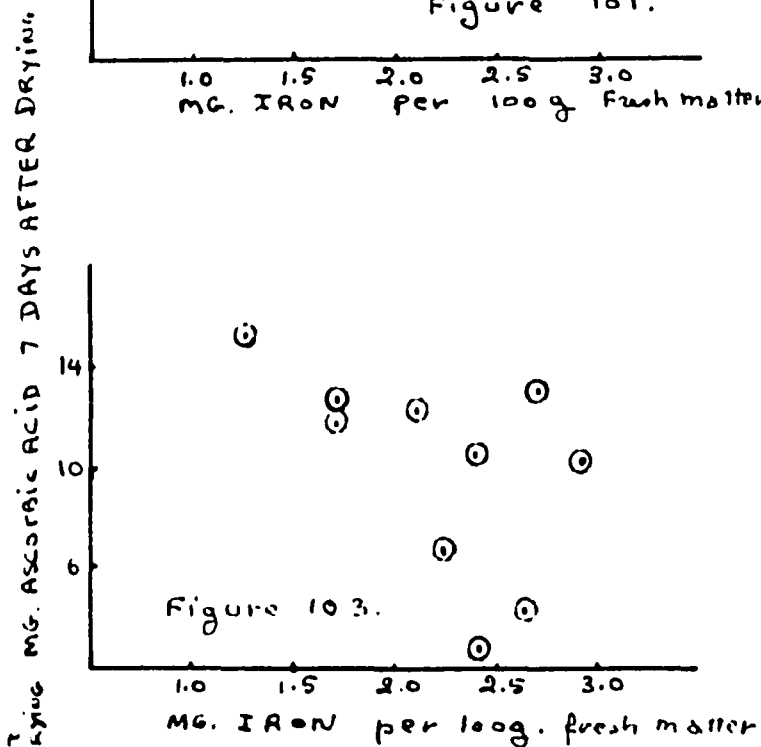
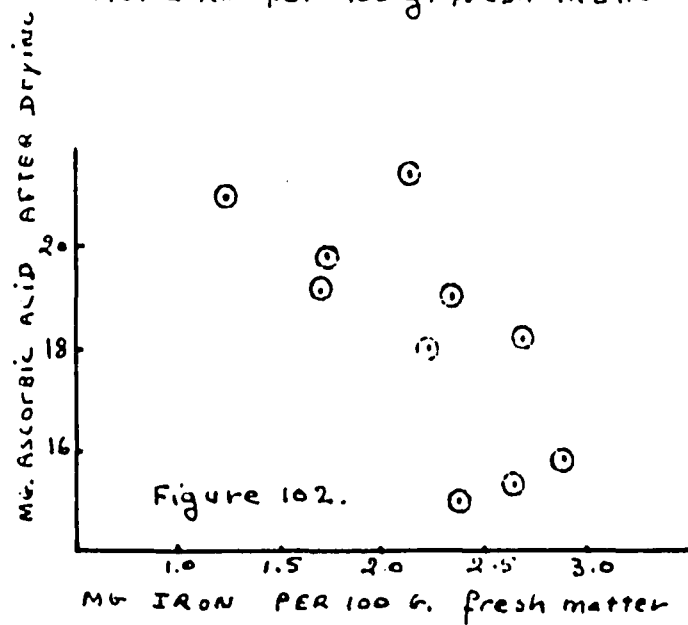
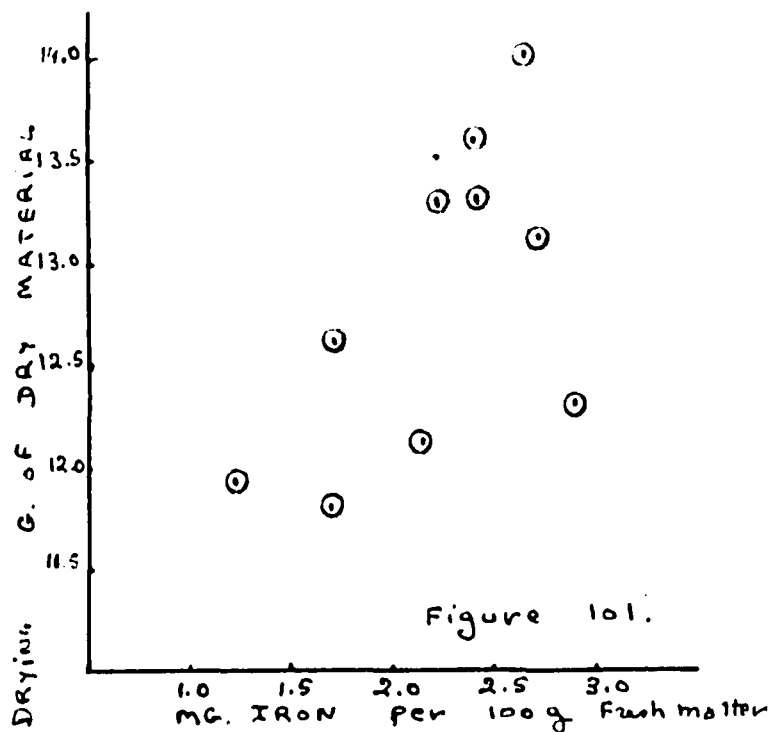
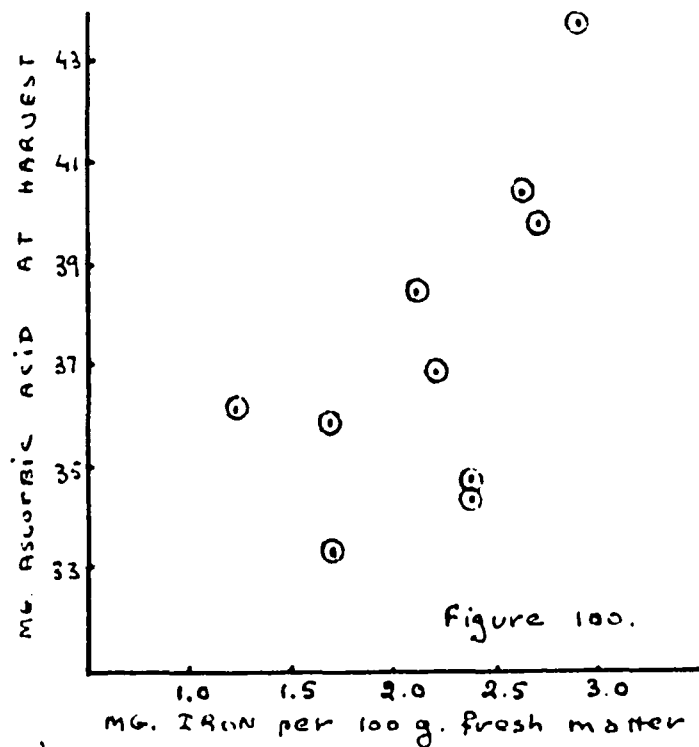




Figure 106. Average amounts of iron, expressed in milligrams per 100 grams of dry matter from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 107. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry matter from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 108. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of dry matter from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 109. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of dry matter from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 110. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 100 grams of dry matter from wheat plants, plotted against the amount of iron in the nutrient solution, expressed in parts per million.

Figure 111. Average amounts of fresh material, expressed in grams per 100 grams of dry matter from wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amount of iron, expressed in parts per million in the nutrient solution.

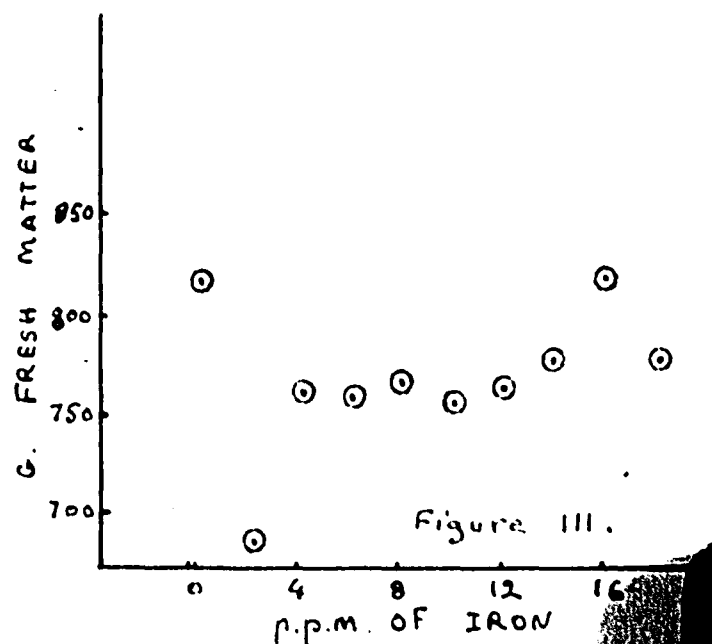
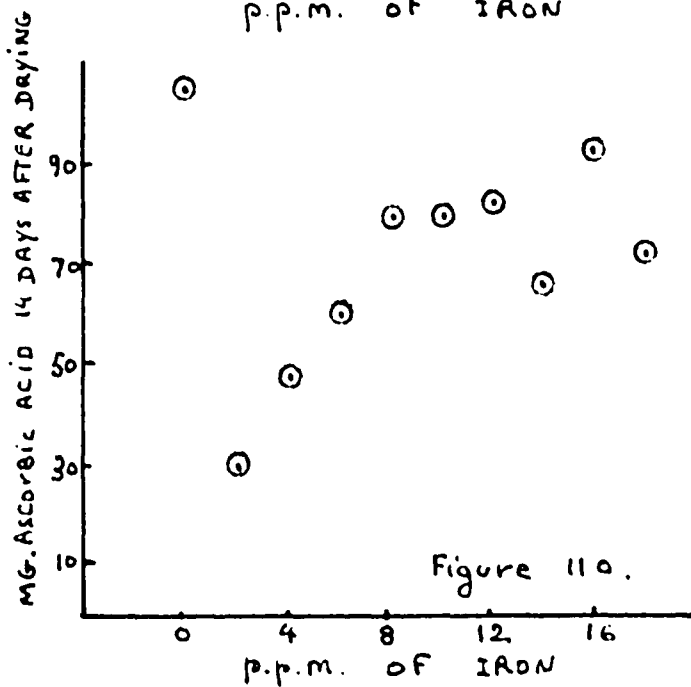
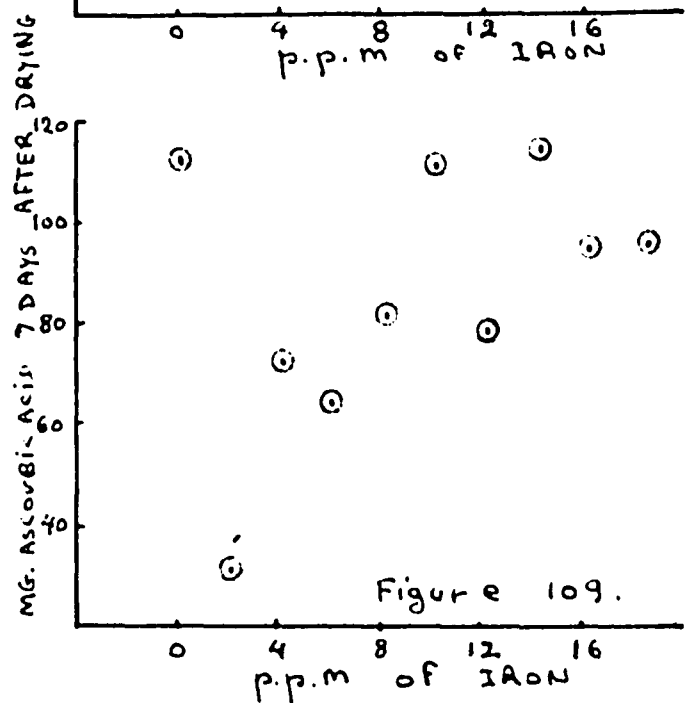
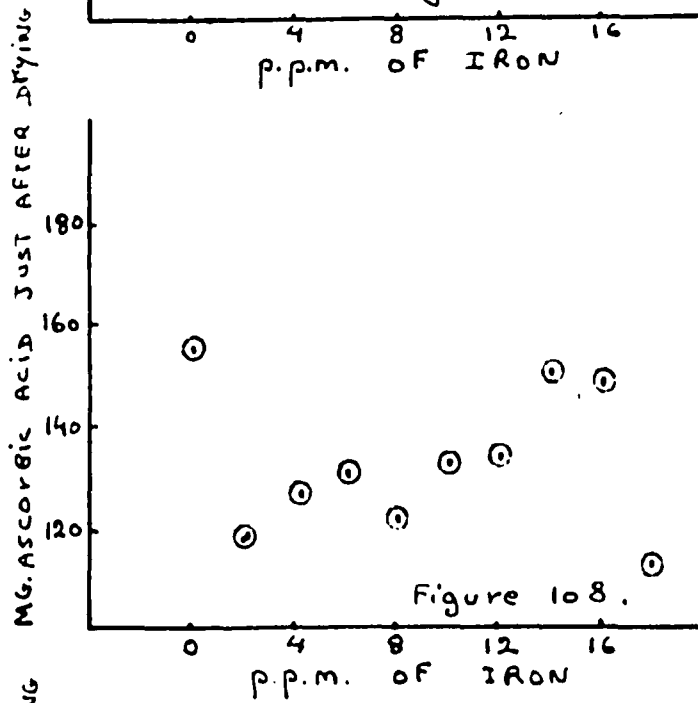
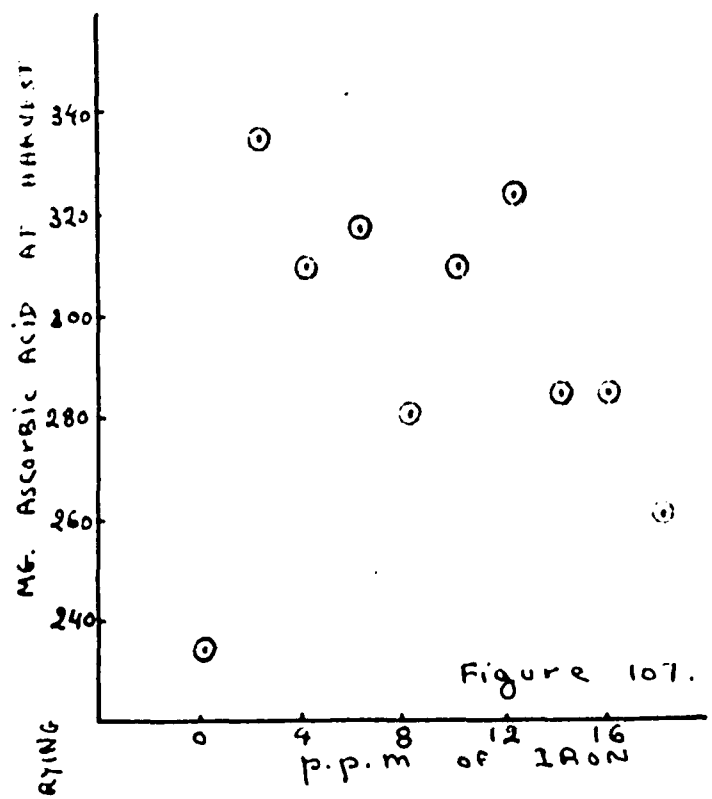
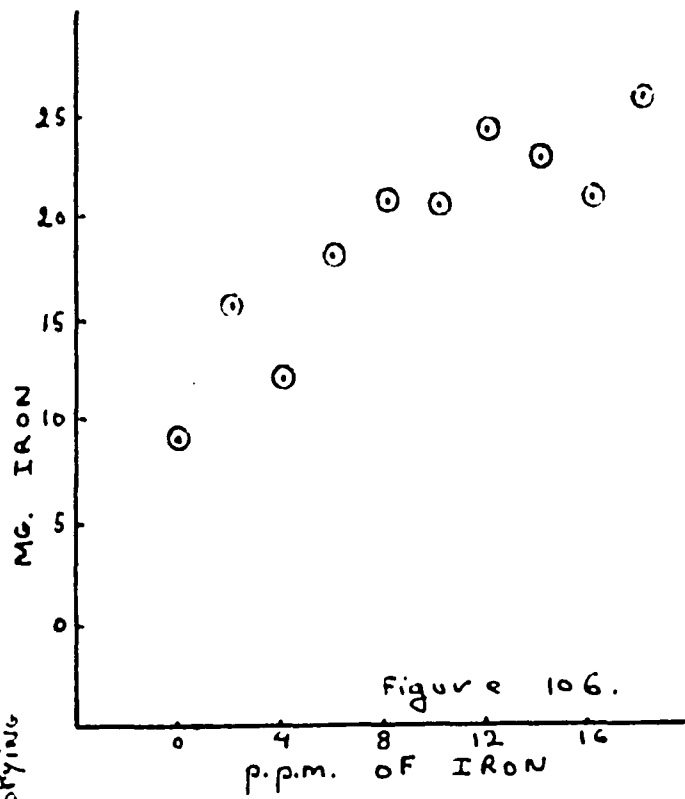


Figure 112. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry material from wheat plants grown on various nutrient solution supplied with different amounts of iron, plotted against the amount of iron , expressed in milligrams per 100 grams of dry material.

Figure 113. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amount of iron in the plant material, expressed in milligrams per 100 grams of dry material.

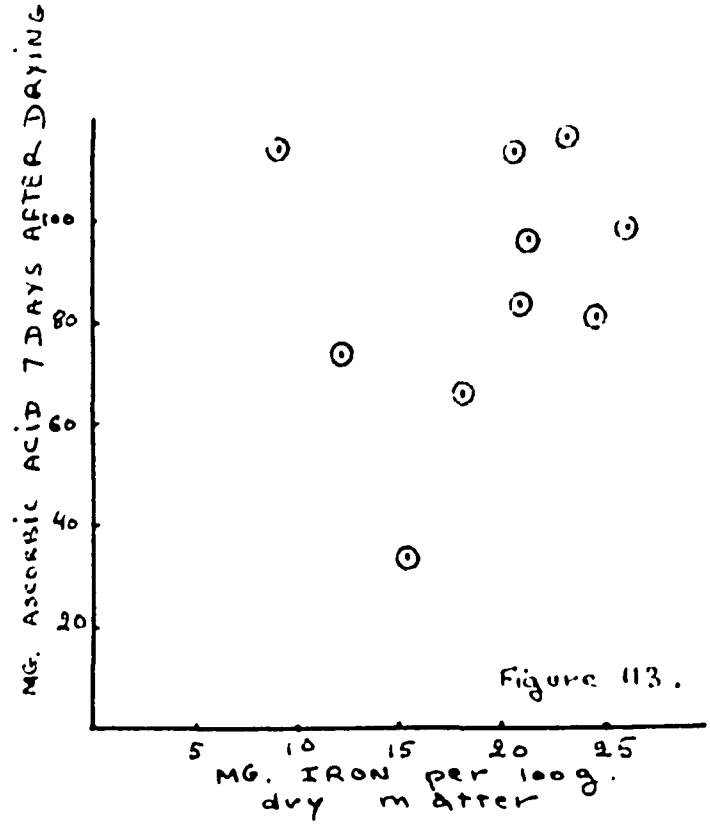
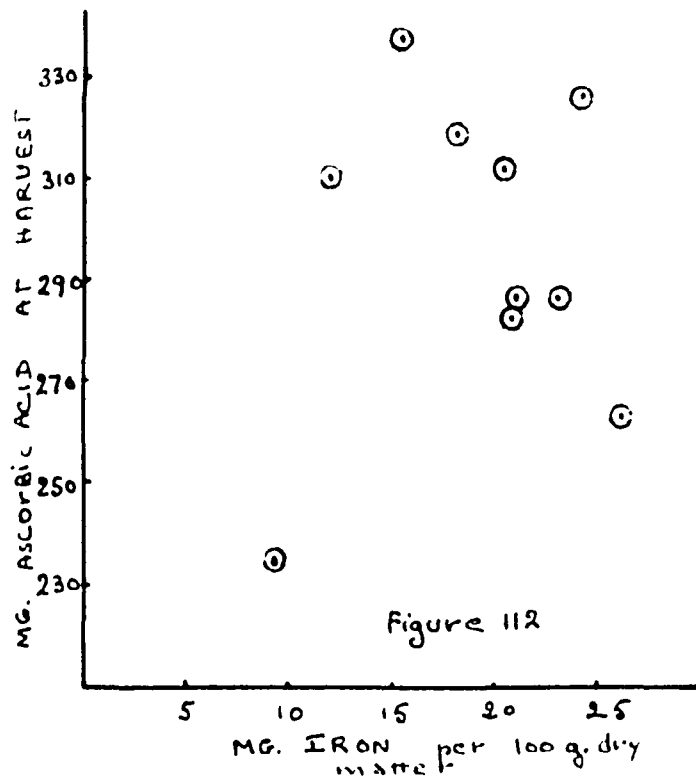


Figure 114. Average amounts of iron, expressed in milligrams per 10 wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.

Figure 115. Average amounts of ascorbic acid at harvest , expressed in milligrams per 10 wheat plants , plotted against the amount of copper in the nutrient solution, expressed in parts per million.

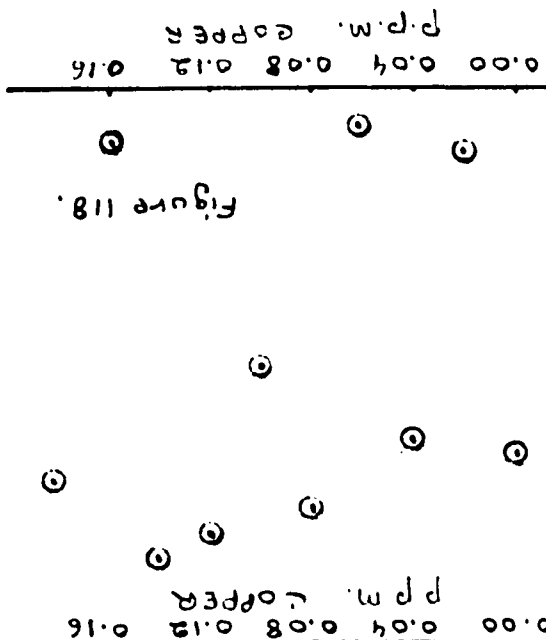
Figure 116. Average amounts of ascorbic acid just after drying, expressed in milligrams per 10 wheat plants , plotted against the amount of copper in the nutrient solution, expressed in parts per million.

Figure 117. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 10 wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.

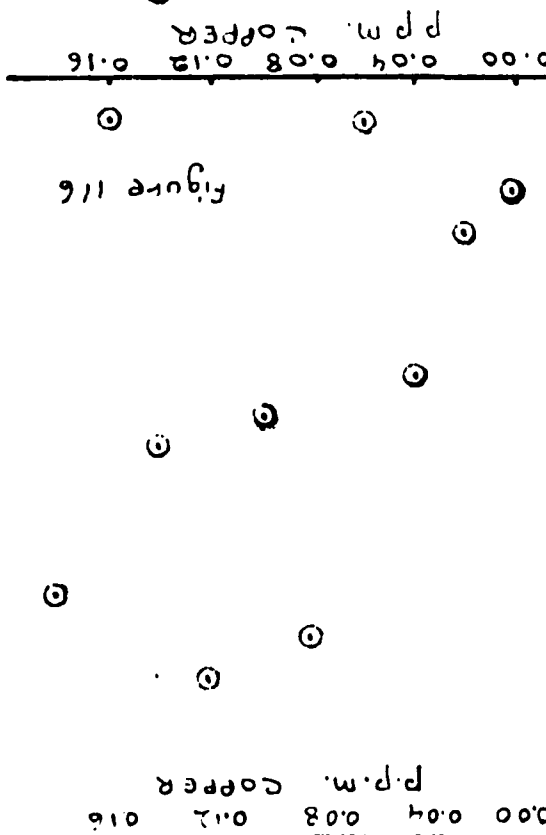
Figure 118. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 10 wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.

Figure 119. Average amounts of fresh matter, expressed in grams per 10 wheat plants , plotted against the amount of copper in the nutrient solution, expressed in parts per million.

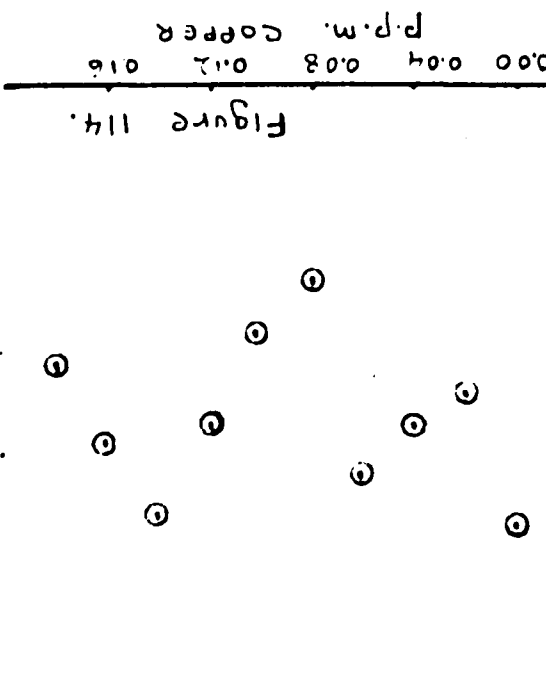
MG. ASCORBIC ACID 14 DAYS AFTER DRYING



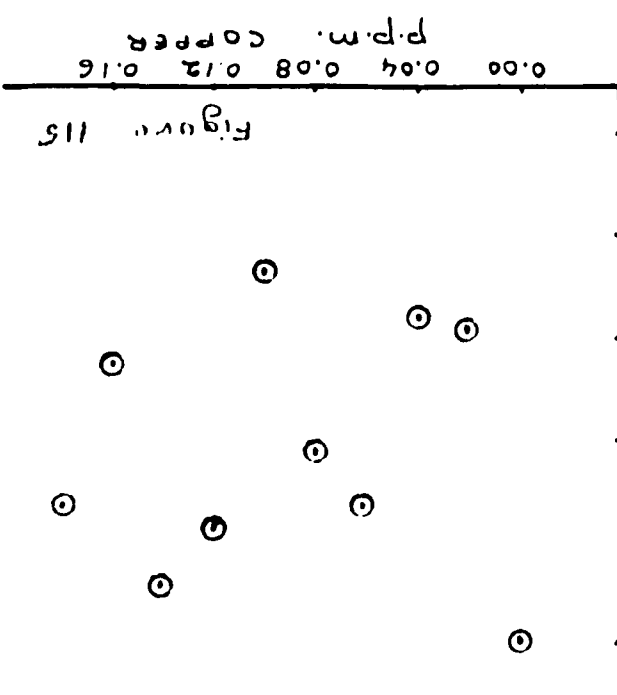
MG. ASCORBIC ACID JUST AFTER DRYING



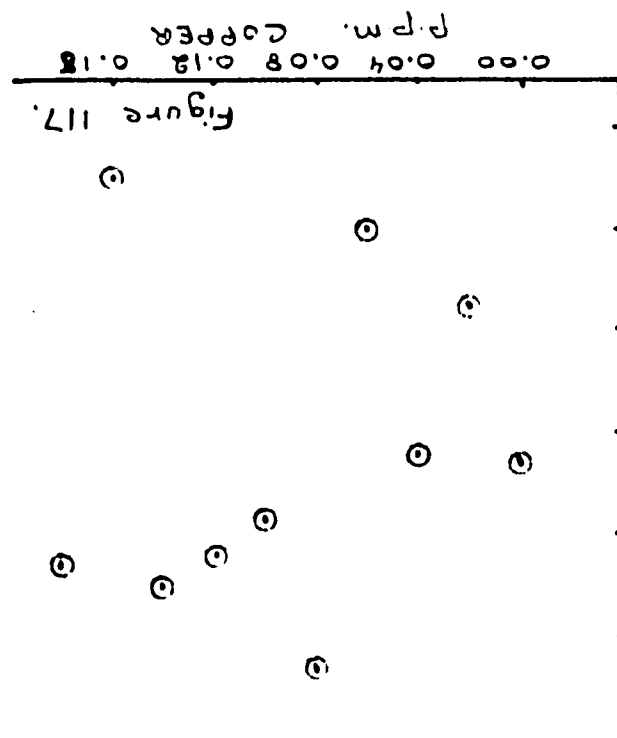
MG. IRON



MG. ASCORBIC ACID AT HARVEST



MG. ASCORBIC ACID 7 DAYS AFTER DRYING



G. FRESH WEIGHT

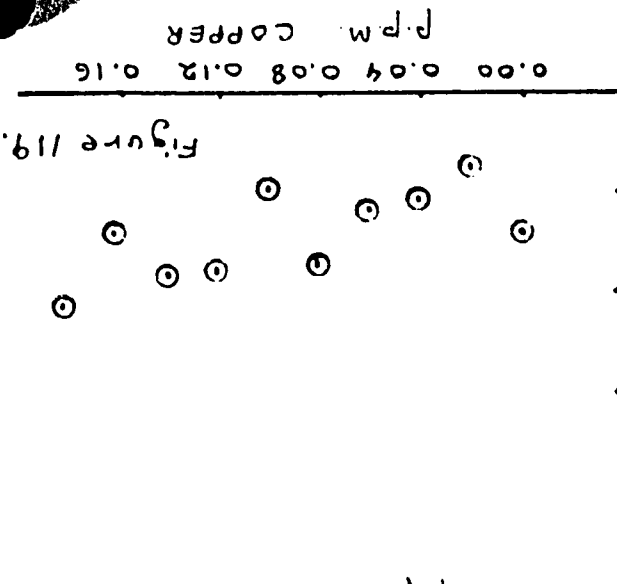


Figure 120. Average amount of dry matter, expressed in grams per 10 wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.

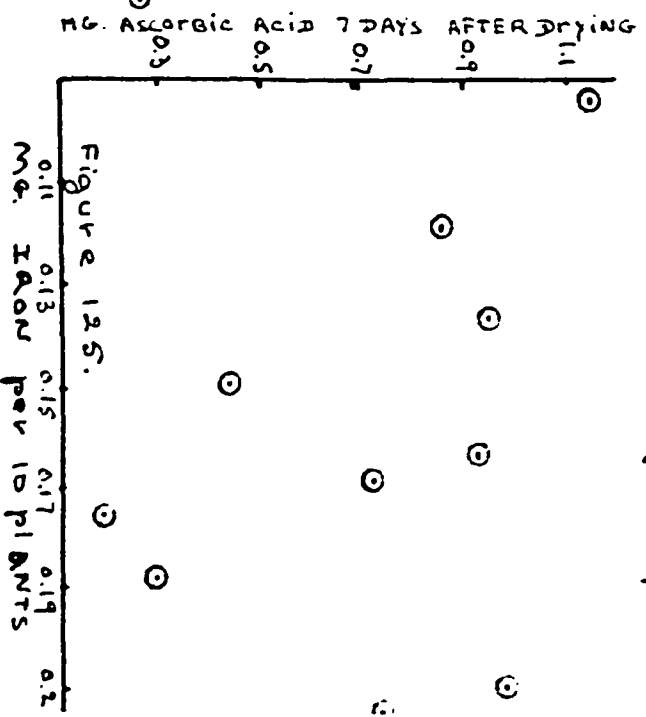
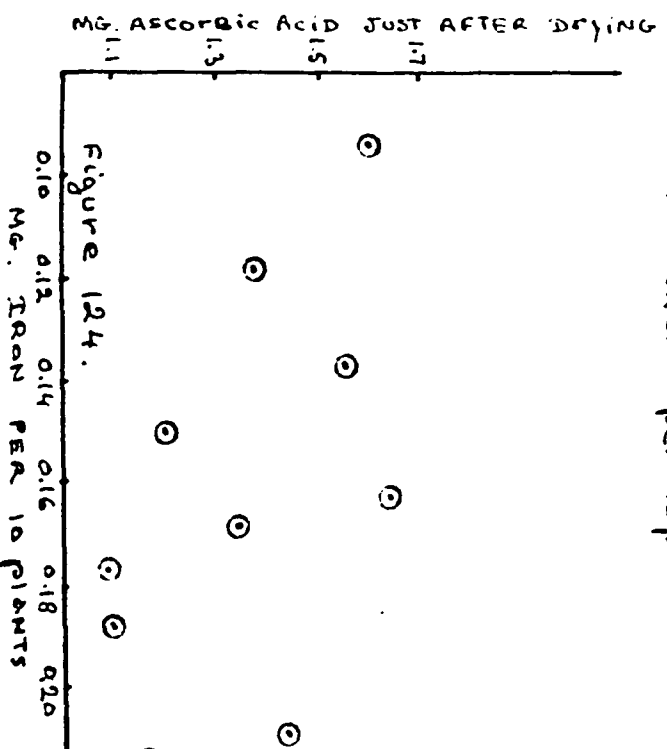
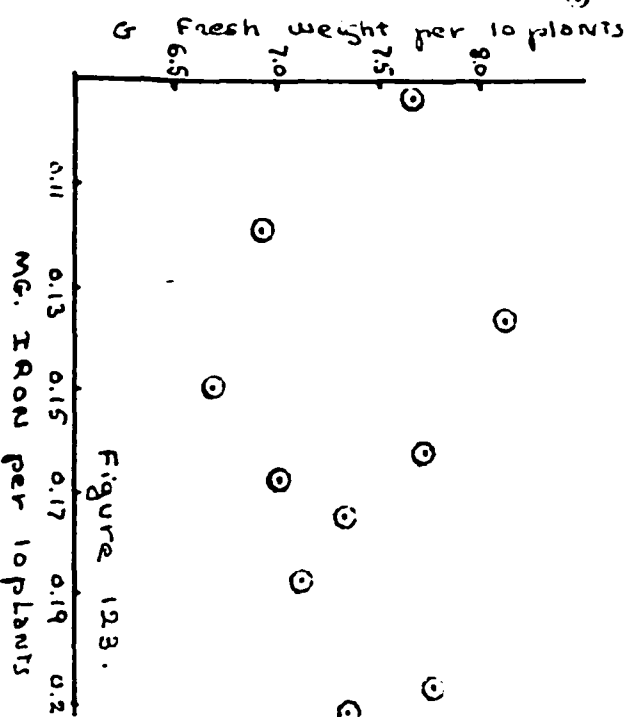
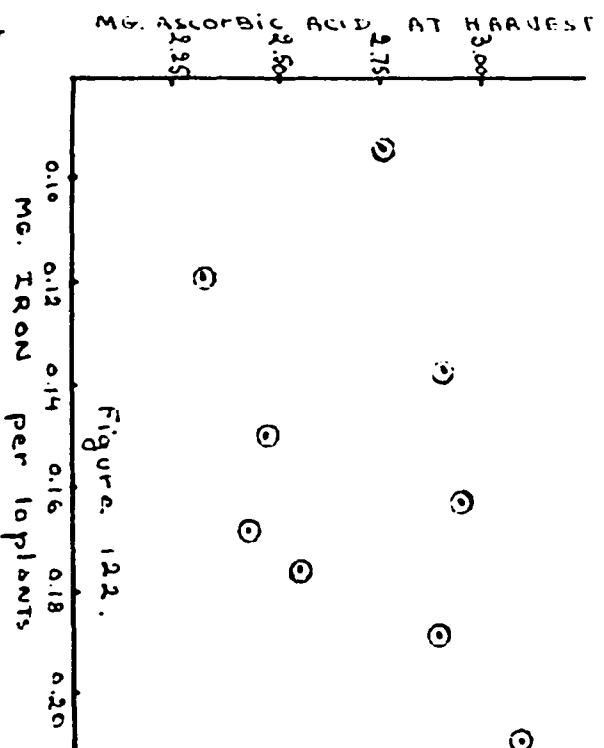
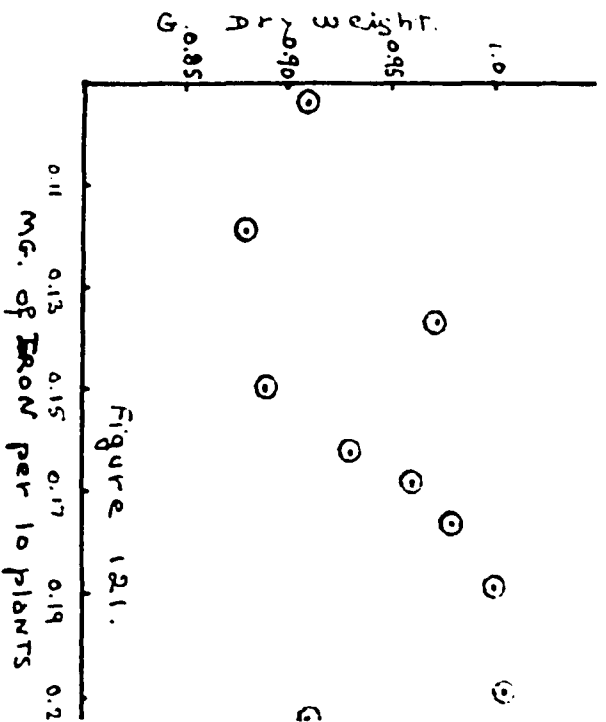
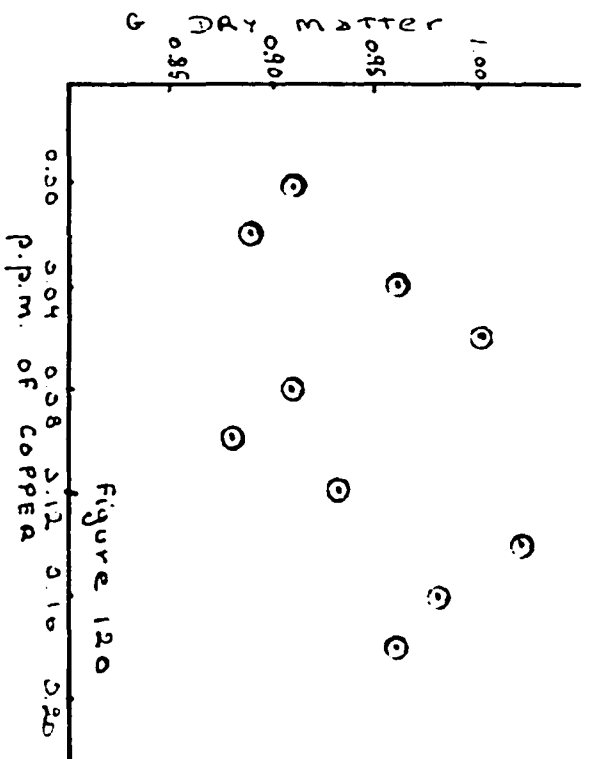
Figure 121. Average amounts of dry weight, expressed in grams per 10 wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.

Figure 122. Average amounts of ascorbic acid, expressed in milligrams per 10 wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.

Figure 123. Average amounts of fresh weight, expressed in grams per 10 wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.

Figure 124. Average amounts of ascorbic acid just after drying, expressed in milligrams per 10 wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 10 wheat plants.

Figure 125. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 10 plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 10 plants.





- Figure 126. Average amounts of iron , expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 127. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh material from wheat plants , plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 128. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 129. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 130. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 100 grams of fresh material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 131. Average amounts of dry matter, expressed in grams per 100 grams of fresh material from wheat plants , plotted against the amount of copper in the nutrient solution, expressed in parts per million.

FIGURE 126.

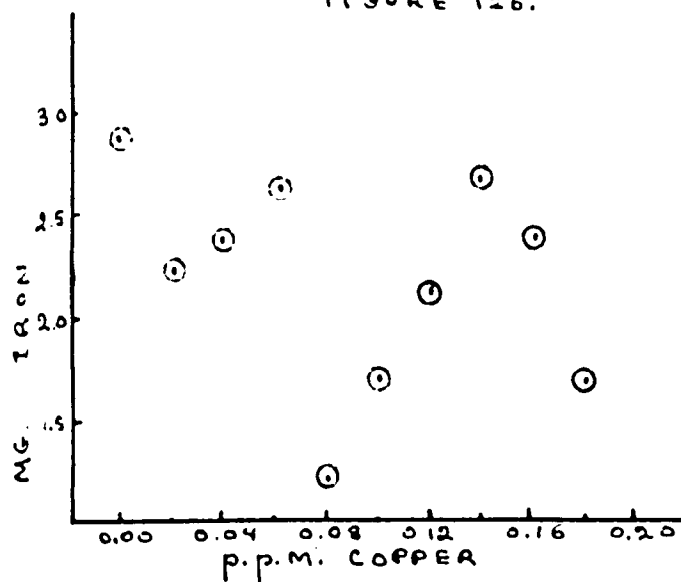


FIGURE 127.

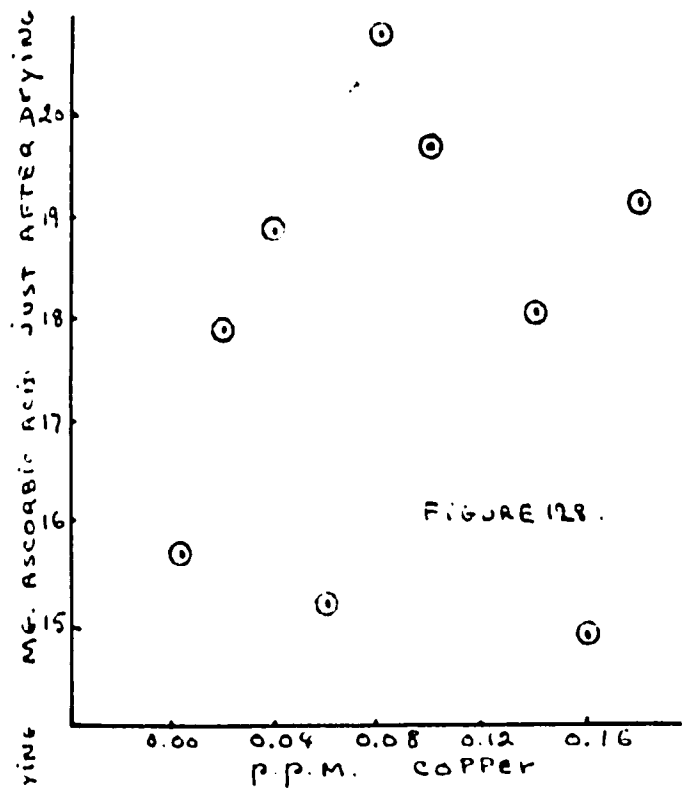
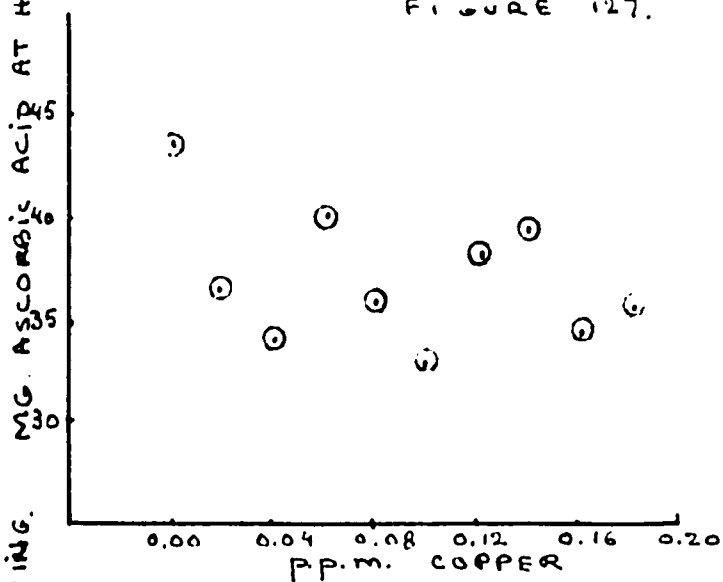


FIGURE 128.

MG. ASCORBIC ACID 7 DAYS AFTER DRYING.

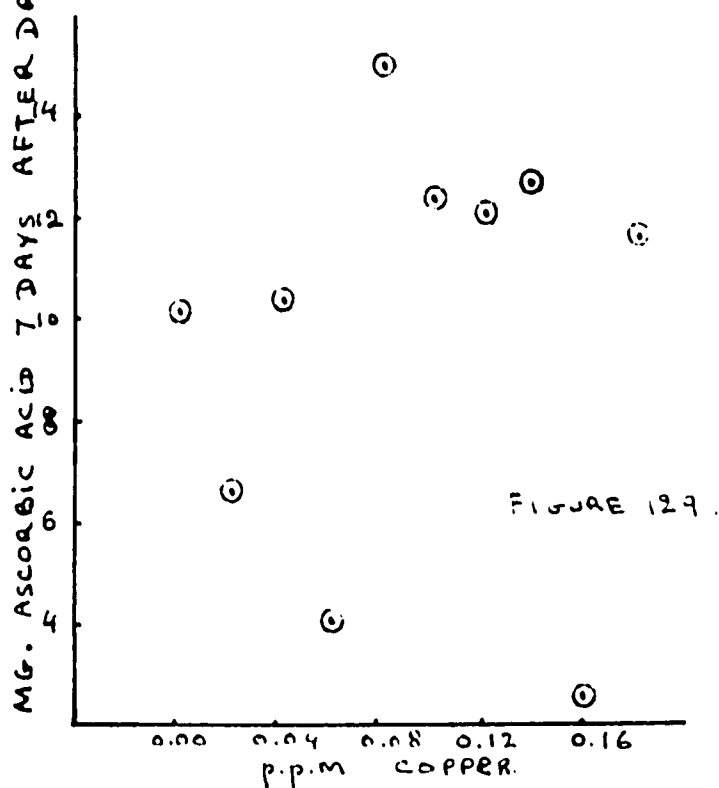


FIGURE 129.

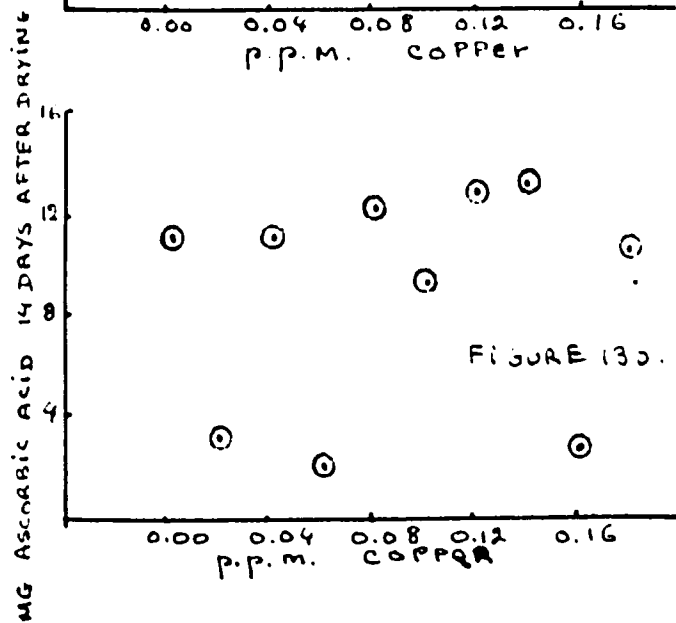


FIGURE 130.

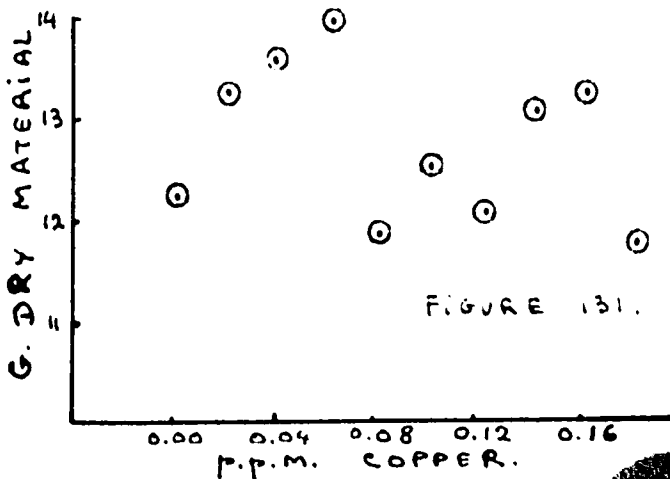
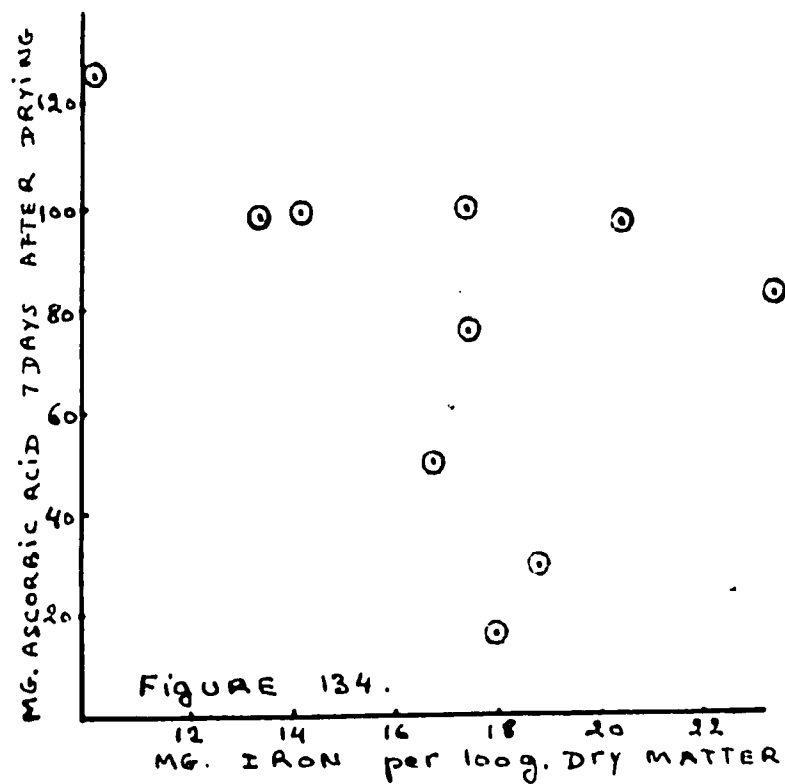
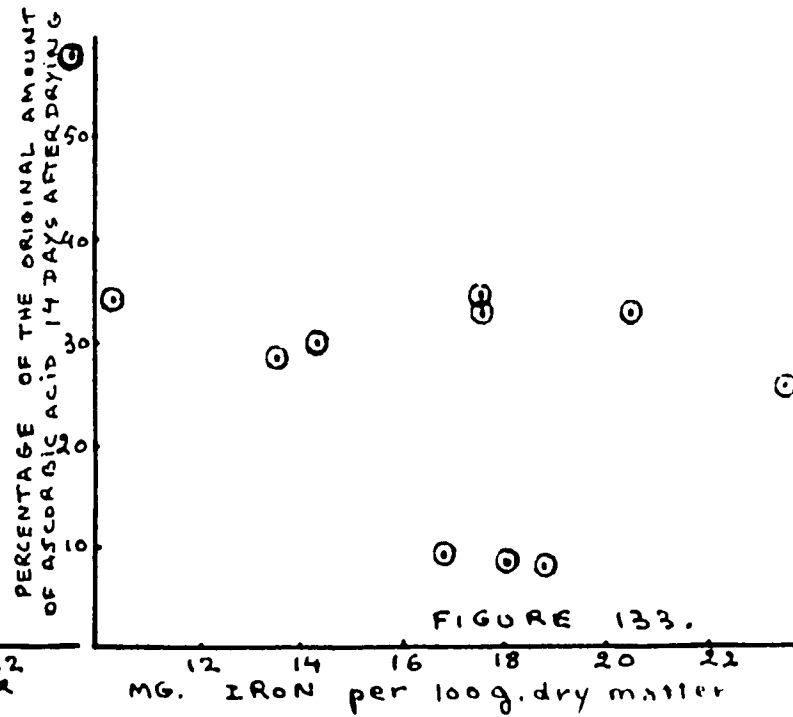
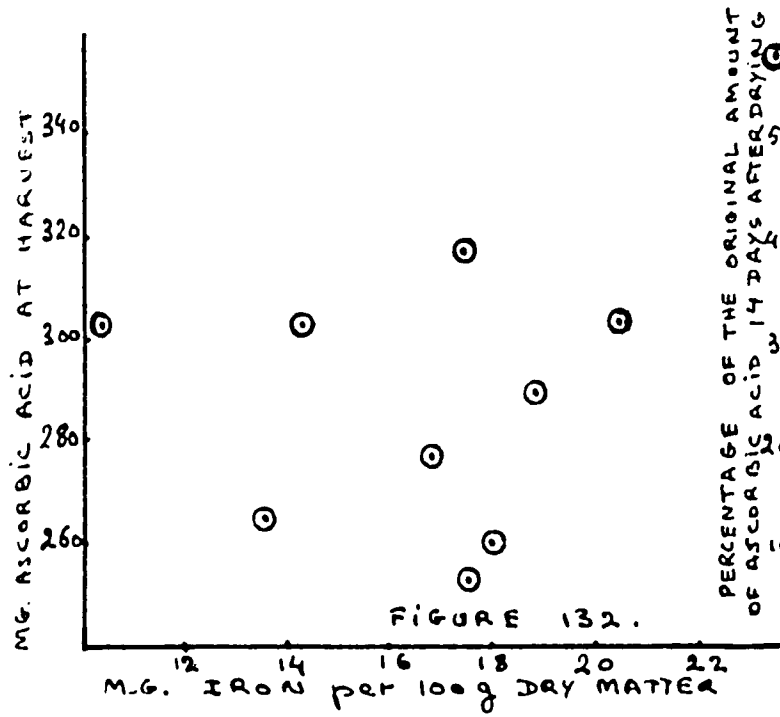


FIGURE 131.

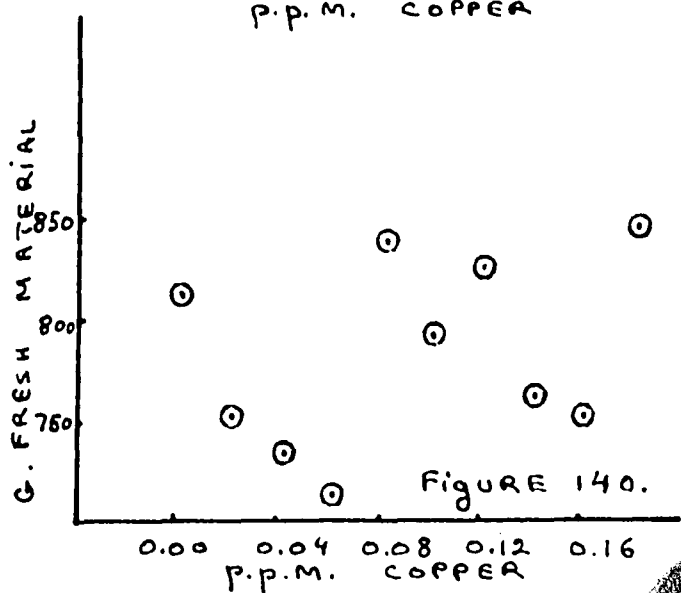
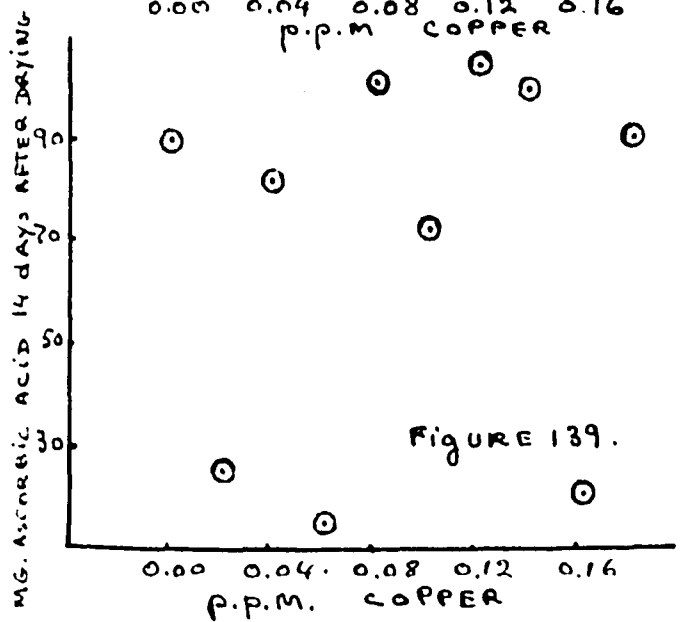
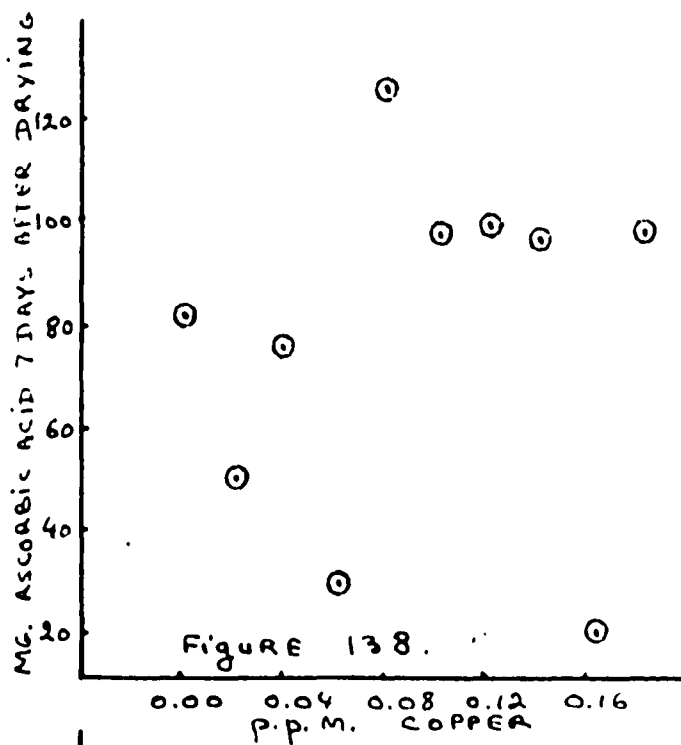
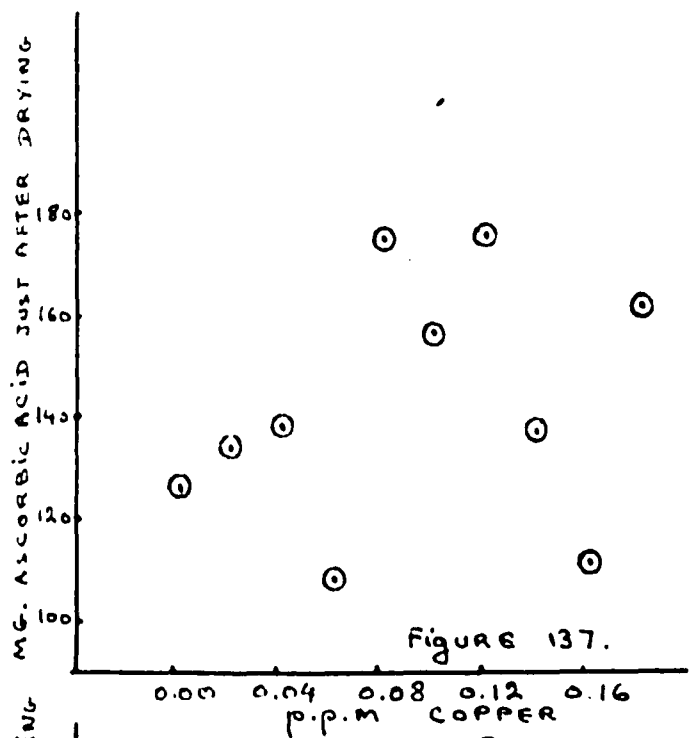
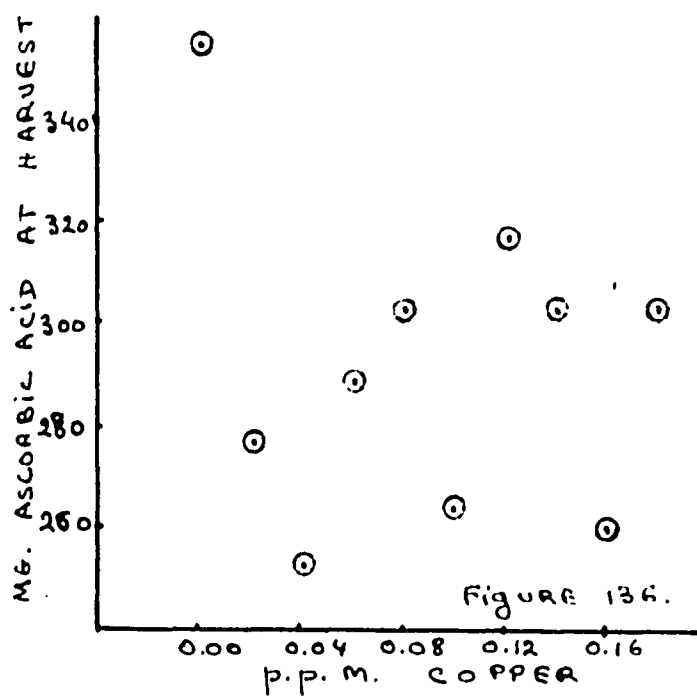
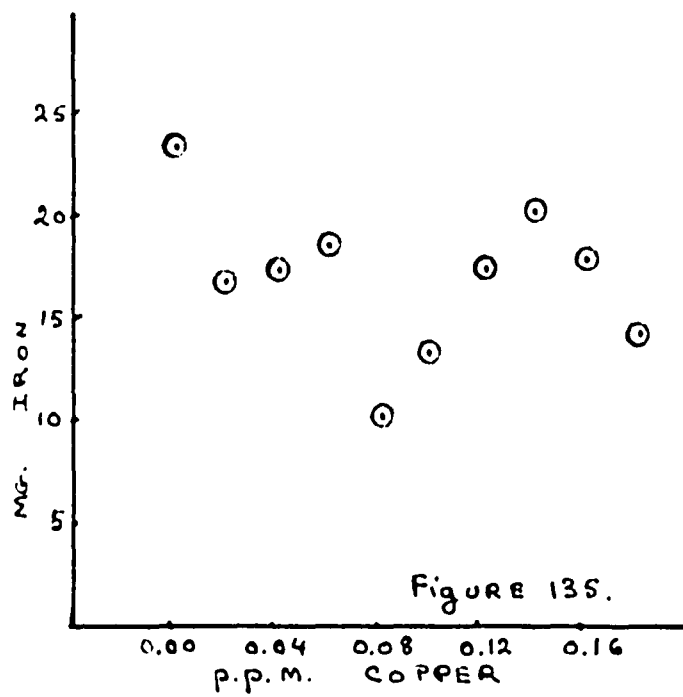
Figure 132. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry matter from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter.

Figure 133. Average percentages of the original amounts of ascorbic acid 14 days after drying, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter from wheat plants grown on nutrient solutions supplied with various amounts of copper.

Figure 134. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of dry matter from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amount of iron, expressed in milligrams per 100 grams of dry matter.



- Figure 135. Average amounts of iron, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 136. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 137. Average amounts of ascorbic acid just after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 138. Average amounts of ascorbic acid 7 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 139. Average amounts of ascorbic acid 14 days after drying, expressed in milligrams per 100 grams of dry material from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.
- Figure 140. Average amounts of fresh material, expressed in grams per 100 grams of dry matter from wheat plants, plotted against the amount of copper in the nutrient solution, expressed in parts per million.



Experiment 3

Table 23. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 10 plants.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>calcium | Milligrams of<br>magnesium | Grams of<br>fresh weight |
|----------------------------------------------------------|----------------------------------------------|--------------------------|----------------------------|--------------------------|
| 0                                                        | 17.5                                         | 14                       | 47                         | 28.1                     |
| 3                                                        | 30.1                                         | 12                       | 64                         | 45.4                     |
| 6                                                        | 26.2                                         | 20                       | 76                         | 46.7                     |
| 9                                                        | 23.0                                         | 12                       | 61                         | 51.3                     |
| 12                                                       | 28.3                                         | 11                       | 59                         | 45.4                     |
| 15                                                       | 29.0                                         | 13                       | 71                         | 48.1                     |
| 18                                                       | 31.9                                         | 15                       | 73                         | 53.0                     |
| 21                                                       | 30.3                                         | 16                       | 63                         | 50.8                     |
| 24                                                       | 31.4                                         | 16                       | 64                         | 54.9                     |
| 27                                                       | 23.2                                         | 18                       | 64                         | 44.5                     |

Table 24. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron ; averages expressed per 10 plants.(continued)

| Parts per million<br>of iron in the<br>nutrient solution | Grams of<br>of dry weight | Milligrams of<br>iron | Milligrams of<br>phosphorus | Milligrams of<br>potassium | Milligrams of<br>nitrogen |
|----------------------------------------------------------|---------------------------|-----------------------|-----------------------------|----------------------------|---------------------------|
| 0                                                        | 2.8                       | 0.41                  | 45                          | 184                        | 126                       |
| 3                                                        | 5.9                       | 0.74                  | 76                          | 405                        | 253                       |
| 6                                                        | 7.8                       | 0.98                  | 105                         | 514                        | 321                       |
| 9                                                        | 6.4                       | 0.83                  | 81                          | 429                        | 268                       |
| 12                                                       | 5.8                       | 0.81                  | 75                          | 392                        | 248                       |
| 15                                                       | 6.3                       | 0.88                  | 81                          | 430                        | 272                       |
| 18                                                       | 7.4                       | 1.04                  | 86                          | 460                        | 287                       |
| 21                                                       | 7.1                       | 0.96                  | 89                          | 451                        | 276                       |
| 24                                                       | 7.1                       | 1.03                  | 92                          | 454                        | 273                       |
| 27                                                       | 6.4                       | 0.80                  | 77                          | 364                        | 234                       |



Experiment 3

Table 25. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 10 plants.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>magnesium | Milligrams of<br>calcium | Grams of<br>fresh weight | Milligrams of<br>copper |
|------------------------------------------------------------|----------------------------------------------|----------------------------|--------------------------|--------------------------|-------------------------|
| 0.0                                                        | 22.0                                         | 61                         | 19                       | 39.7                     | 0.072                   |
| 0.3                                                        | 18.6                                         | 54                         | 10                       | 34.1                     | 0.114                   |
| 0.6                                                        | 20.4                                         | 54                         | 12                       | 41.6                     | 0.143                   |
| 0.9                                                        | 15.9                                         | 47                         | 11                       | 36.4                     | 0.121                   |
| 1.2                                                        | 17.1                                         | 41                         | 11                       | 31.6                     | 0.114                   |
| 1.5                                                        | 14.0                                         | 41                         | 15                       | 30.8                     | 0.095                   |
| 1.8                                                        | 11.5                                         | 31                         | 11                       | 18.7                     | 0.086                   |
| 2.1                                                        | 8.2                                          | 21                         | 11                       | 13.3                     | 0.069                   |
| 2.4                                                        | 6.4                                          | 19                         | 10                       | 9.9                      | 0.055                   |
| 2.7                                                        | 4.9                                          | 16                         | 9                        | 7.7                      | 0.047                   |

Experiment 3

Table 26. Experimental results obtained from wheat plants grown on nutrient solution varying in concentrations of copper; averages expressed per 10 plants. (continued)

| Parts per million<br>of copper in the<br>nutrient solution | Grams of<br>dry weight | Milligrams of<br>iron | Milligrams of<br>phosphorus | Milligrams of<br>potassium | Milligrams of<br>nitrogen |
|------------------------------------------------------------|------------------------|-----------------------|-----------------------------|----------------------------|---------------------------|
| 0.0                                                        | 4.7                    | 0.68                  | 75                          | 351                        | 210                       |
| 0.3                                                        | 4.3                    | 0.58                  | 57                          | 318                        | 189                       |
| 0.6                                                        | 5.4                    | 0.68                  | 57                          | 409                        | 244                       |
| 0.9                                                        | 3.9                    | 0.51                  | 41                          | 325                        | 184                       |
| 1.2                                                        | 3.5                    | 0.42                  | 36                          | 276                        | 169                       |
| 1.5                                                        | 3.6                    | 0.38                  | 39                          | 273                        | 167                       |
| 1.8                                                        | 2.3                    | 0.31                  | 28                          | 173                        | 106                       |
| 2.1                                                        | 1.6                    | 0.19                  | 23                          | 117                        | 73                        |
| 2.4                                                        | 1.3                    | 0.14                  | 22                          | 92                         | 57                        |
| 2.7                                                        | 1.1                    | 0.13                  | 20                          | 77                         | 49                        |

Experiment 3

Table 27. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of fresh plant material.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>phosphorus | Milligrams of<br>potassium | Milligrams of<br>nitrogen | Milligrams of<br>calcium | Milligrams of<br>magnesium |
|----------------------------------------------------------|-----------------------------|----------------------------|---------------------------|--------------------------|----------------------------|
| 0                                                        | 160                         | 657                        | 452                       | 50                       | 169                        |
| 3                                                        | 169                         | 900                        | 561                       | 26                       | 141                        |
| 6                                                        | 227                         | 1107                       | 692                       | 42                       | 165                        |
| 9                                                        | 156                         | 832                        | 520                       | 22                       | 119                        |
| 12                                                       | 165                         | 857                        | 542                       | 24                       | 130                        |
| 15                                                       | 168                         | 893                        | 565                       | 26                       | 148                        |
| 18                                                       | 161                         | 863                        | 539                       | 28                       | 136                        |
| 21                                                       | 175                         | 889                        | 545                       | 31                       | 123                        |
| 24                                                       | 168                         | 832                        | 499                       | 30                       | 117                        |
| 27                                                       | 173                         | 819                        | 526                       | 40                       | 145                        |

Experiment 3

Table 28. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of fresh plant material (continued).

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Grams of<br>dry matter | Milligrams of<br>iron |
|----------------------------------------------------------|----------------------------------------------|------------------------|-----------------------|
| 0                                                        | 62.2                                         | 10.0                   | 1.45                  |
| 3                                                        | 66.4                                         | 13.1                   | 1.64                  |
| 6                                                        | 56.1                                         | 16.8                   | 2.10                  |
| 9                                                        | 54.1                                         | 12.4                   | 1.61                  |
| 12                                                       | 62.4                                         | 12.7                   | 1.78                  |
| 15                                                       | 60.3                                         | 13.1                   | 1.83                  |
| 18                                                       | 60.2                                         | 13.9                   | 1.95                  |
| 21                                                       | 59.7                                         | 14.0                   | 1.89                  |
| 24                                                       | 57.2                                         | 13.0                   | 1.89                  |
| 27                                                       | 52.1                                         | 14.4                   | 1.80                  |

Experiment 3

Table 29. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 100 grams of fresh material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>phosphorus | Milligrams of<br>potassium | Milligrams of<br>nitrogen | Milligrams of<br>calcium | Milligrams of<br>magnesium |
|------------------------------------------------------------|-----------------------------|----------------------------|---------------------------|--------------------------|----------------------------|
| 0.0                                                        | 189                         | 889                        | 532                       | 48                       | 154                        |
| 0.3                                                        | 166                         | 925                        | 550                       | 30                       | 158                        |
| 0.6                                                        | 137                         | 978                        | 583                       | 30                       | 129                        |
| 0.9                                                        | 111                         | 884                        | 500                       | 30                       | 127                        |
| 1.2                                                        | 114                         | 874                        | 536                       | 33                       | 130                        |
| 1.5                                                        | 124                         | 880                        | 538                       | 50                       | 132                        |
| 1.8                                                        | 154                         | 939                        | 578                       | 59                       | 169                        |
| 2.1                                                        | 172                         | 861                        | 540                       | 84                       | 155                        |
| 2.4                                                        | 216                         | 929                        | 578                       | 98                       | 193                        |
| 2.7                                                        | 259                         | 1007                       | 638                       | 111                      | 215                        |

Experiment 3

Table 30. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of copper; averages expressed per 100 grams of fresh plant material. (continued).

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>ascorbic acid<br>at harvest | Grams of<br>dry matter | Milligrams of<br>iron | Milligrams of<br>copper |
|------------------------------------------------------------|----------------------------------------------|------------------------|-----------------------|-------------------------|
| 0.0                                                        | 55.5                                         | 11.9                   | 1.73                  | 0.18                    |
| 0.3                                                        | 55.1                                         | 12.5                   | 1.69                  | 0.33                    |
| 0.6                                                        | 49.0                                         | 12.9                   | 1.61                  | 0.34                    |
| 0.9                                                        | 43.7                                         | 10.6                   | 1.38                  | 0.33                    |
| 1.2                                                        | 54.2                                         | 11.1                   | 1.33                  | 0.36                    |
| 1.5                                                        | 45.6                                         | 11.6                   | 1.22                  | 0.31                    |
| 1.8                                                        | 61.5                                         | 12.5                   | 1.69                  | 0.47                    |
| 2.1                                                        | 61.5                                         | 11.8                   | 1.42                  | 0.51                    |
| 2.4                                                        | 64.4                                         | 13.1                   | 1.38                  | 0.56                    |
| 2.7                                                        | 63.8                                         | 14.4                   | 1.73                  | 0.61                    |

Experiment 3

Table 31. Experimental results obtained from wheat plants grown on nutrient solutions varying in the concentrations of iron; averages expressed per 100 grams of dry plant material.

| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>iron | Grams of<br>phosphorus | Grams of<br>Potassium | Grams of<br>nitrogen | Grams of<br>calcium | Grams of<br>magnesium |
|----------------------------------------------------------|-----------------------|------------------------|-----------------------|----------------------|---------------------|-----------------------|
| 0                                                        | 14.5                  | 1.60                   | 6.57                  | 4.52                 | 0.50                | 1.69                  |
| 3                                                        | 12.5                  | 1.29                   | 6.87                  | 4.28                 | 0.20                | 1.08                  |
| 6                                                        | 12.5                  | 1.35                   | 6.59                  | 4.12                 | 0.25                | 0.98                  |
| 9                                                        | 13.0                  | 1.26                   | 6.71                  | 4.19                 | 0.18                | 0.96                  |
| 12                                                       | 14.0                  | 1.30                   | 6.75                  | 4.27                 | 0.19                | 1.02                  |
| 15                                                       | 14.0                  | 1.28                   | 6.82                  | 4.31                 | 0.20                | 1.13                  |
| 18                                                       | 14.0                  | 1.16                   | 6.21                  | 3.88                 | 0.20                | 0.98                  |
| 21                                                       | 13.5                  | 1.25                   | 6.35                  | 3.89                 | 0.22                | 0.88                  |
| 24                                                       | 14.5                  | 1.29                   | 6.40                  | 3.84                 | 0.23                | 0.90                  |
| 27                                                       | 12.5                  | 1.20                   | 5.69                  | 3.65                 | 0.28                | 1.01                  |

Experiment 3.

Table 32. Experimental results obtained from wheat plants grown on nutrient solutions varying in the concentrations of copper; averages expressed per 100 grams of dry plant material.

| Parts per million<br>of copper in the<br>nutrient solution | Milligrams of<br>iron | Grams of<br>phosphorus | Grams of<br>potassium | Grams of<br>nitrogen | Grams of<br>calcium | Grams of<br>magnesium | Milligrams of<br>copper |
|------------------------------------------------------------|-----------------------|------------------------|-----------------------|----------------------|---------------------|-----------------------|-------------------------|
| 0.0                                                        | 14.5                  | 1.59                   | 7.47                  | 4.47                 | 0.40                | 1.29                  | 1.53                    |
| 0.3                                                        | 13.5                  | 1.33                   | 7.40                  | 4.40                 | 0.24                | 1.26                  | 2.64                    |
| 0.6                                                        | 12.5                  | 1.06                   | 7.58                  | 4.52                 | 0.23                | 1.00                  | 2.64                    |
| 0.9                                                        | 13.0                  | 1.05                   | 8.34                  | 4.72                 | 0.28                | 1.20                  | 3.10                    |
| 1.2                                                        | 12.0                  | 1.03                   | 7.87                  | 4.83                 | 0.30                | 1.17                  | 3.25                    |
| 1.5                                                        | 10.5                  | 1.07                   | 7.59                  | 4.64                 | 0.43                | 1.14                  | 2.64                    |
| 1.8                                                        | 13.5                  | 1.23                   | 7.51                  | 4.62                 | 0.47                | 1.35                  | 3.74                    |
| 2.1                                                        | 12.0                  | 1.46                   | 7.30                  | 4.58                 | 0.71                | 1.31                  | 4.30                    |
| 2.4                                                        | 10.5                  | 1.65                   | 7.09                  | 4.41                 | 0.75                | 1.47                  | 4.24                    |
| 2.7                                                        | 12.0                  | 1.80                   | 6.99                  | 4.43                 | 0.77                | 1.49                  | 4.23                    |



Experiment 3

Table 33. Percentages of the original amounts of ascorbic acid found in wheat plants grown on nutrient solutions varying in the concentrations of iron, at each determination during the drying of the plants at room temperature.

| Parts per million<br>of iron in the<br>nutrient solution | Percentages of<br>ascorbic acid<br>at harvest | Percentages of<br>ascorbic acid<br>14 hours after<br>harvest | Percentages of<br>ascorbic acid<br>43 hours after<br>harvest | Percentages of<br>ascorbic acid<br>62 hours after<br>harvest | Percentages of<br>ascorbic acid<br>110 hours<br>after harvest | Percentages of<br>ascorbic acid<br>157 hours after<br>harvest |
|----------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| 0                                                        | 100                                           | 58.5                                                         | 35.5                                                         | 21.7                                                         | 9.3                                                           | 5.6                                                           |
| 3                                                        | 100                                           | 46.4                                                         | 26.9                                                         | 17.2                                                         | 8.3                                                           | 2.4                                                           |
| 6                                                        | 100                                           | 76.2                                                         | 44.9                                                         | 31.1                                                         | 13.0                                                          | 6.6                                                           |
| 9                                                        | 100                                           | 65.0                                                         | 41.4                                                         | 28.7                                                         | 10.4                                                          | 5.5                                                           |
| 12                                                       | 100                                           | 65.5                                                         | 36.1                                                         | 25.5                                                         | 9.5                                                           | 4.2                                                           |
| 15                                                       | 100                                           | 72.5                                                         | 36.0                                                         | 25.9                                                         | 7.8                                                           | 4.8                                                           |
| 18                                                       | 100                                           | 77.6                                                         | 41.7                                                         | 27.4                                                         | 11.1                                                          | 5.8                                                           |
| 21                                                       | 100                                           | 72.5                                                         | ----                                                         | 27.0                                                         | 8.7                                                           | 5.0                                                           |
| 24                                                       | 100                                           | 78.0                                                         | 34.8                                                         | 22.7                                                         | 9.3                                                           | 4.9                                                           |
| 27                                                       | 100                                           | 62.6                                                         | 39.2                                                         | 22.1                                                         | 8.3                                                           | 5.6                                                           |

Experiment 3

Table 34. Percentages of the original amounts of ascorbic acid found in wheat plants grown on nutrient solutions varying in the concentrations of copper, at each determination during the drying of the plants at room temperature.

| Parts per million<br>of copper in the<br>nutrient solution | Percentages of<br>ascorbic acid<br>at harvest | Percentages of<br>ascorbic acid<br>13 hours after<br>harvest | Percentages of<br>ascorbic acid<br>30 hours after<br>harvest | Percentages of<br>ascorbic acid<br>52 hours after<br>harvest | Percentages of<br>ascorbic acid<br>100 hours after<br>harvest | Percentages of<br>ascorbic acid<br>147 hours<br>after harvest |
|------------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| 0.0                                                        | 100                                           | 56.6                                                         | 41.3                                                         | 25.9                                                         | 13.0                                                          | 5.8                                                           |
| 0.3                                                        | 100                                           | 59.0                                                         | 44.6                                                         | 27.6                                                         | 14.5                                                          | 5.4                                                           |
| 0.6                                                        | 100                                           | 69.0                                                         | 47.1                                                         | 28.4                                                         | 13.1                                                          | 6.3                                                           |
| 0.9                                                        | 100                                           | 75.3                                                         | 38.9                                                         | 28.6                                                         | 14.4                                                          | 6.4                                                           |
| 1.2                                                        | 100                                           | 60.2                                                         | 42.6                                                         | 22.8                                                         | 10.2                                                          | 5.7                                                           |
| 1.5                                                        | 100                                           | 62.7                                                         | 45.0                                                         | 28.1                                                         | 11.0                                                          | 6.6                                                           |
| 1.8                                                        | 100                                           | 77.2                                                         | 32.8                                                         | 22.0                                                         | ----                                                          | ---                                                           |
| 2.1                                                        | 100                                           | 85.5                                                         | 36.1                                                         | ----                                                         | ----                                                          | ---                                                           |
| 2.4                                                        | 100                                           | 79.2                                                         | 37.7                                                         | ----                                                         | ----                                                          | ---                                                           |
| 2.7                                                        | 100                                           | 89.3                                                         | 39.7                                                         | ----                                                         | ----                                                          | ---                                                           |

Table 35. Percentages of the original amounts of water found in the wheat plants grown on nutrient solutions varying in the concentrations of iron, at each determination during the drying of the plants at room temperature.

| Parts per million<br>of iron in the<br>nutrient solution | Percentages of<br>water at<br>harvest | Percentages of<br>water 14 hours<br>after harvest | Percentages of<br>water 43 hours<br>after harvest | Percentages of<br>water 62 hours<br>after harvest | Percentages of<br>water 110 hours<br>after harvest | Percentages of<br>water 157 hours<br>after harvest |
|----------------------------------------------------------|---------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| 0                                                        | 100                                   | 75.8                                              | 41.6                                              | 33.7                                              | 15.4                                               | 9.3                                                |
| 3                                                        | 100                                   | 77.0                                              | 46.1                                              | 34.1                                              | 16.5                                               | 10.0                                               |
| 6                                                        | 100                                   | 79.3                                              | 52.4                                              | 42.3                                              | 23.5                                               | 13.1                                               |
| 9                                                        | 100                                   | 78.3                                              | 51.4                                              | 41.7                                              | 20.9                                               | 11.9                                               |
| 12                                                       | 100                                   | 80.4                                              | 53.5                                              | 42.6                                              | 23.8                                               | 16.4                                               |
| 15                                                       | 100                                   | 77.7                                              | 49.8                                              | 38.4                                              | 19.1                                               | 11.5                                               |
| 18                                                       | 100                                   | 79.2                                              | 51.2                                              | 39.6                                              | 19.7                                               | 11.8                                               |
| 21                                                       | 100                                   | 77.6                                              | 51.4                                              | 39.0                                              | 19.0                                               | 11.7                                               |
| 24                                                       | 100                                   | 77.0                                              | 49.0                                              | 37.9                                              | 18.0                                               | 10.4                                               |
| 27                                                       | 100                                   | 73.0                                              | 43.5                                              | 33.8                                              | 14.8                                               | 8.9                                                |

Experiment 3.

Table 36. Percentages of the original amounts of water found in the wheat plants grown on nutrient solutions varying in concentrations of copper, at each determination during the drying of the plants at room temperature.

| Parts per million<br>of copper in the<br>nutrient solution | Percentages of<br>water at<br>harvest | Percentages of<br>water 13 hours<br>after harvest | Percentages of<br>water 30 hours<br>after harvest | Percentages of<br>water 52 hours<br>after harvest | Percentages of<br>water 100 hours<br>after harvest | Percentages of<br>water 147 hours<br>after harvest |
|------------------------------------------------------------|---------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| 0.0                                                        | 100                                   | 78.4                                              | 52.2                                              | 39.5                                              | 21.9                                               | 13.4                                               |
| 0.3                                                        | 100                                   | 78.6                                              | 51.3                                              | 39.2                                              | 21.2                                               | 12.6                                               |
| 0.6                                                        | 100                                   | 78.7                                              | 47.2                                              | 35.6                                              | 18.4                                               | 11.5                                               |
| 0.9                                                        | 100                                   | 79.6                                              | 48.1                                              | 35.5                                              | 18.3                                               | 10.1                                               |
| 1.2                                                        | 100                                   | 79.1                                              | 45.4                                              | 33.0                                              | 16.9                                               | 10.6                                               |
| 1.5                                                        | 100                                   | 77.4                                              | 42.8                                              | 30.5                                              | 15.0                                               | 8.7                                                |
| 1.8                                                        | 100                                   | 73.7                                              | 32.9                                              | 21.2                                              | 8.7                                                | 5.3                                                |
| 2.1                                                        | 100                                   | 73.5                                              | 36.8                                              | 25.2                                              | 10.0                                               | 6.4                                                |
| 2.4                                                        | 100                                   | 70.5                                              | 35.1                                              | 21.9                                              | 7.4                                                | 2.8                                                |
| 2.7                                                        | 100                                   | 68.7                                              | 31.8                                              | 19.9                                              | 5.7                                                | 1.9                                                |

Figure 143. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 144. Average dry weight, expressed in grams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 145. Average amounts of iron, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 146. Average amounts of phosphorus, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 147. Average amounts of potassium, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million .

Figure 148. Average amounts of total nitrogen, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

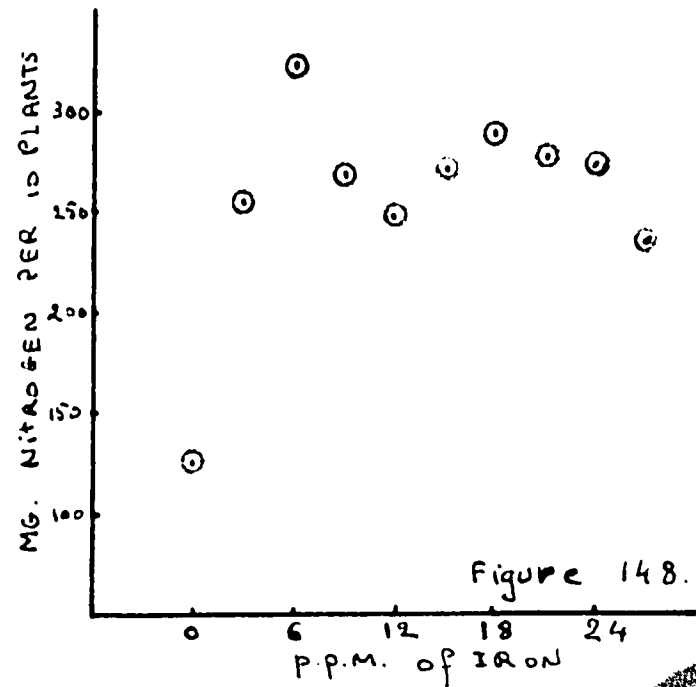
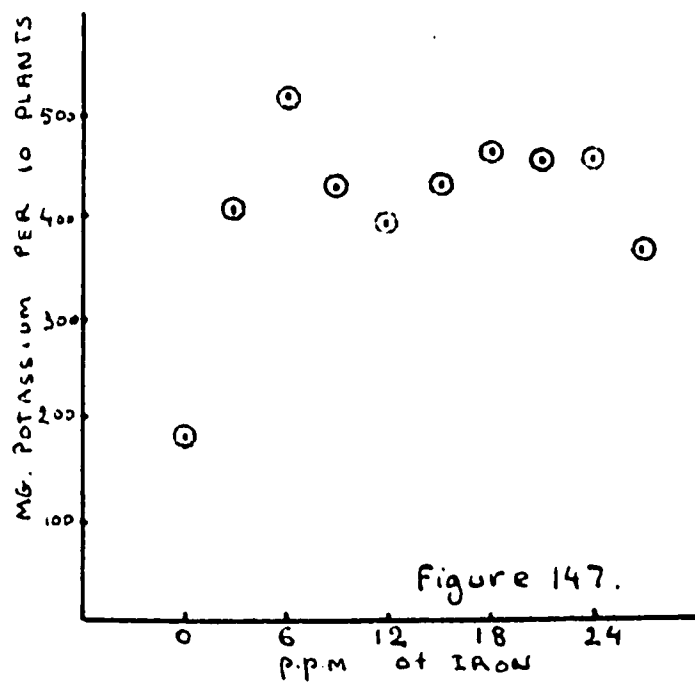
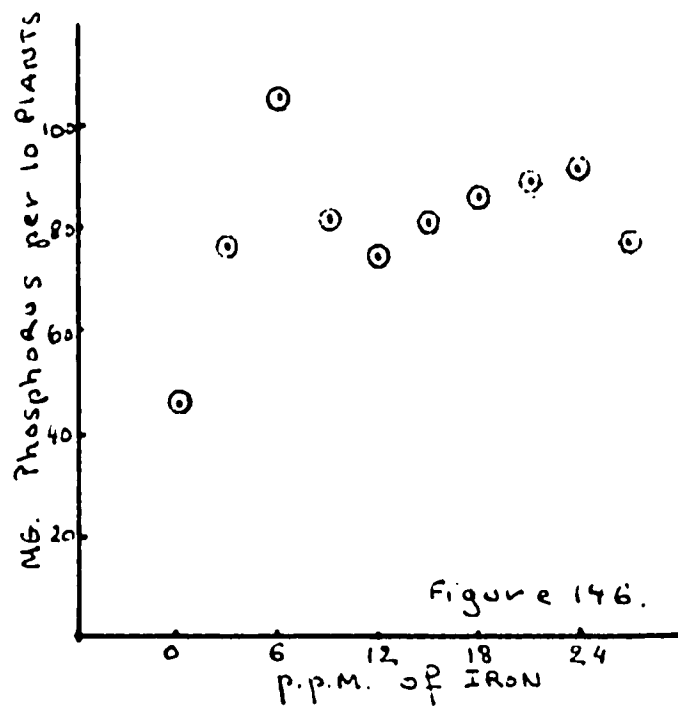
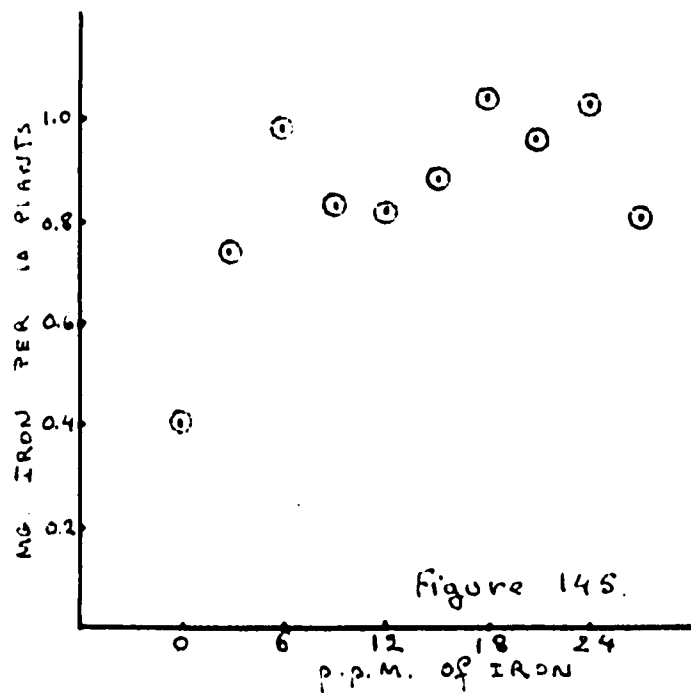
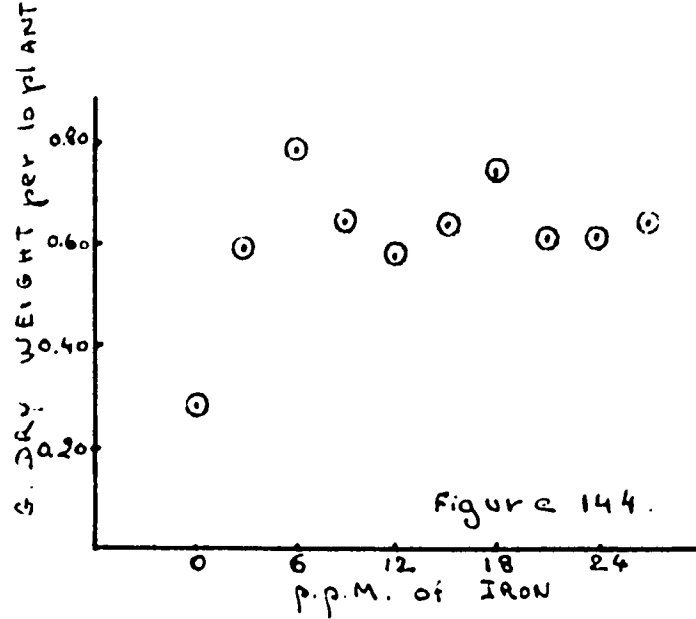
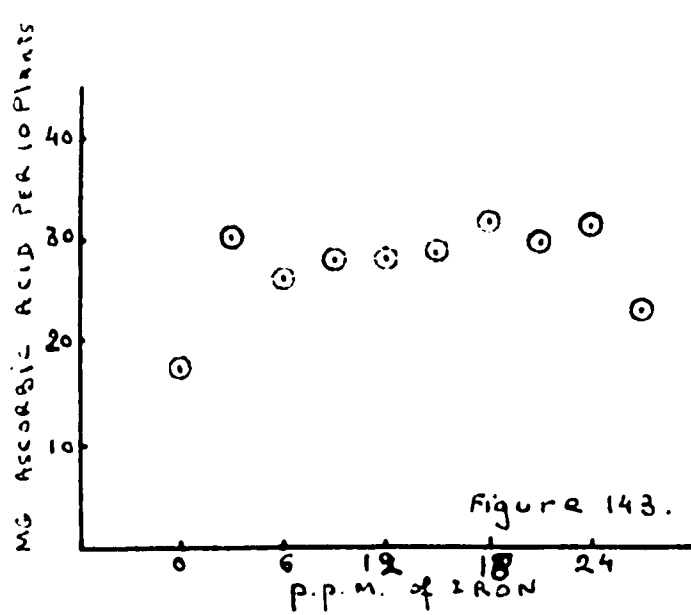


Figure 143. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 149. Average amounts of calcium, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 150. Average amounts of magnesium, expressed in milligrams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 151. Average fresh weight, expressed in grams per 10 wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

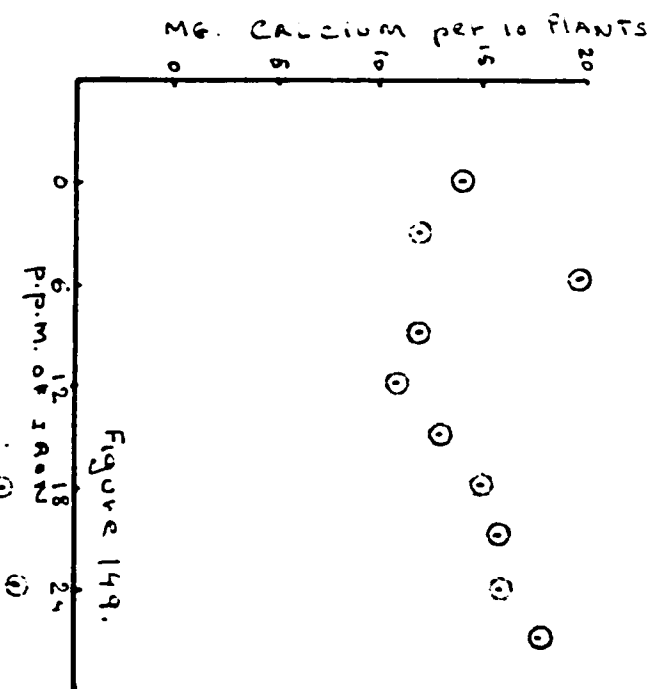
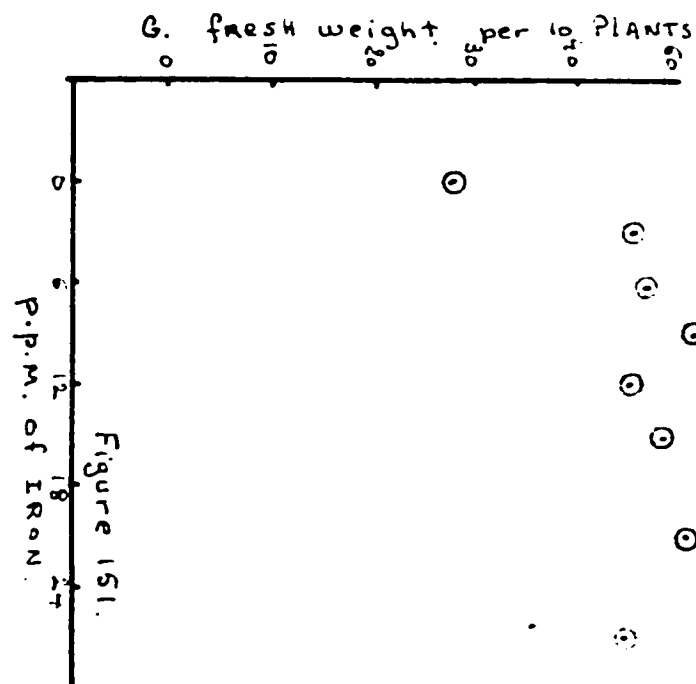
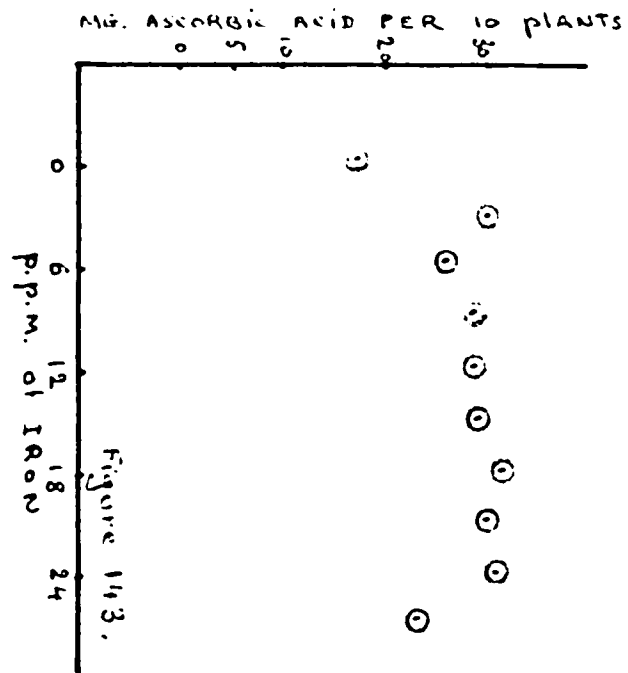
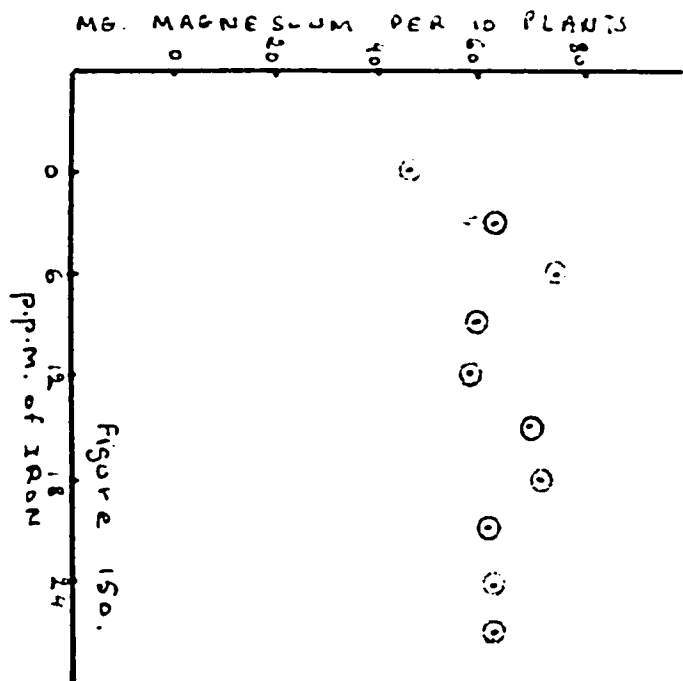




Figure 152. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 153. Average amounts of phosphorus, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 154. Average amounts of potassium, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 155. Average amounts of total nitrogen, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 156. Average amounts of calcium, expressed in milligrams per 100 grams of fresh matter from wheat plants plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 157. Average amounts of magnesium, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

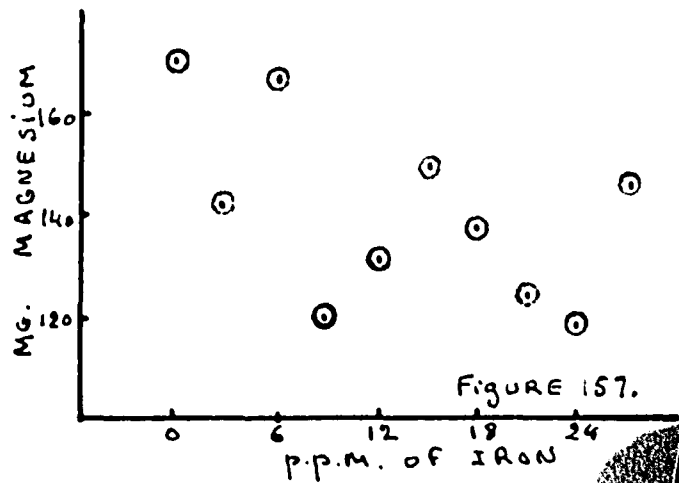
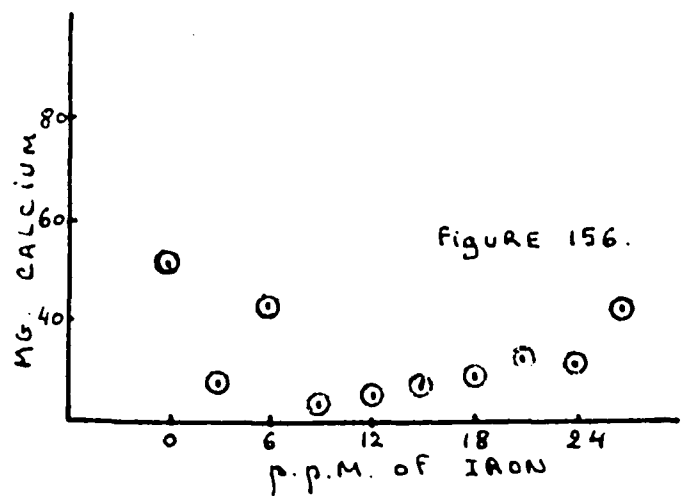
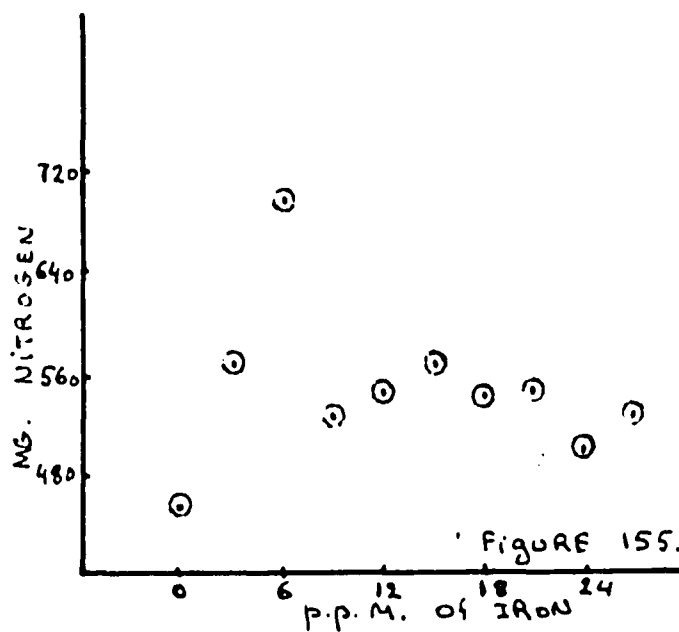
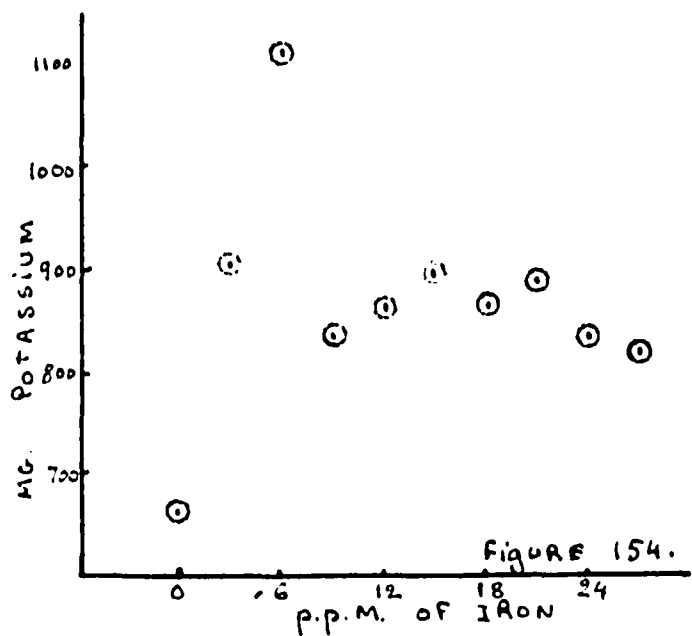
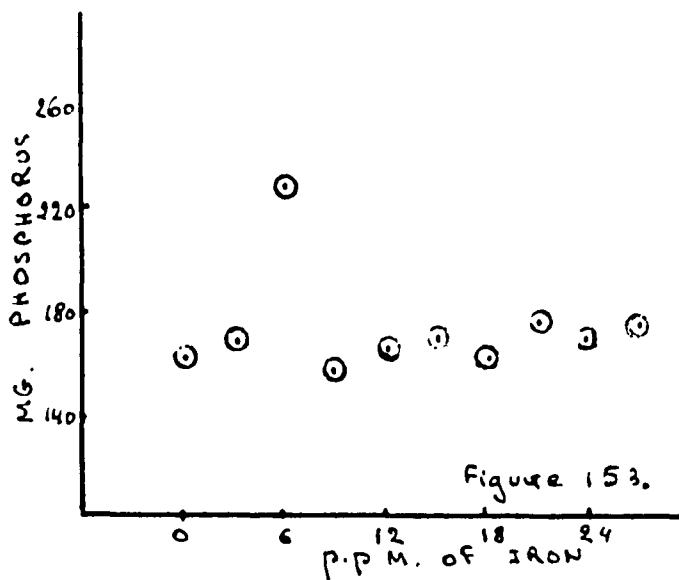
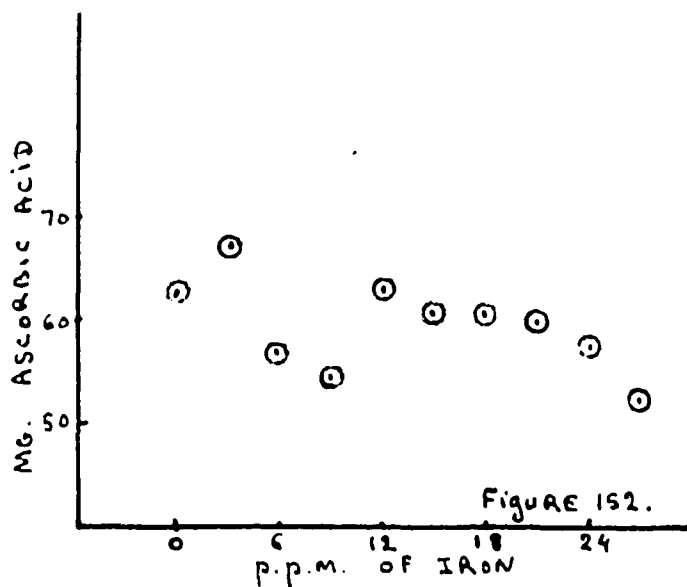
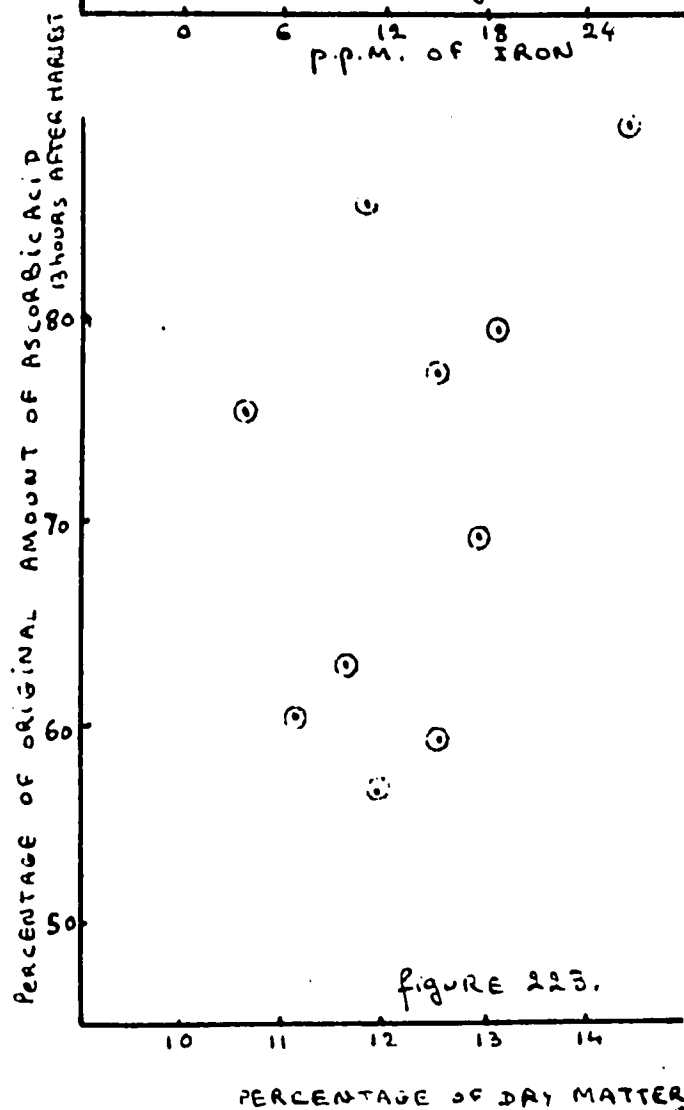
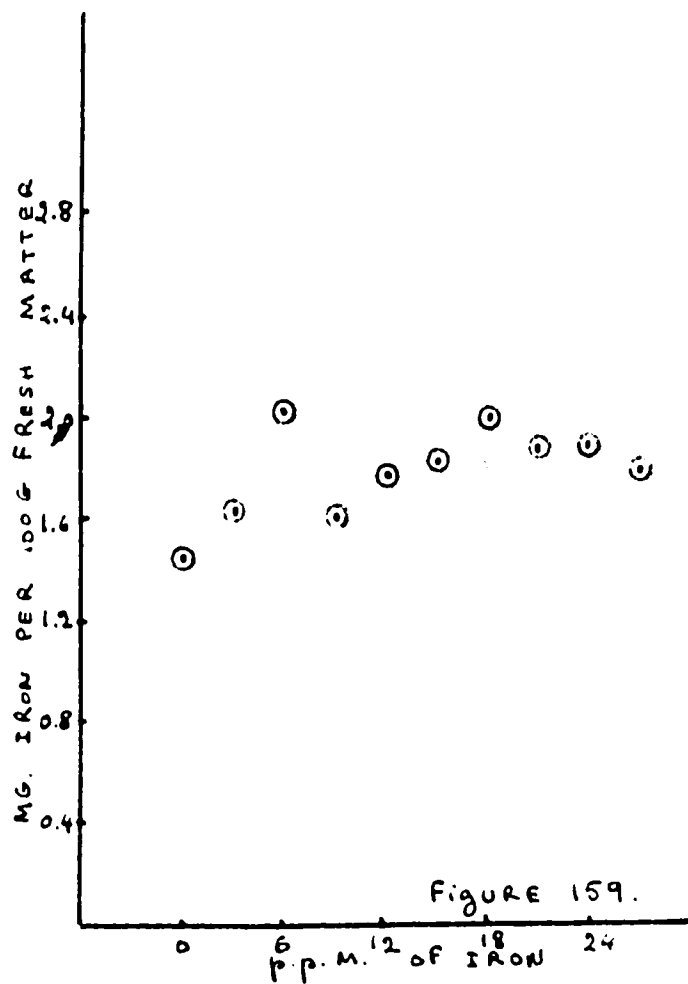
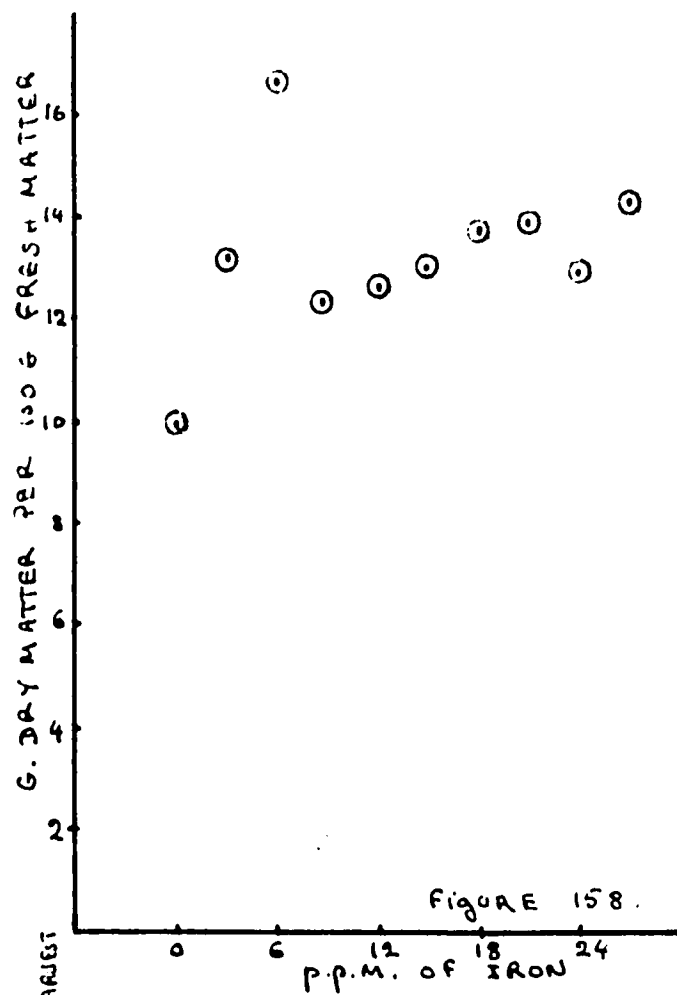
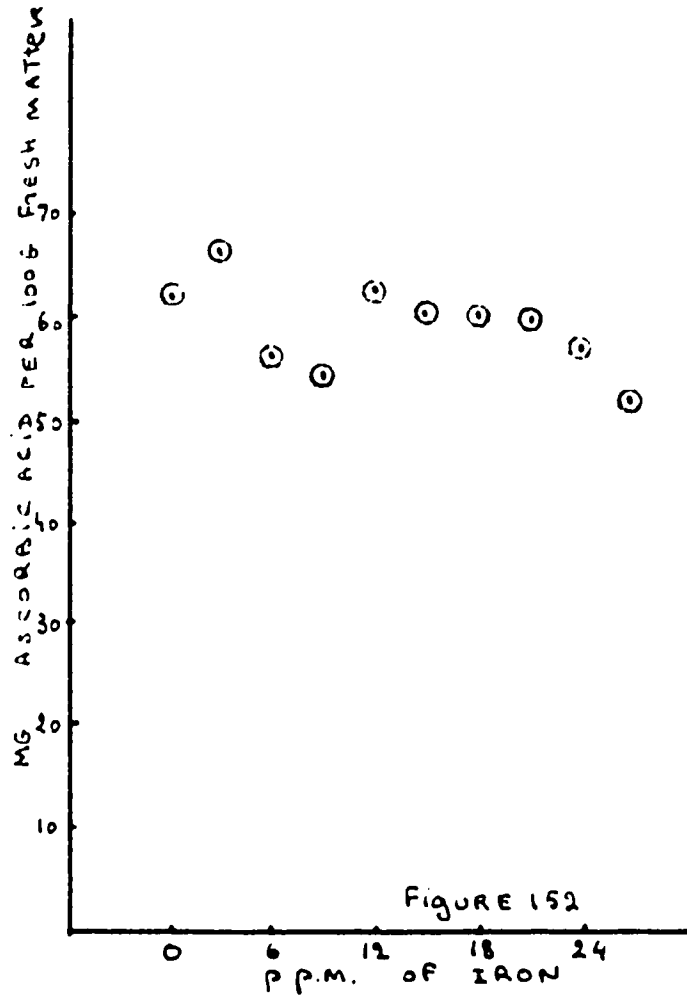


Figure 152. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 158. Average amounts of dry matter, expressed in grams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 159. Average amounts of iron, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 223. Percentage of the original amounts of ascorbic acid, 13 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various concentration of copper, plotted against the amount of dry matter, expressed in grams per hundred grams of fresh matter.



- Figure 152. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 160. Average amounts of ascorbic acid, 14 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 161. Average amounts of ascorbic acid, 43 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 162. Average amounts of ascorbic acid, 62 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 163. Average amounts of ascorbic acid, 110 hours after harvest, expressed in milligrams per hundred grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 164. Average amounts of ascorbic acid, 157 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

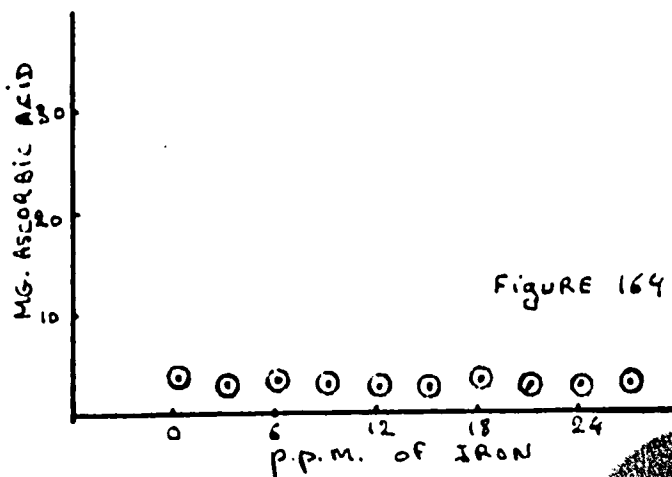
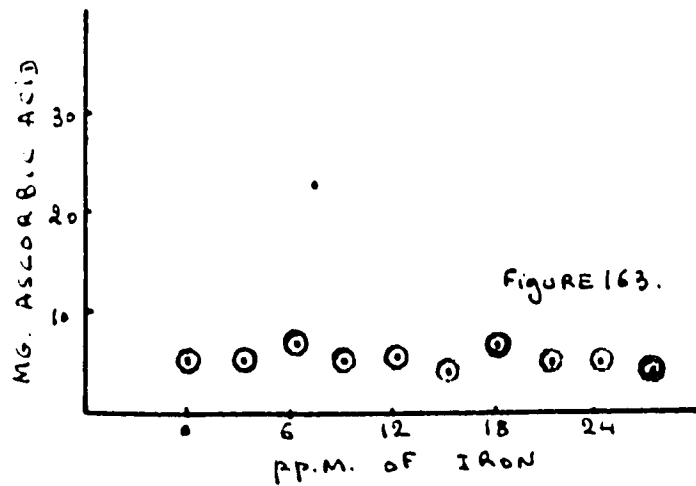
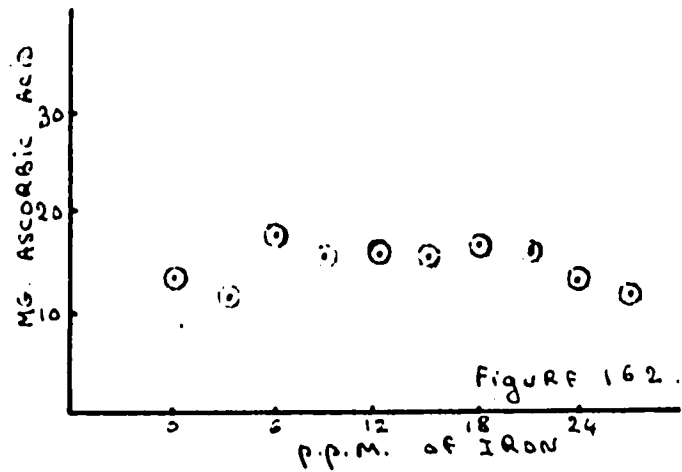
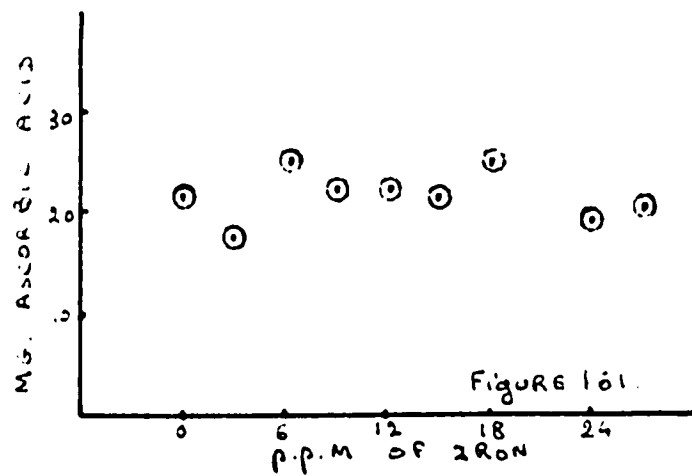
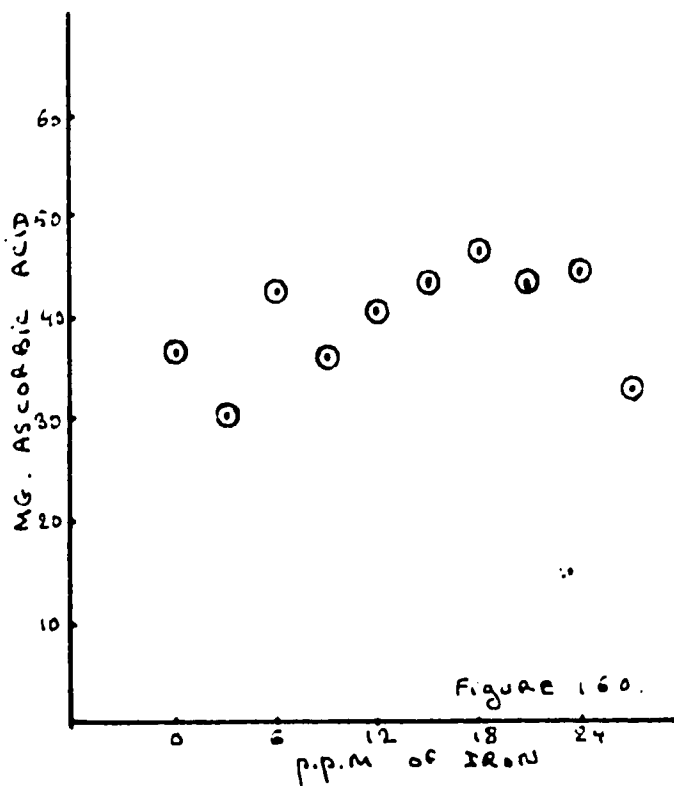
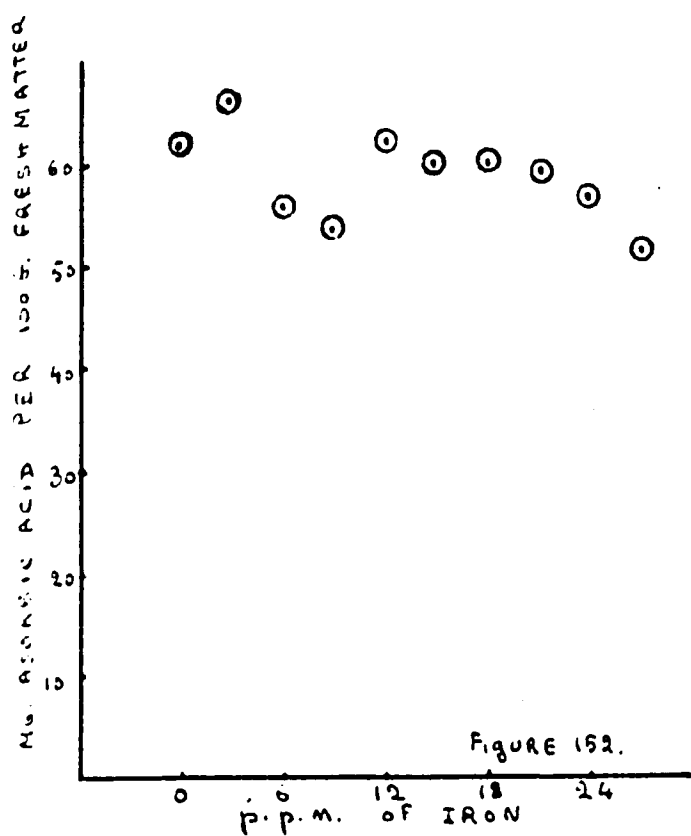


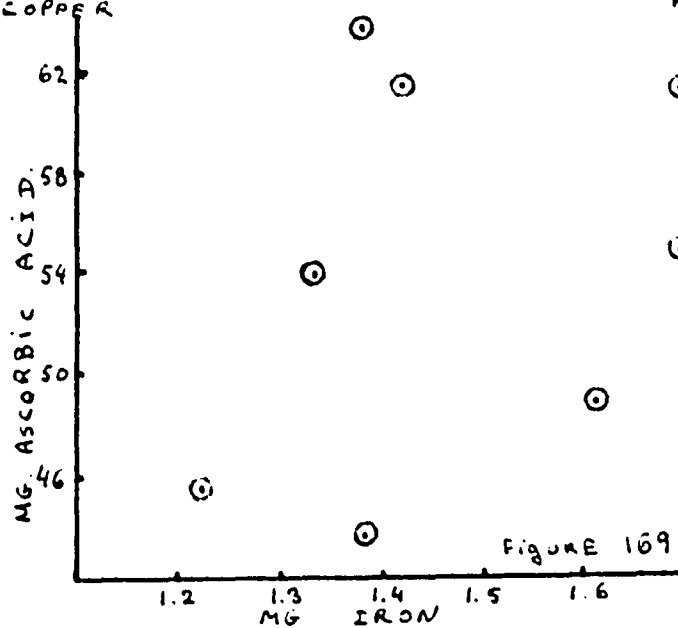
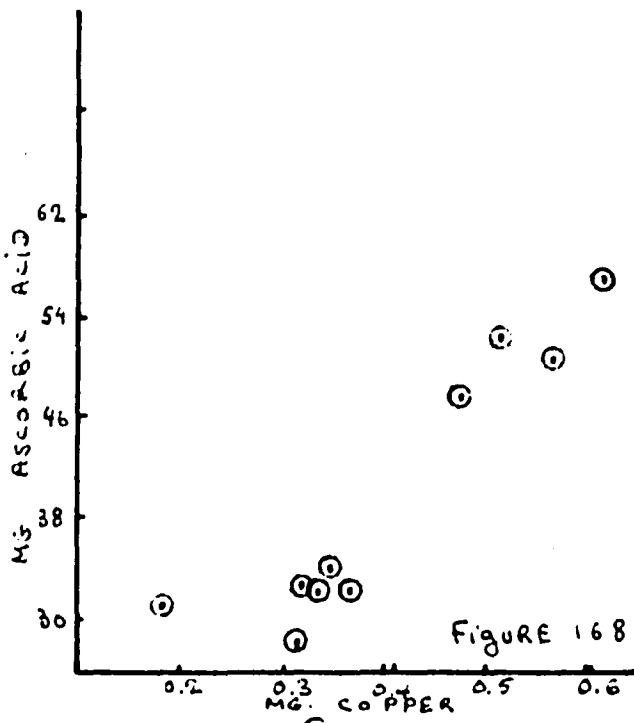
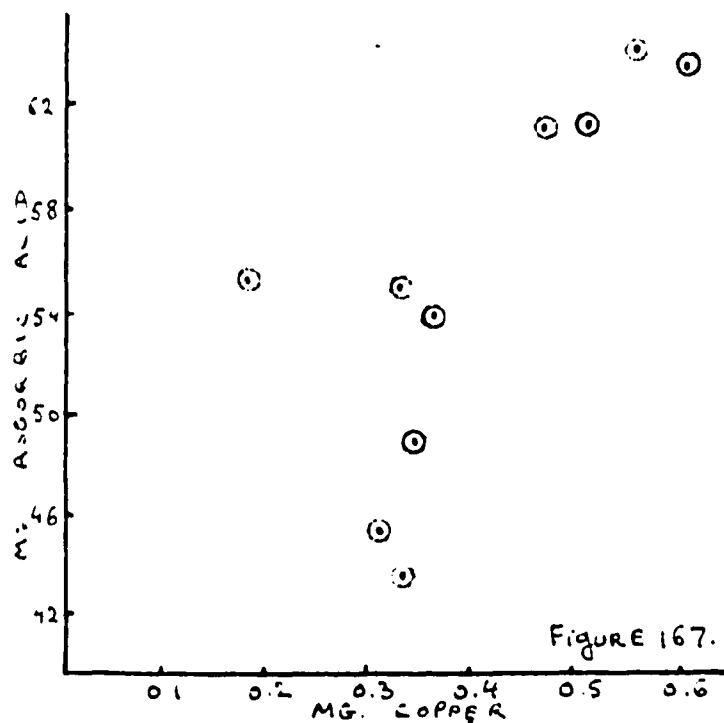
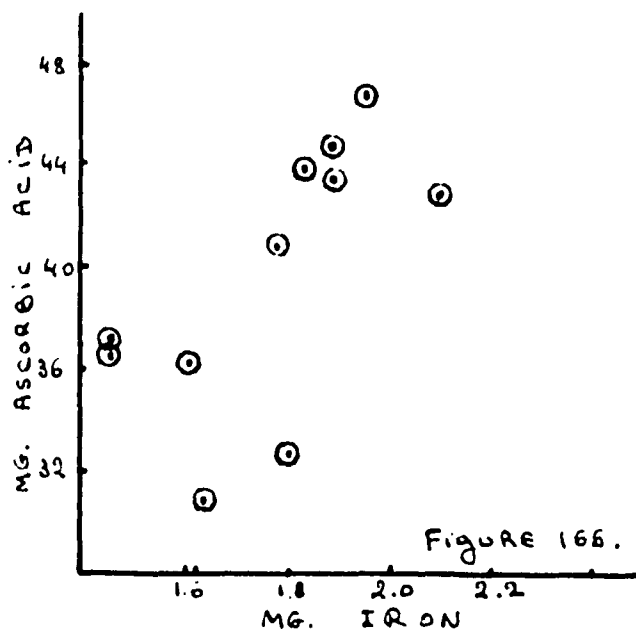
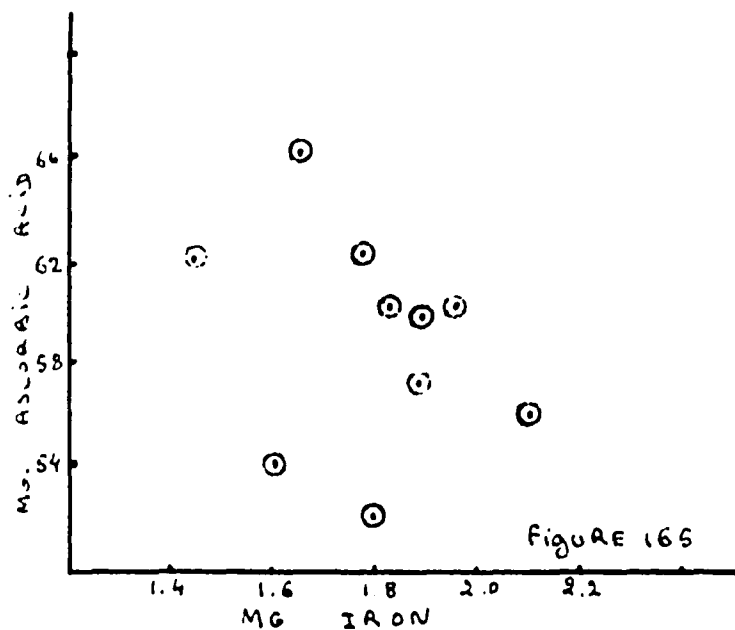
Figure 165. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 166. Average amounts of ascorbic acid, 14 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solution supplied with various concentrations of iron, plotted against the concentration of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 167. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various concentrations of copper, plotted against the amounts of copper, expressed in milligrams per 100 grams of fresh matter.

Figure 168. Average amounts of ascorbic acid, 13 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solution supplied with various concentrations of copper, plotted against the amounts of copper, expressed in milligrams per 100 grams of fresh matter.

Figure 169. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solution supplied with various concentrations of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.





- Figure 170. Average amounts of iron, expressed in milligrams per 100 grams of oven dry tissue from wheat plants grown nutrient solutions supplied with various concentrations of iron, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 171. Average amounts of phosphorus, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 172. Average amounts of potassium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 173. Average amounts of nitrogen, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 174. Average amounts of calcium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.
- Figure 175. Average amounts of magnesium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

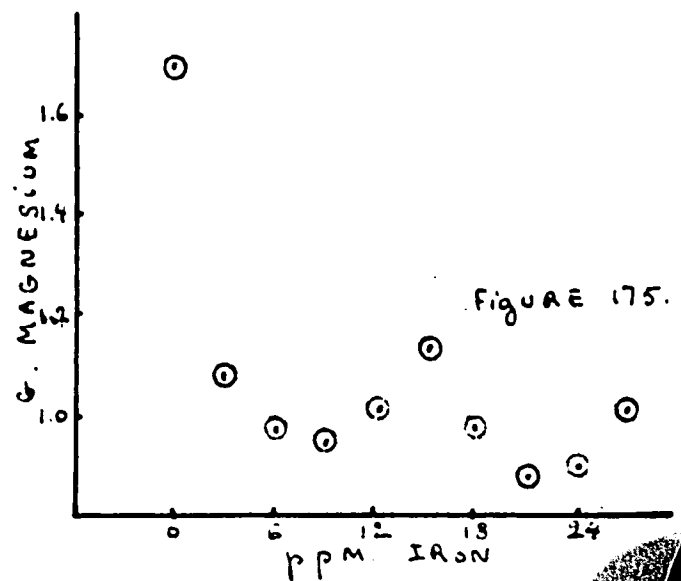
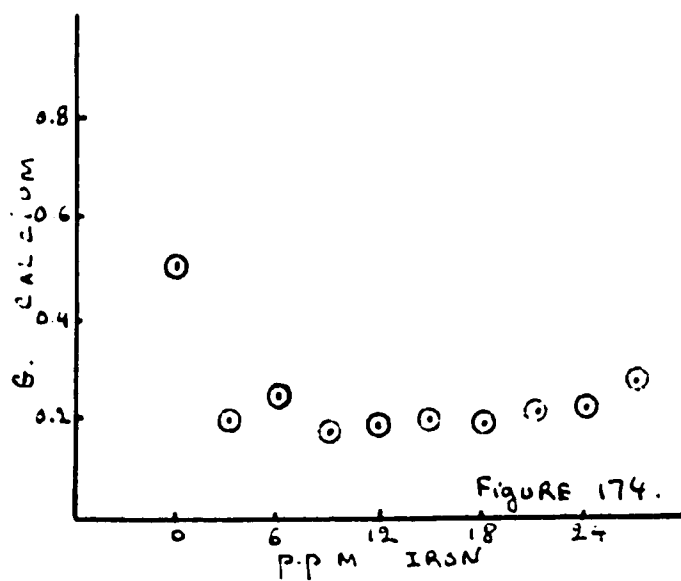
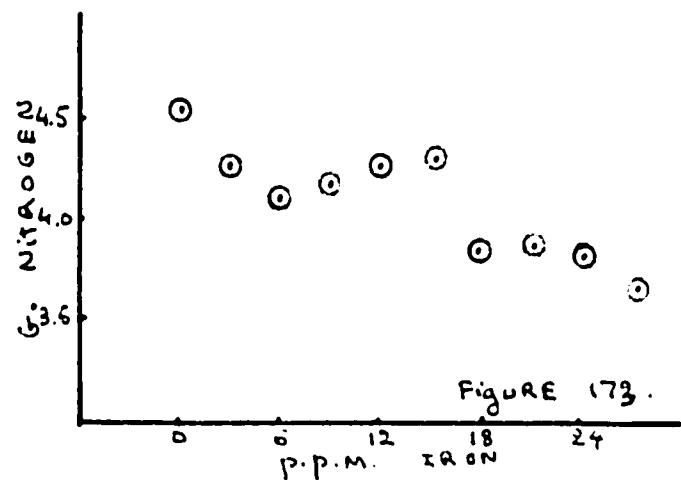
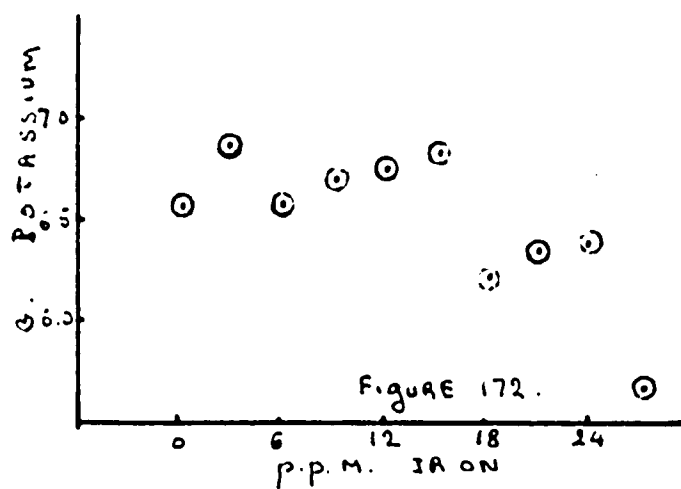
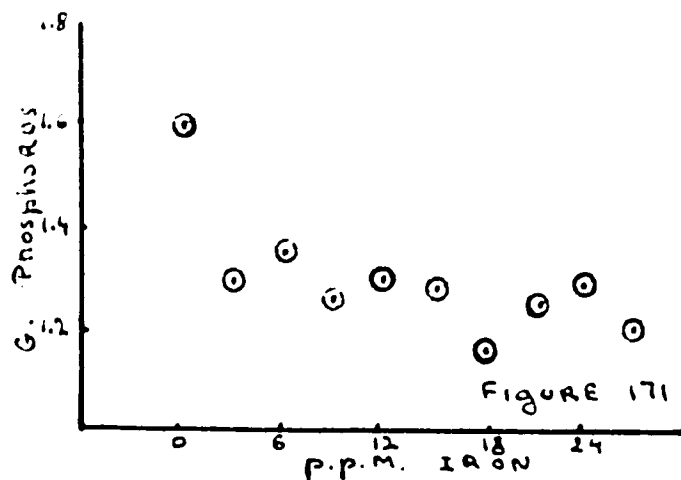
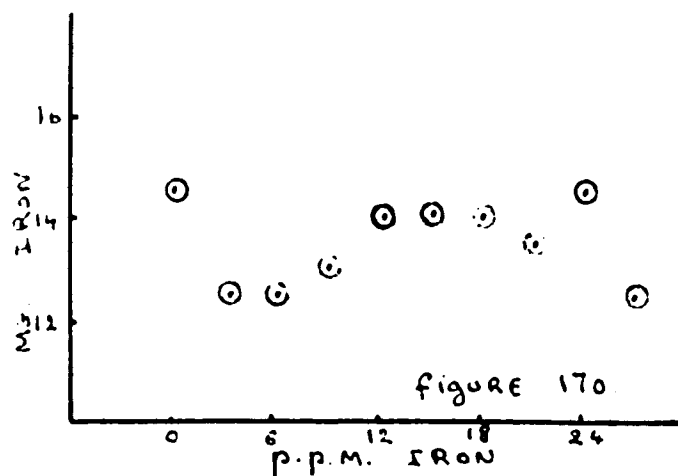


Figure 176. Percentages of the original amounts of ascorbic acid, 14 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various concentrations of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 177. Percentages of the original amounts of ascorbic acid, 43 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various concentrations of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 178. Percentages of the original amounts of ascorbic acid, 62 hours after harvest, in the tissues from wheat plant grown on nutrient solution supplied with various concentrations of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 179. Percentages of the original amounts of ascorbic acid, 110 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various concentrations of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

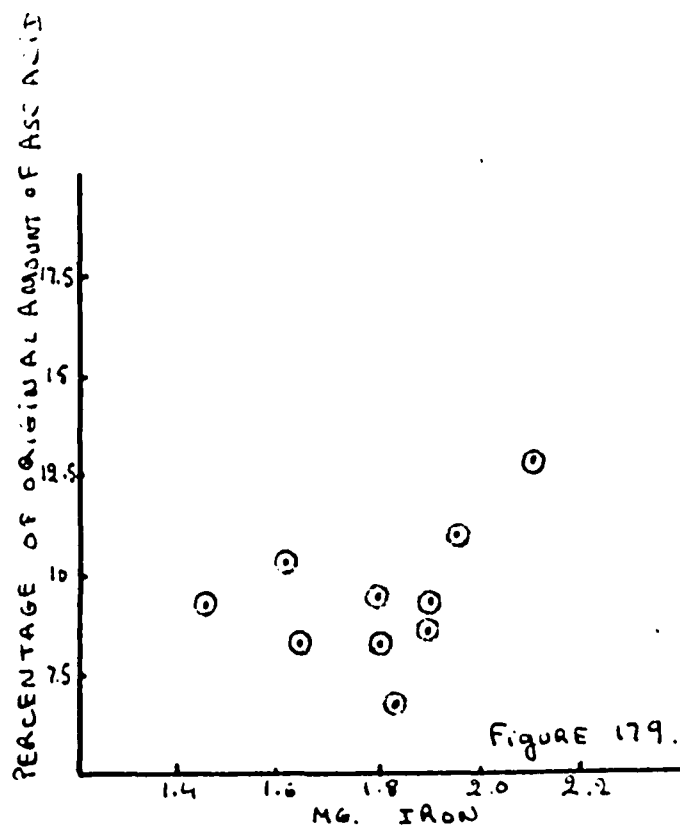
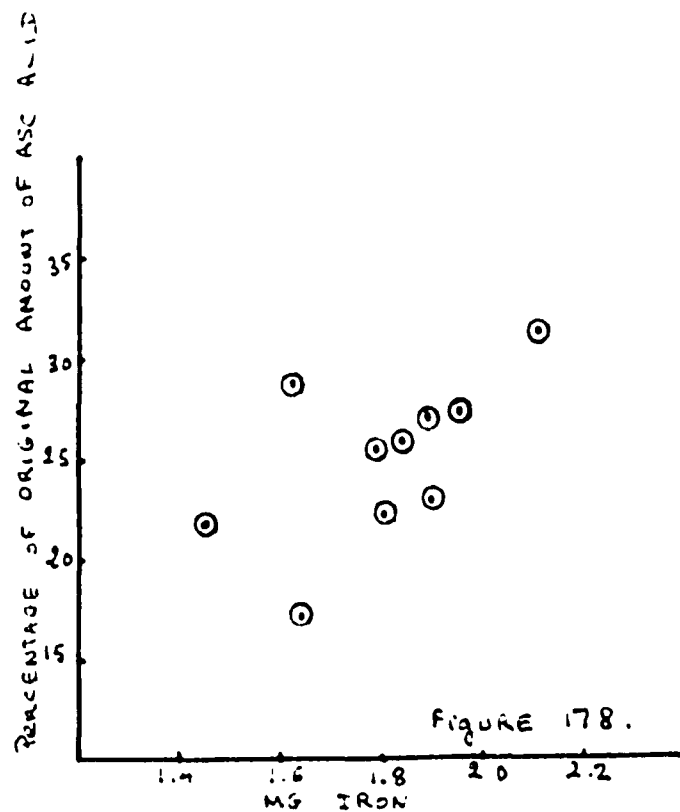
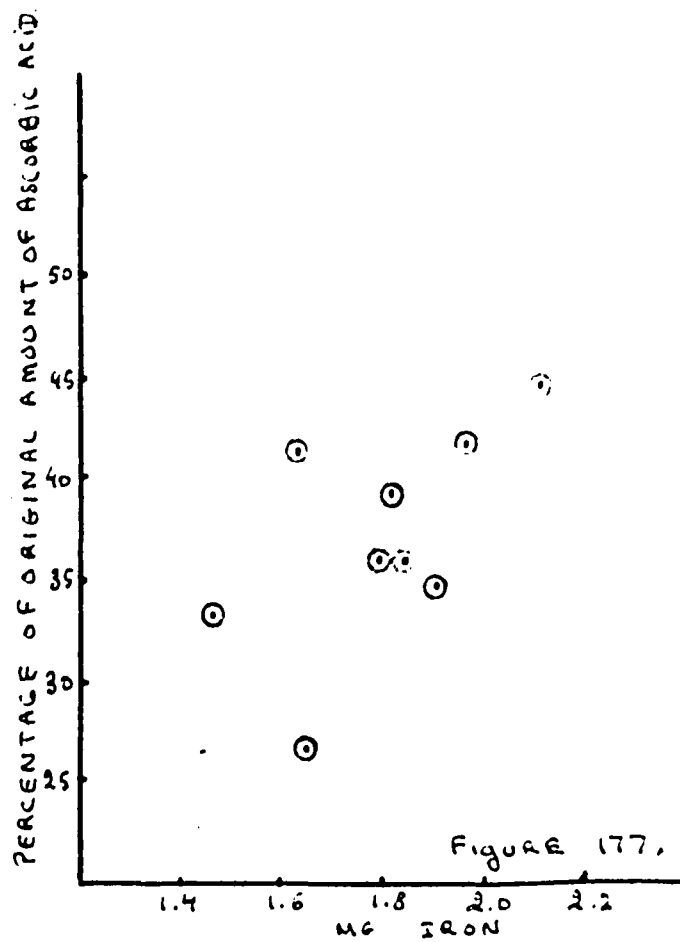
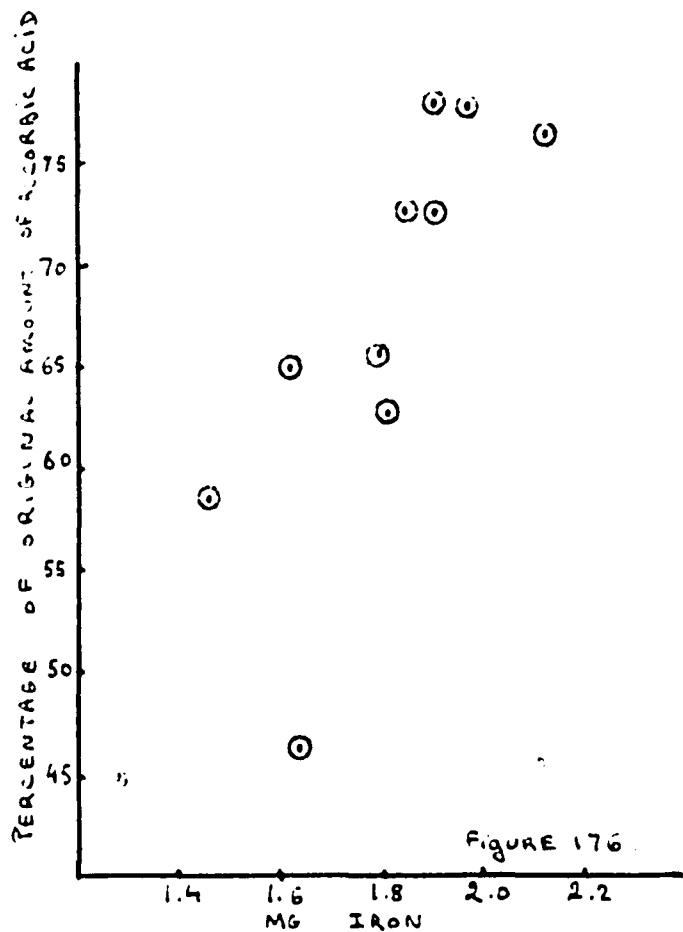


Figure 180. Percentages of the original amounts of water, 14 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various concentrations of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 181. Percentages of the original amounts of water, 43 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 182. Percentages of the original amounts of water, 62 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 183. Percentages of the original amounts of water, 110 hours after harvest, in the tissues from wheat plants grown on nutrient supplied with various amounts of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

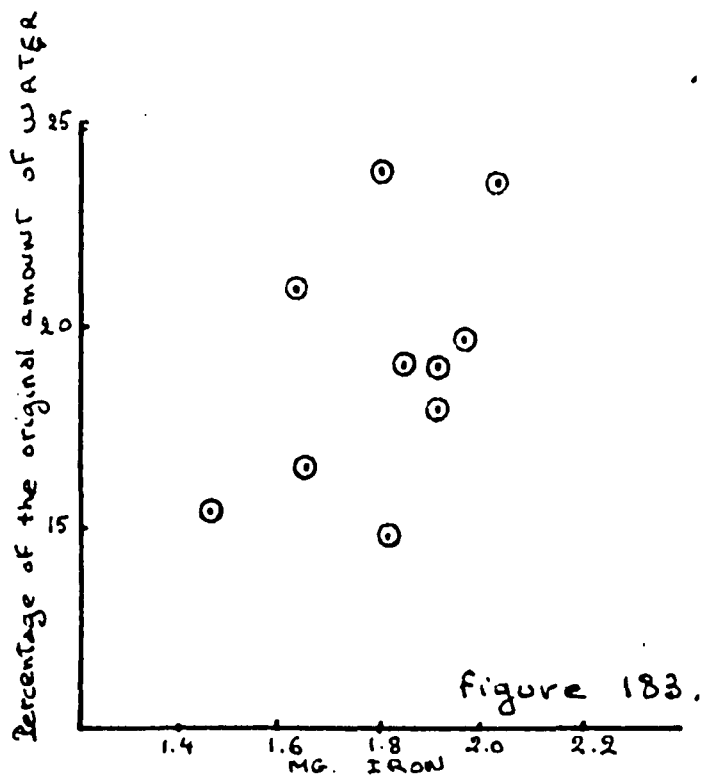
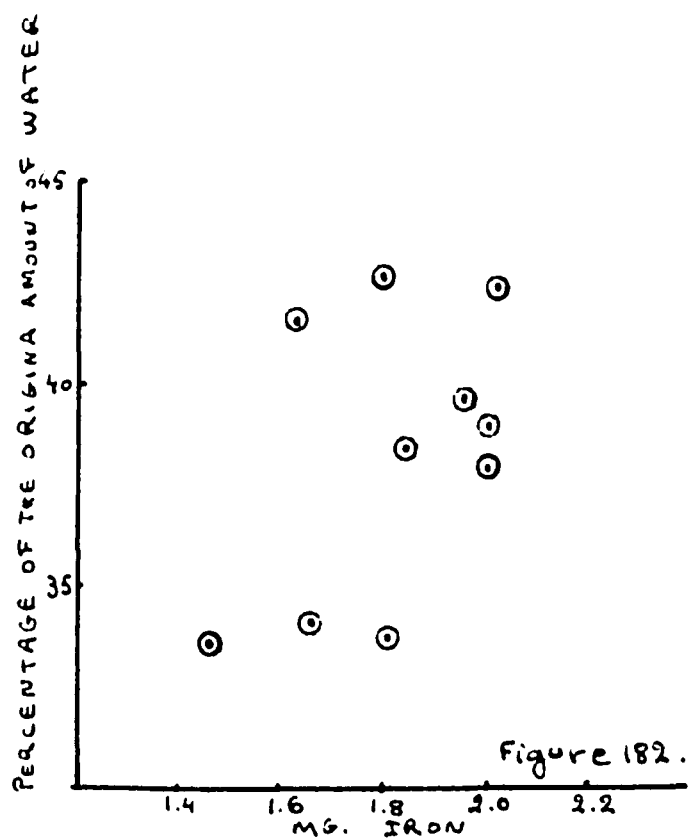
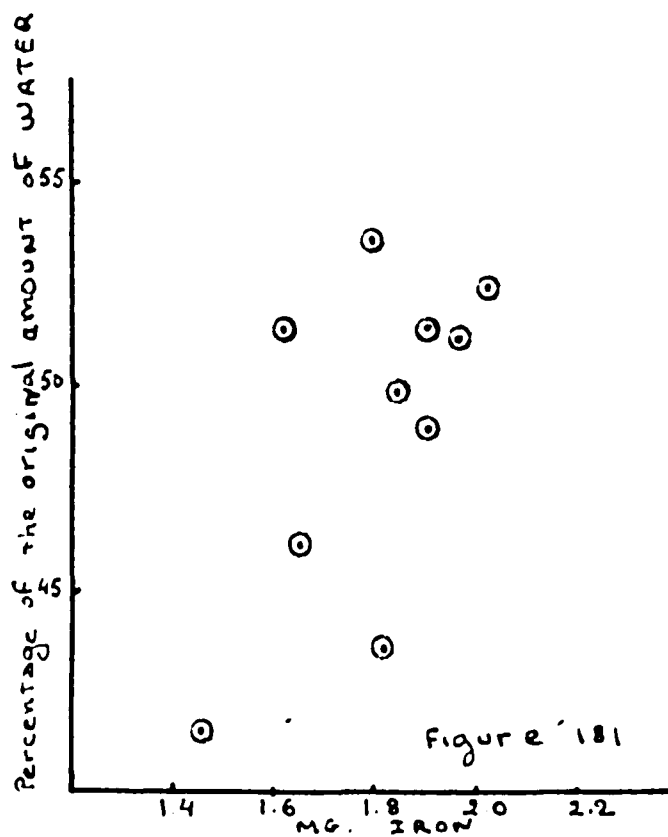
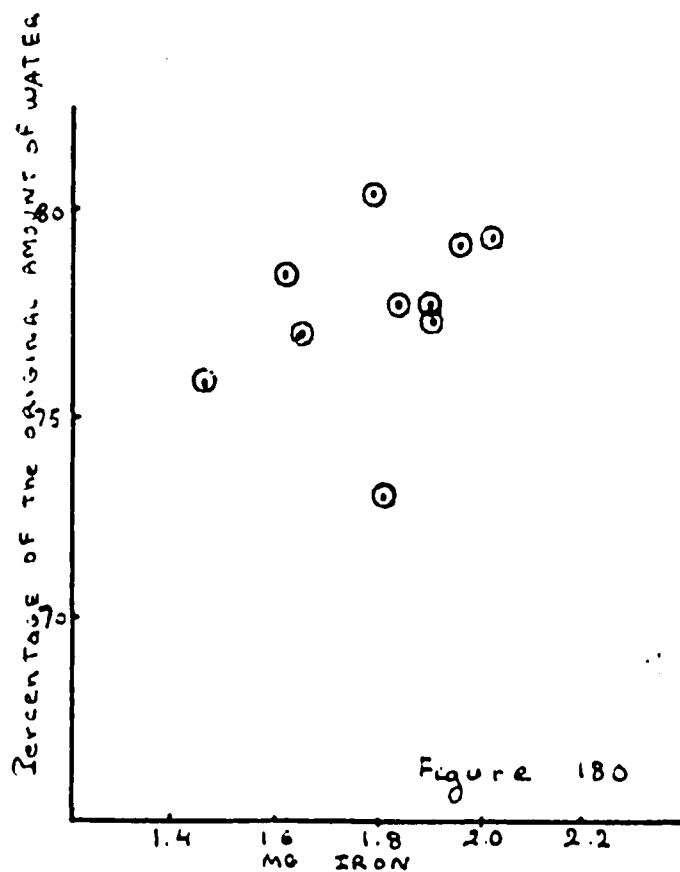


Figure 184. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 185. Average dry weight, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 186. Average amounts of iron, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 187. Average amounts of phosphorus, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 188. Average amounts of potassium, expressed in milligrams per 10 wheat plants grown on nutrient solution supplied with various amounts of copper, plotted against the concentration of copper in the nutrient solution, expressed in parts per million

Figure 189. Average amounts of total nitrogen, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

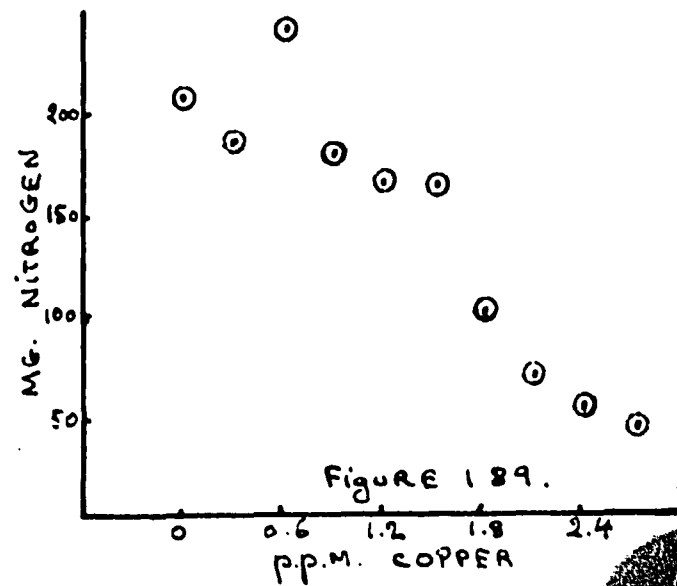
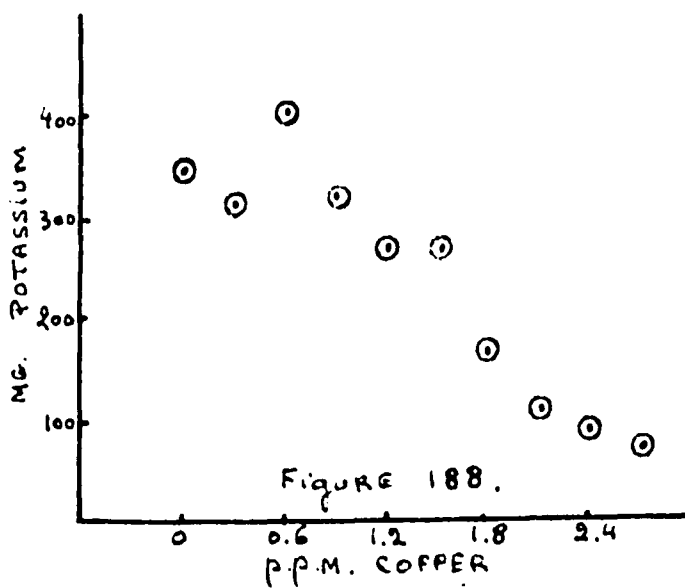
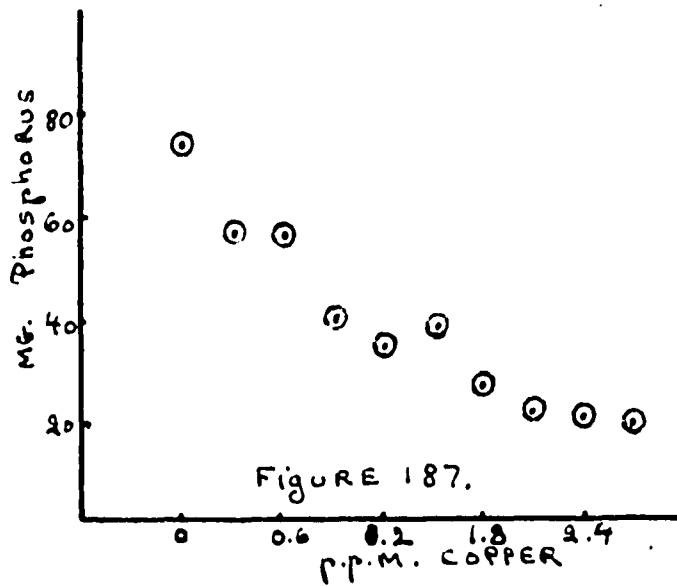
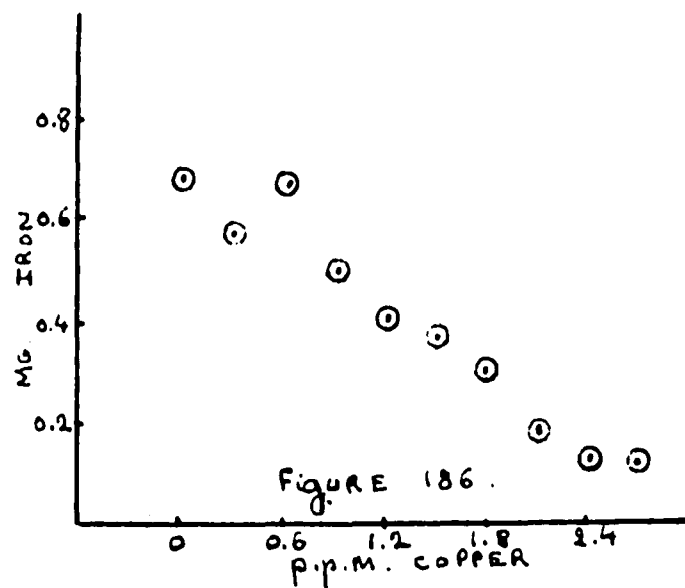
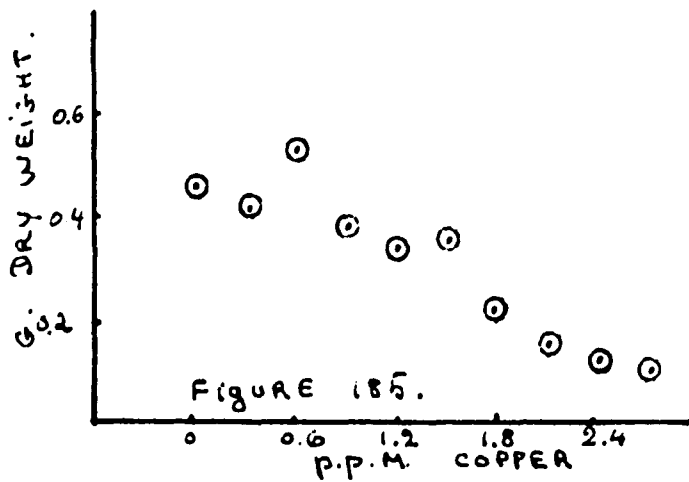
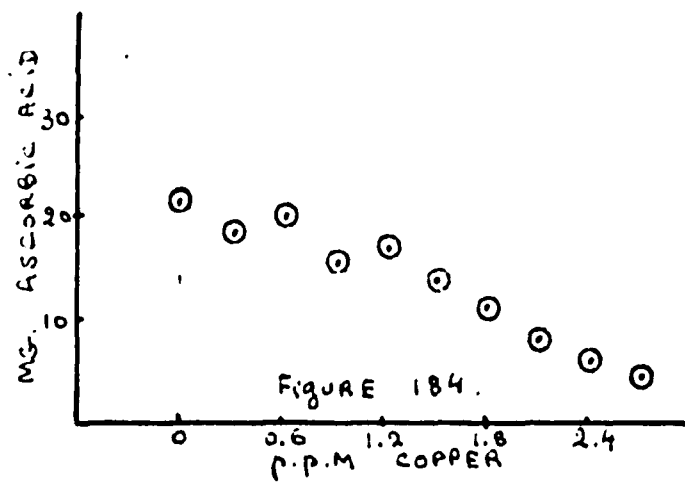




Figure 184. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 190. Average amounts of calcium, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 191. Average amounts of magnesium, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 192. Average amounts of copper, expressed in milligrams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 193. Average fresh weight, expressed in grams per 10 wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 214. Average amounts of copper, expressed in milligrams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

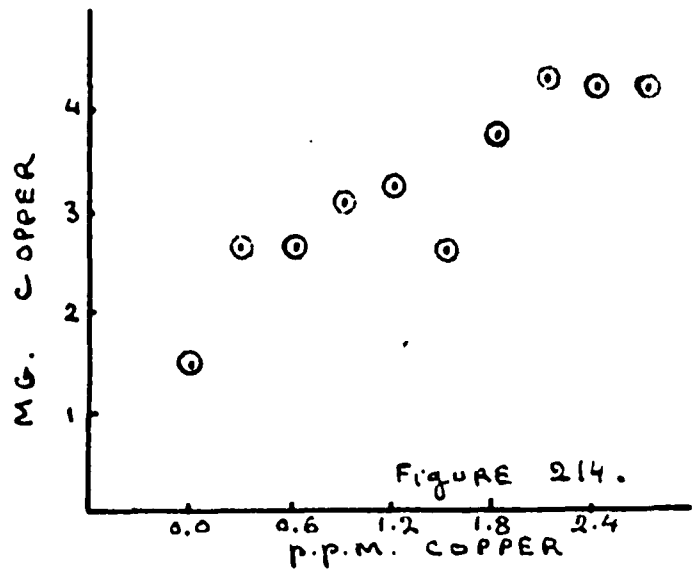
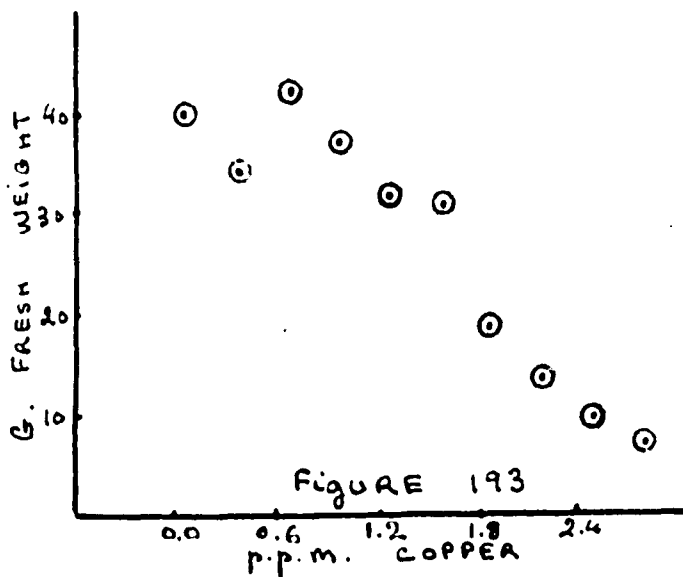
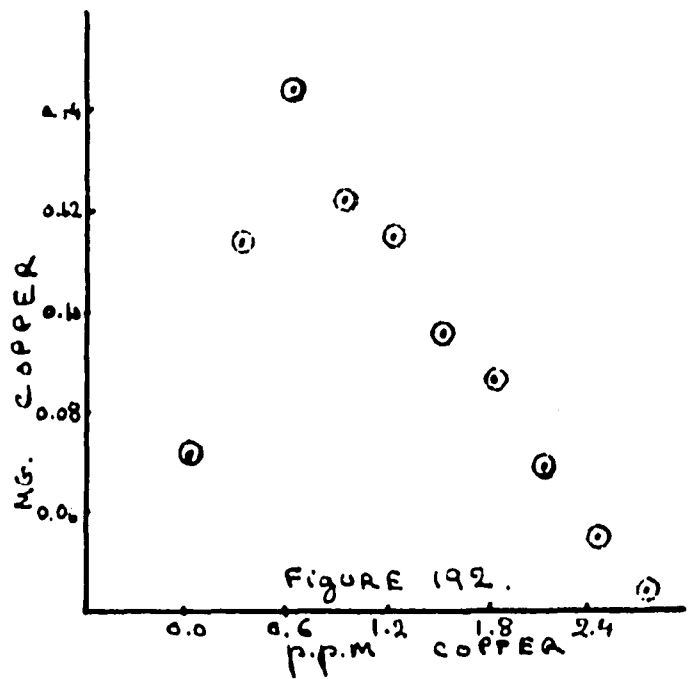
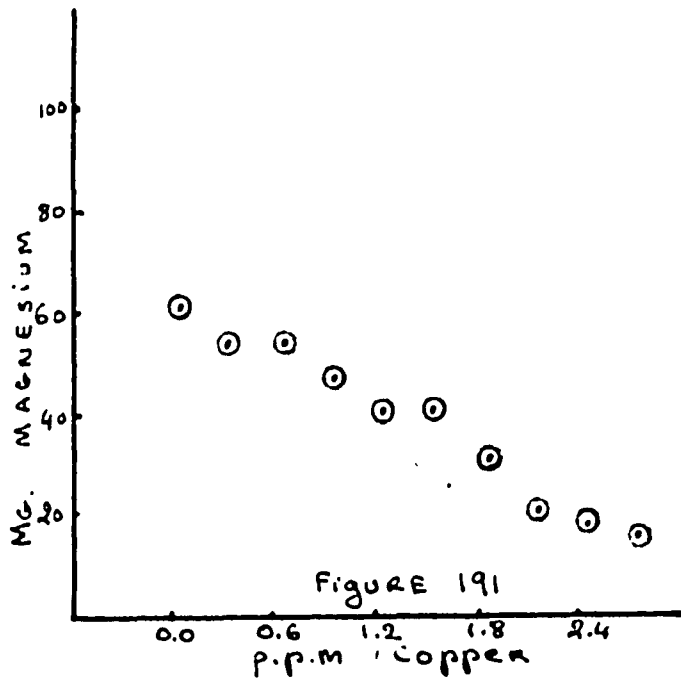
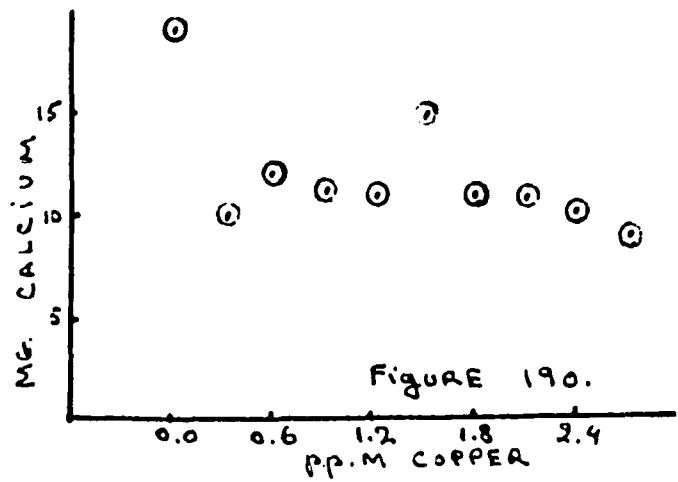
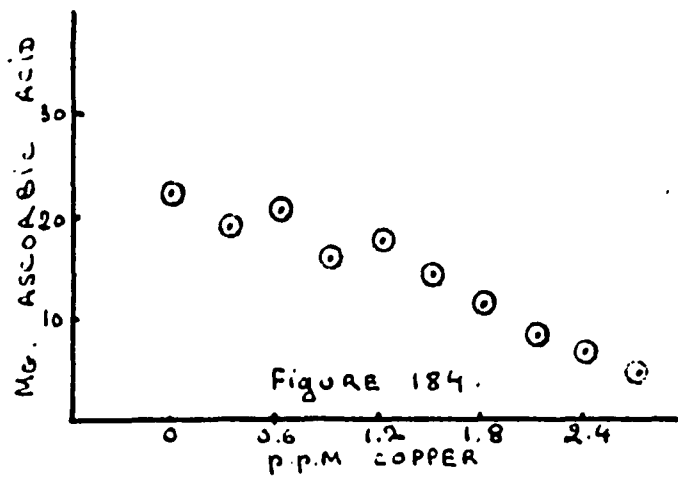


Figure 194. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 195. Average amounts of phosphorus, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 196. Average amounts of potassium, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 197. Average amounts of total nitrogen, expressed in milligrams per 100 grams of fresh matter, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 198. Average amounts of calcium, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 199. Average amounts of magnesium, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

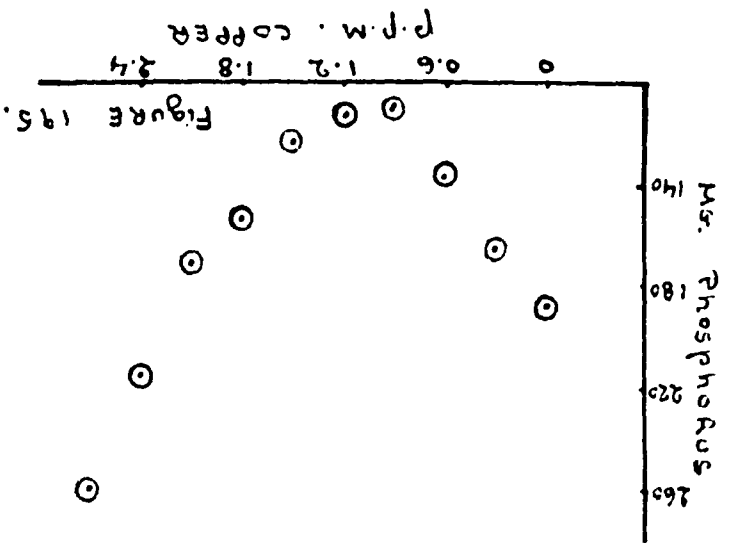
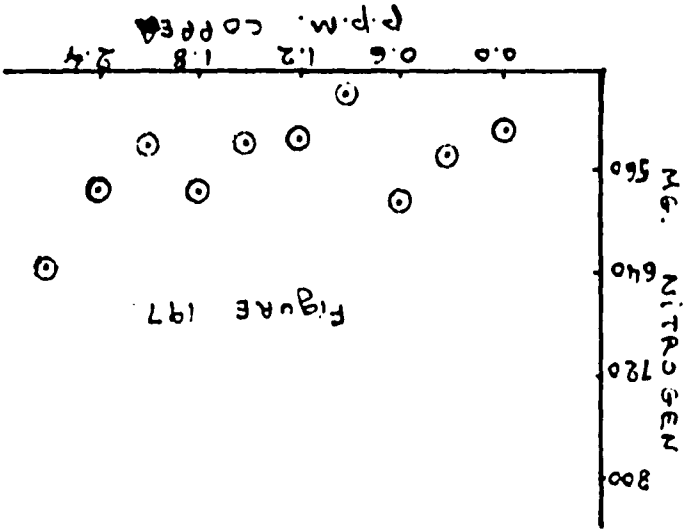
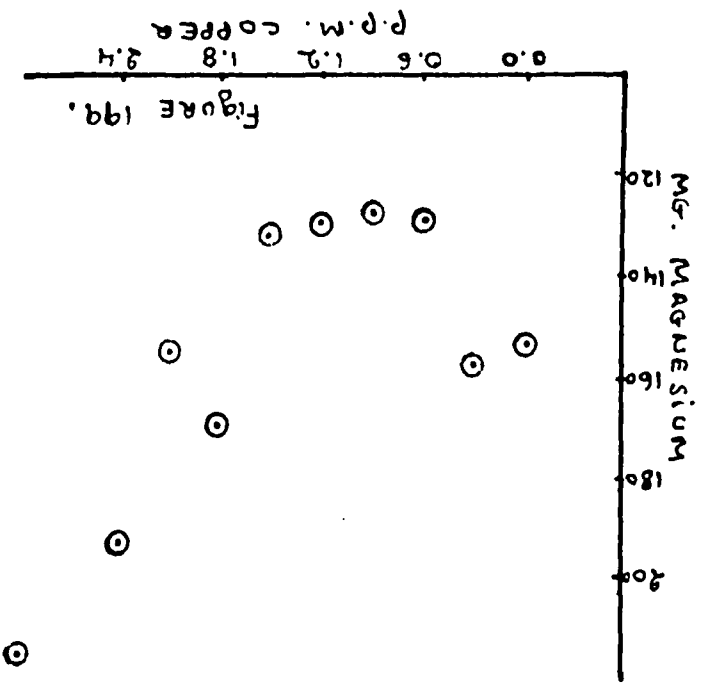
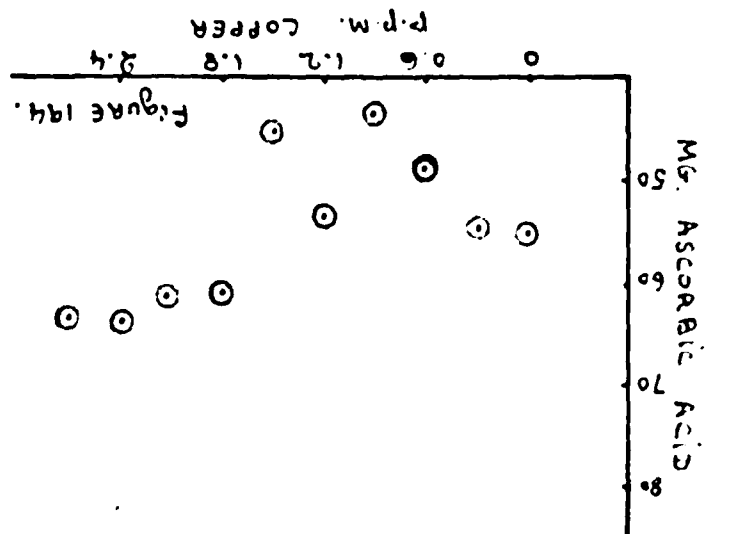
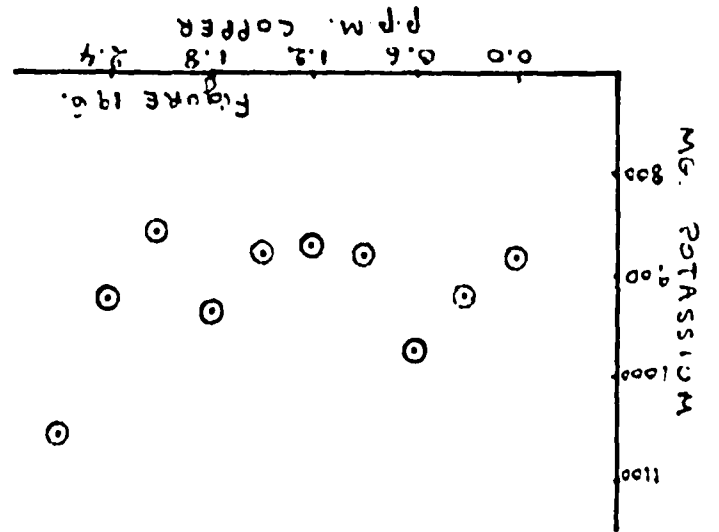
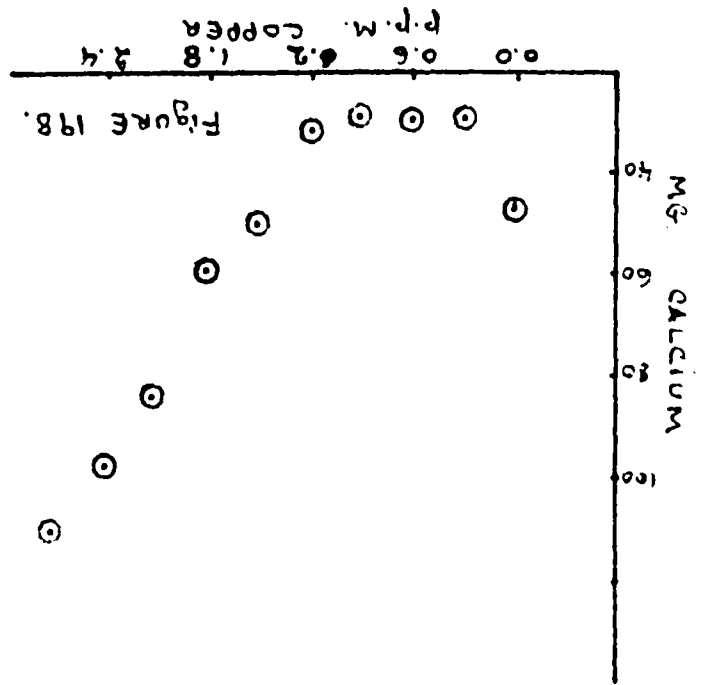


Figure 194. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 200. Average amounts of dry matter, expressed in grams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 201. Average amounts of iron, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 202. Average amounts of copper, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

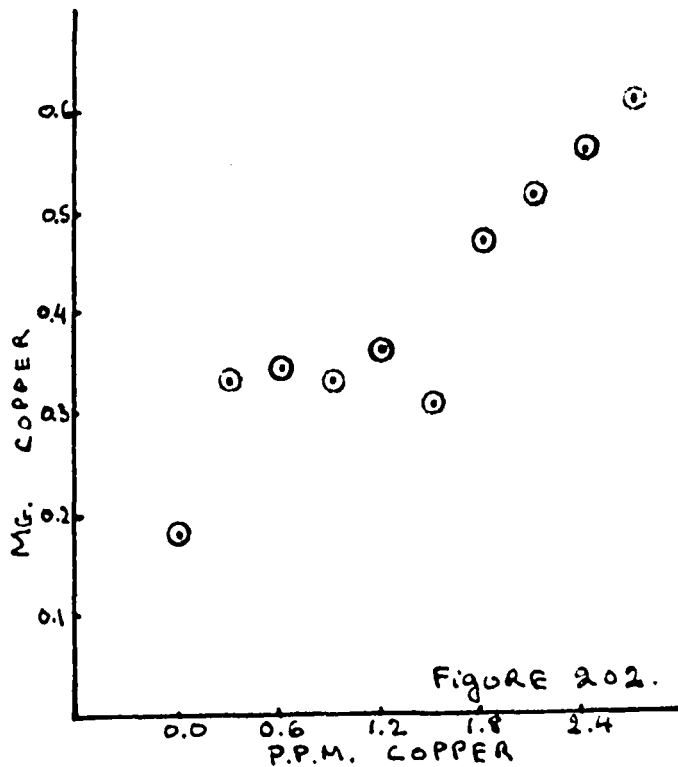
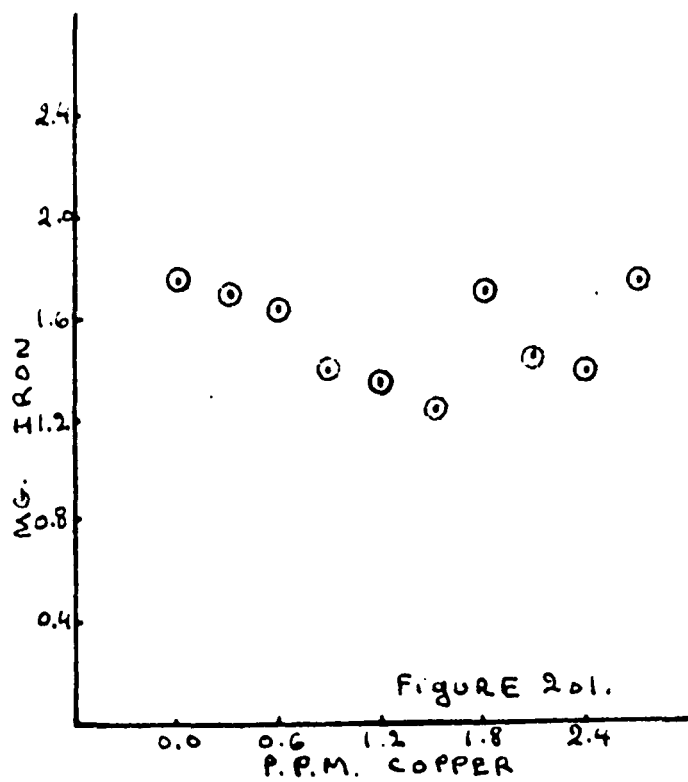
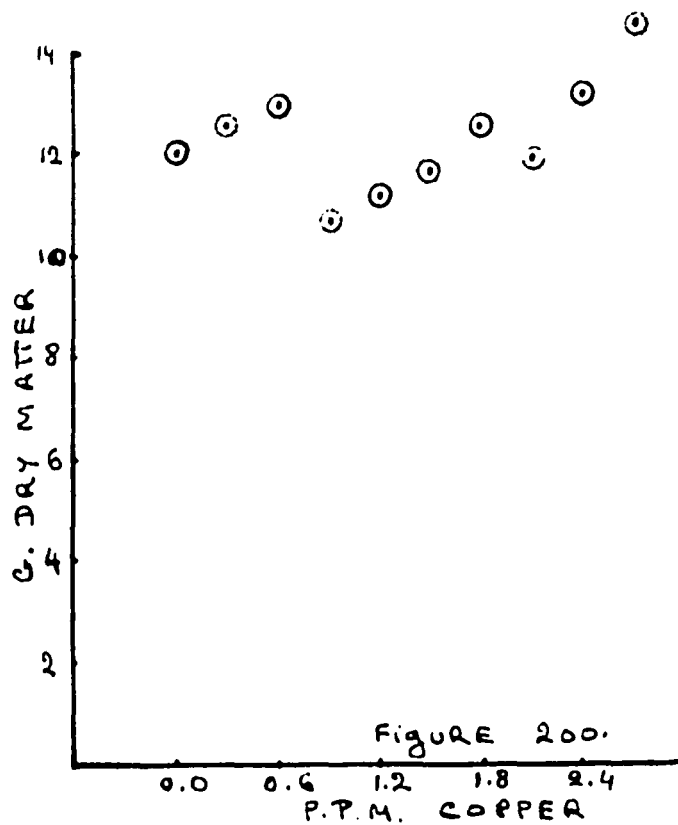
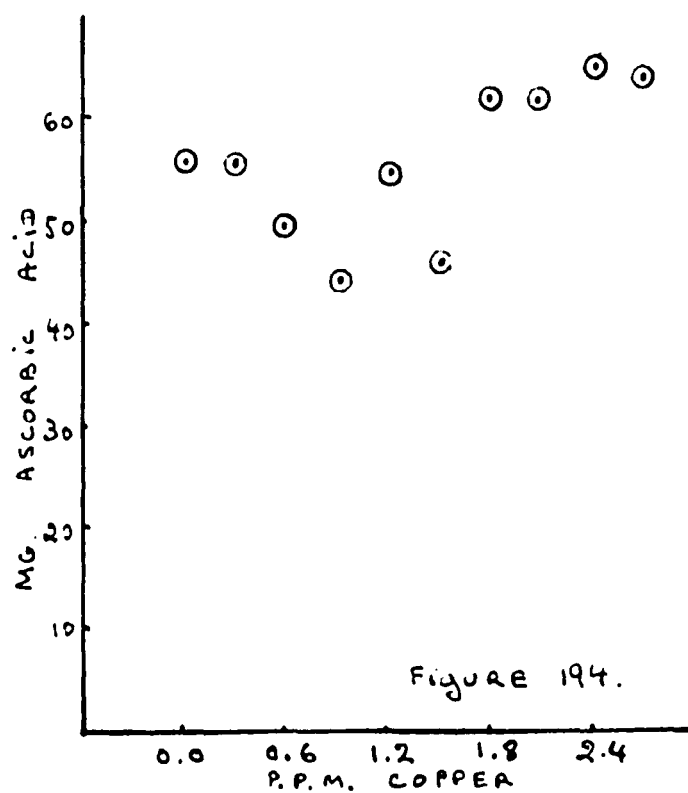


Figure 194. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 203. Average amounts of ascorbic acid, 13 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 204. Average amounts of ascorbic acid, 30 hours after harvest expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 205. Average amounts of ascorbic acid, 52 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 206. Average amounts of ascorbic acid, 100 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution., expressed in parts per million.

Figure 207. Average amounts of ascorbic acid, 147 hours after harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

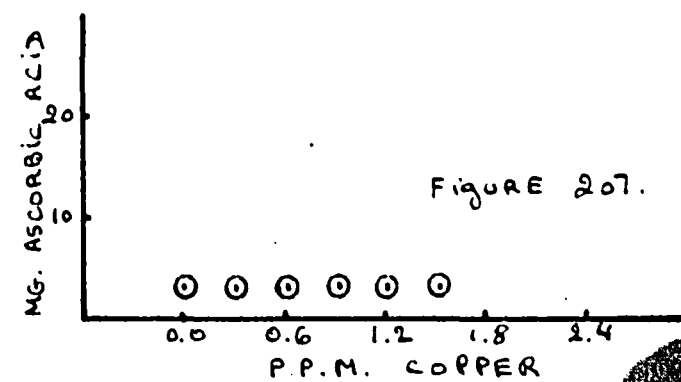
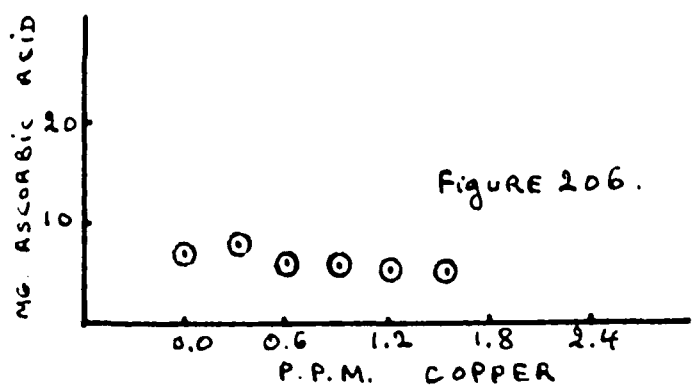
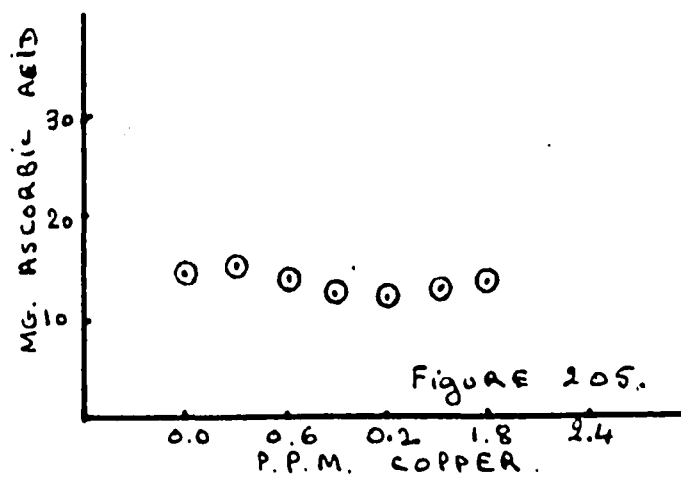
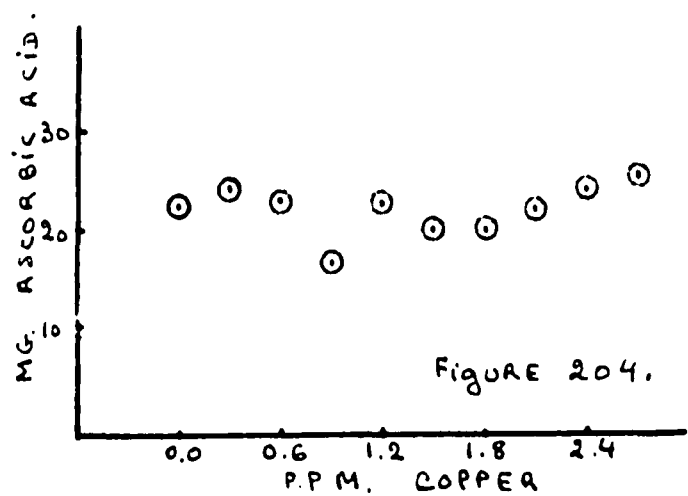
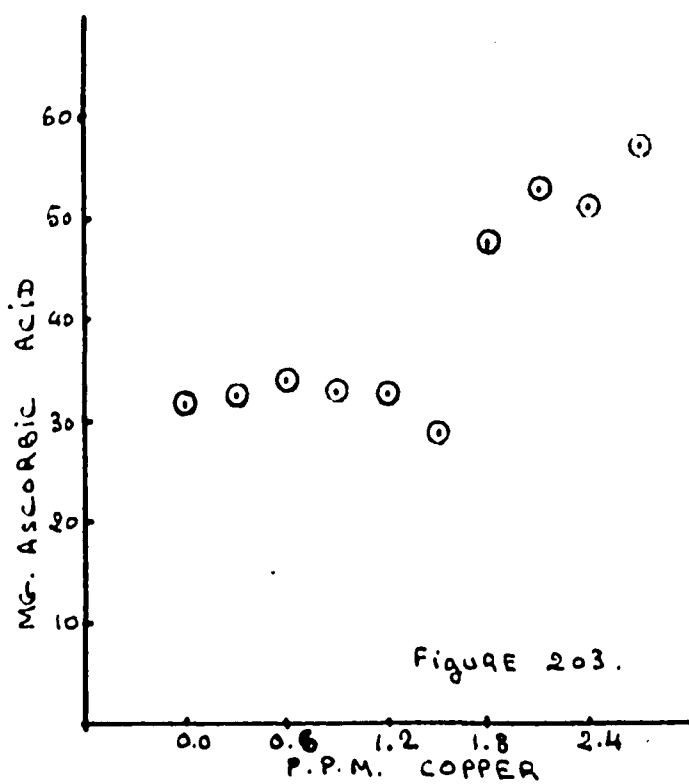
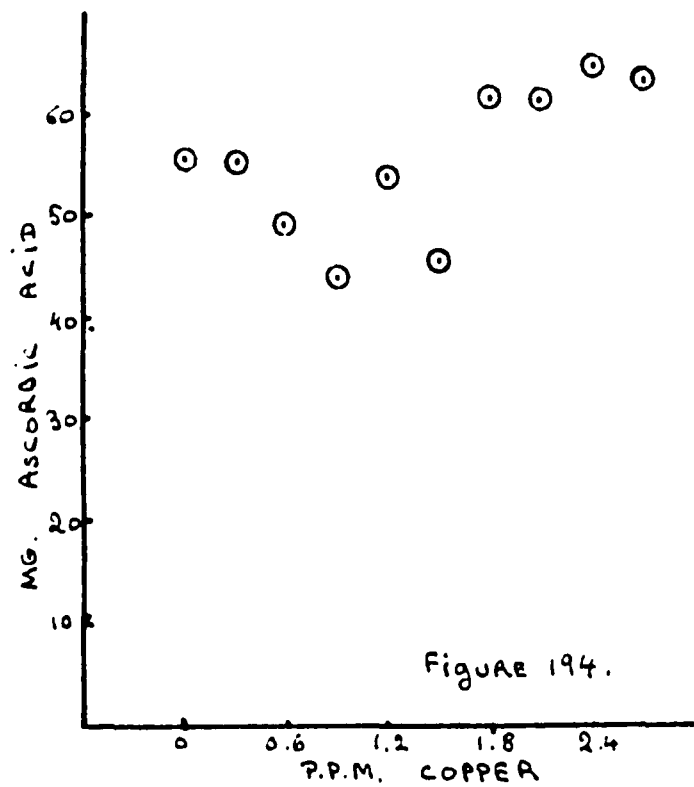




Figure 206. Average amounts of iron, expressed in milligrams per hundred grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 209. Average amounts of phosphorus, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 210. Average amounts of potassium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 211. Average amounts of total nitrogen, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 212. Average amounts of calcium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 213. Average amounts of magnesium, expressed in grams per 100 grams of oven dry tissue from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

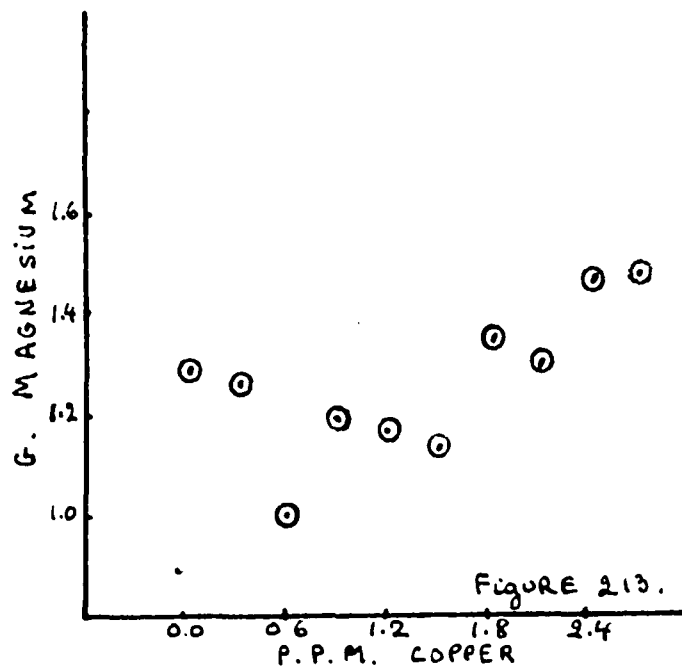
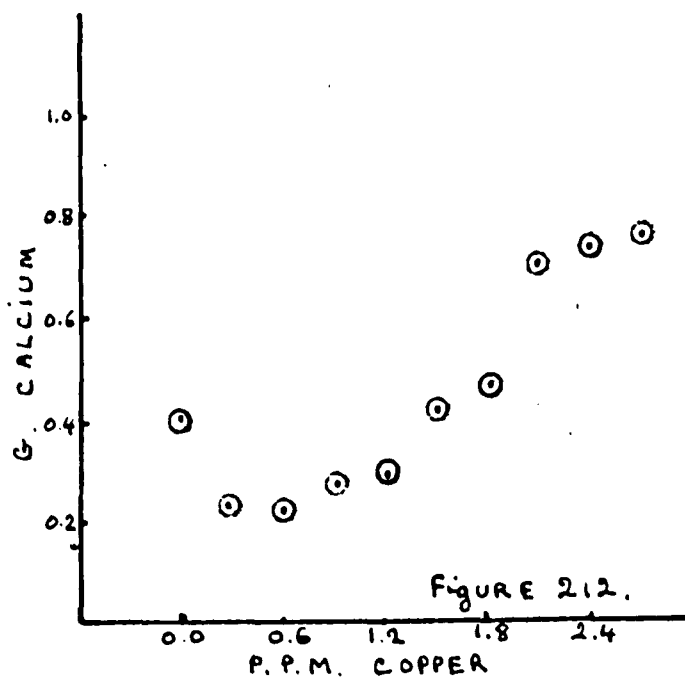
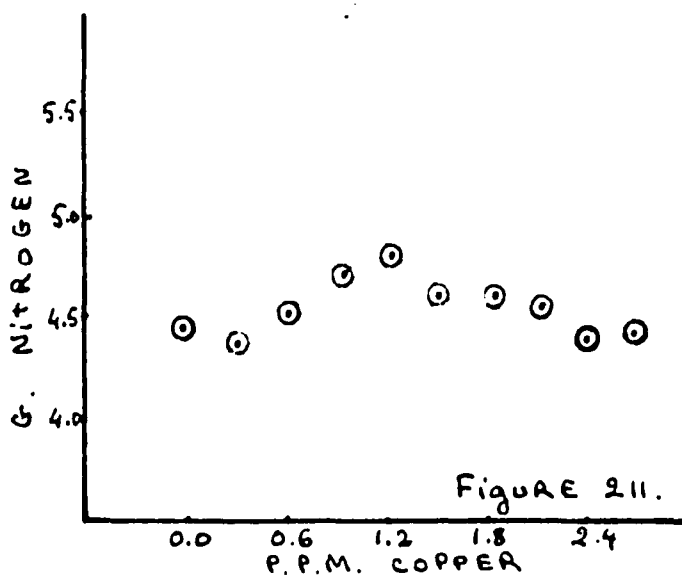
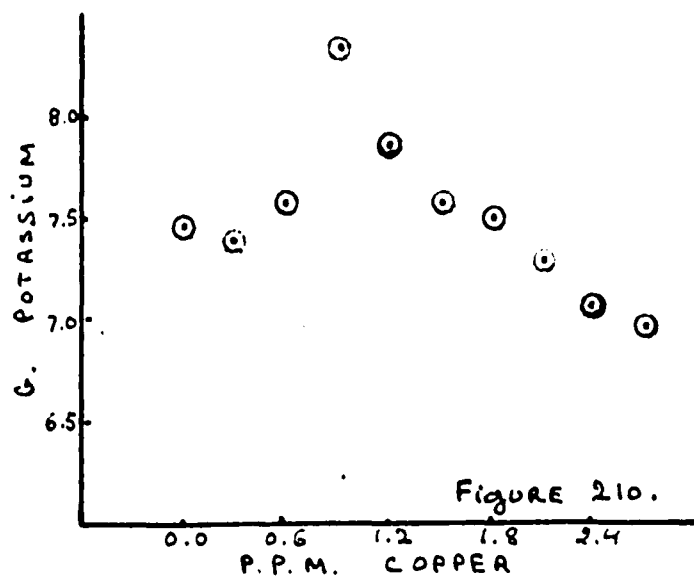
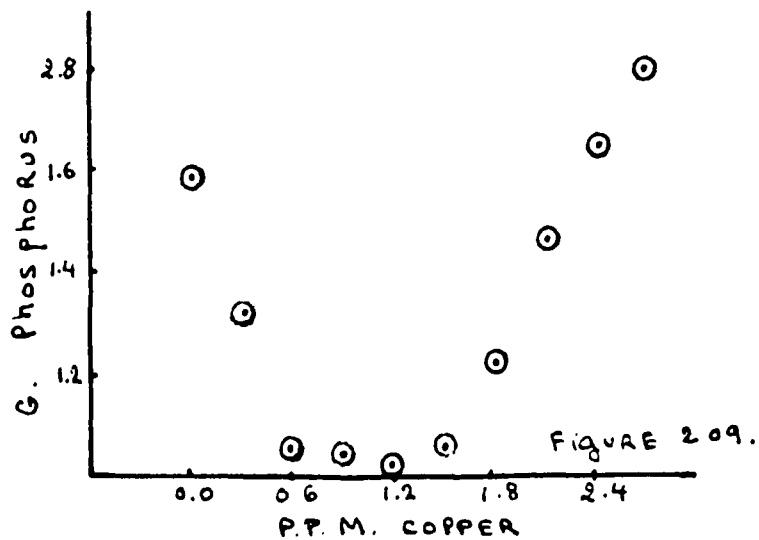
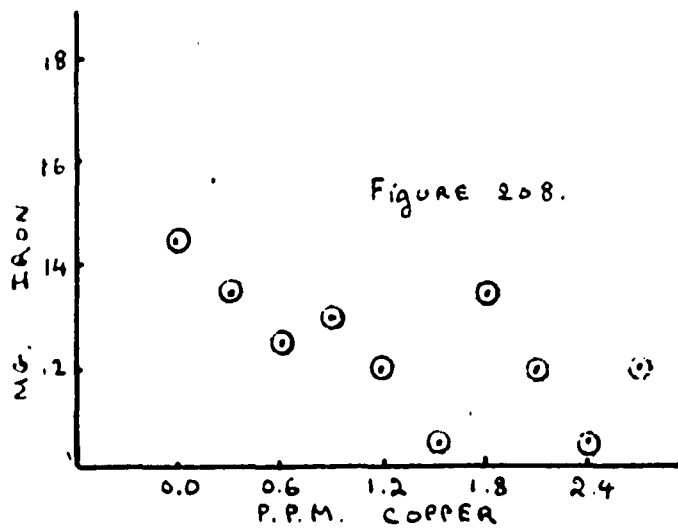


Figure 214. This figure may be found after figure 193.

Figure 215. Percentages of the original amounts of water, 13 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various amounts of copper, plotted against the amounts of copper, expressed in milligrams per 100 grams of fresh matter.

Figure 216. Percentages of the original amounts of water, 30 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of copper, expressed in milligrams per 100 grams of fresh matter.

Figure 217. Percentages of the original amounts of water, 13 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 218. Percentages of the original amounts of water, 30 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

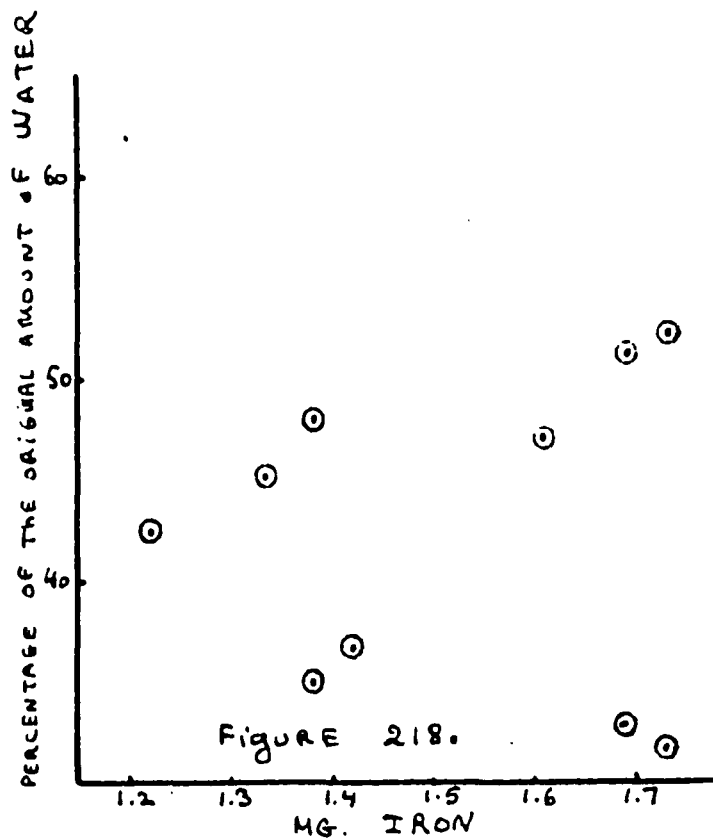
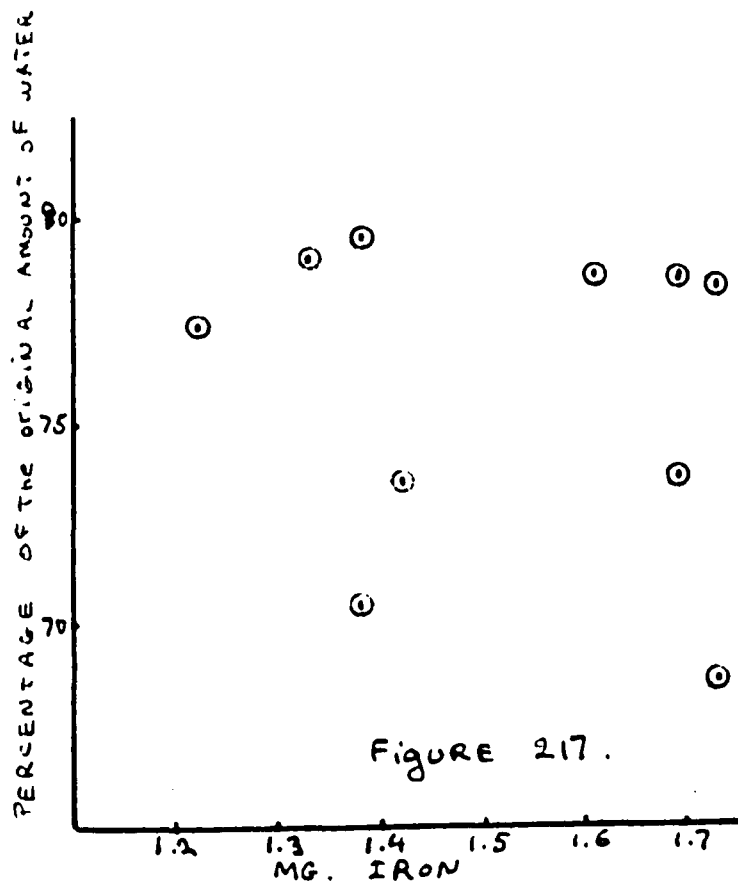
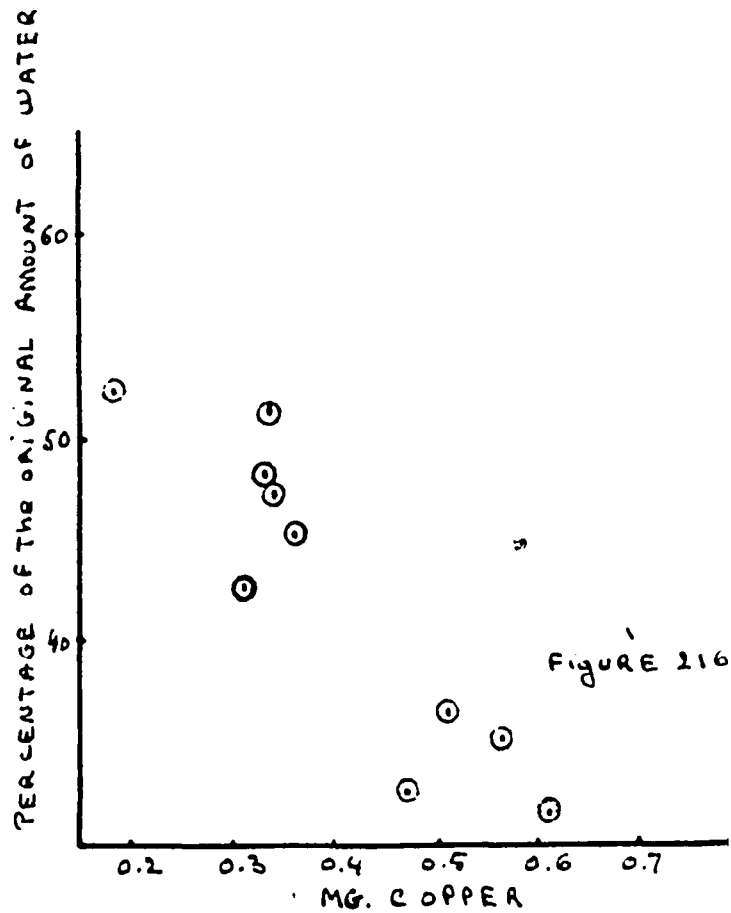
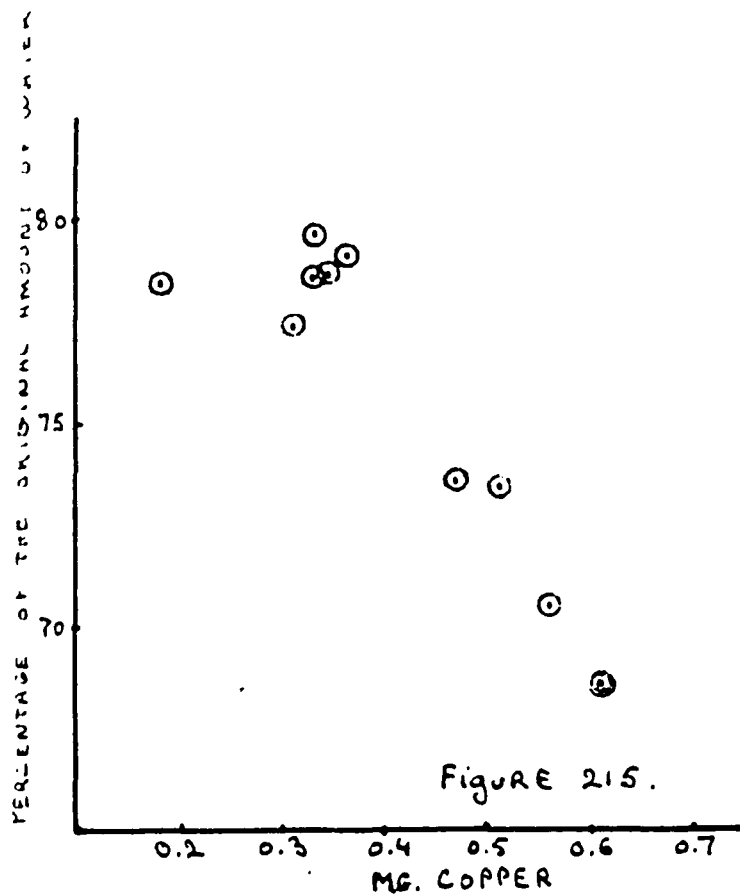


Figure 219. Percentages of the original amounts of ascorbic acid, 13 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various amounts of copper, plotted against the amounts of copper, expressed in milligrams per 100 grams of fresh matter.

Figure 220. Percentages of the original amounts of ascorbic acid, 30 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of copper in the fresh tissue, expressed in milligrams per 100 grams of fresh matter.

Figure 221. Percentages of the original amounts of ascorbic acid, 13 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 222. Percentages of the original amounts of ascorbic acid, 30 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, expressed in milligrams per 100 grams of fresh matter.

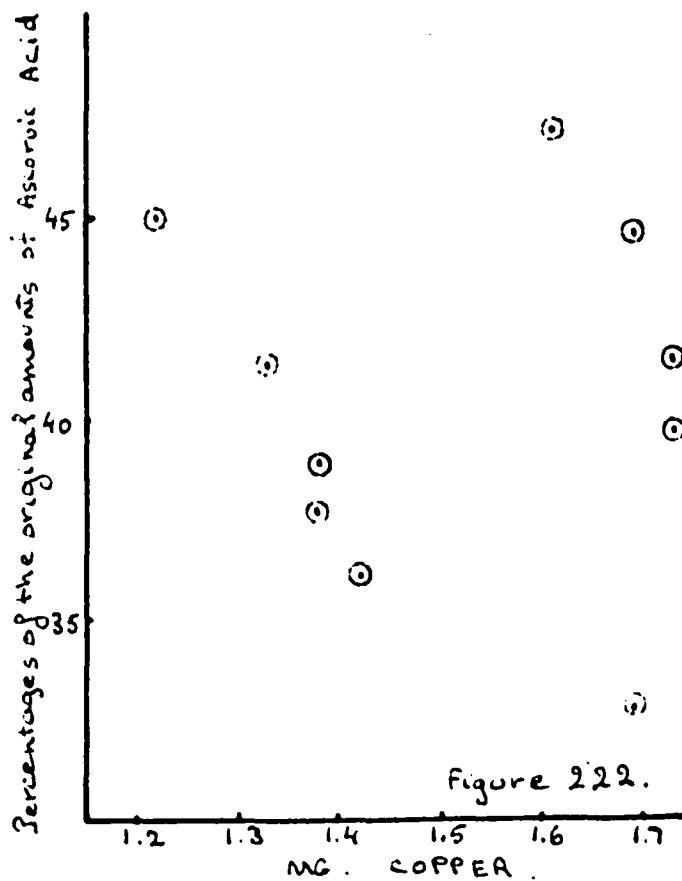
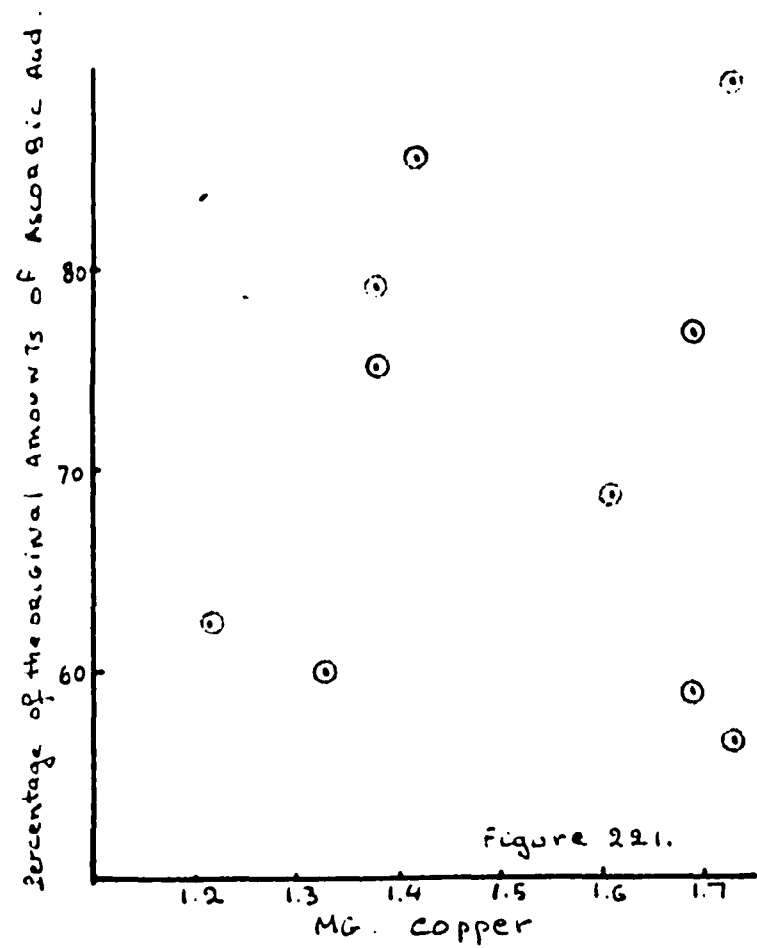
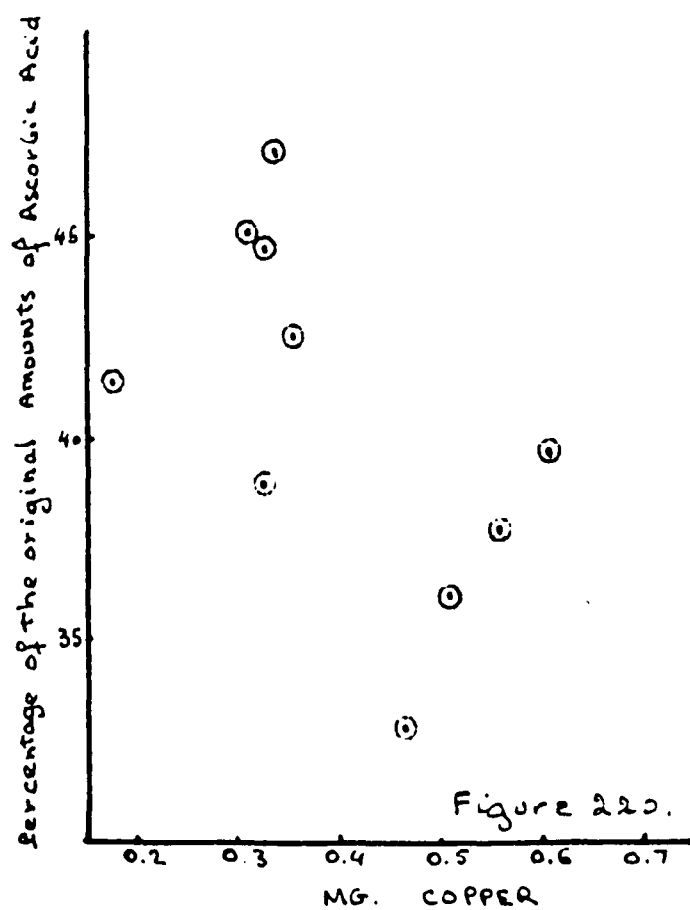
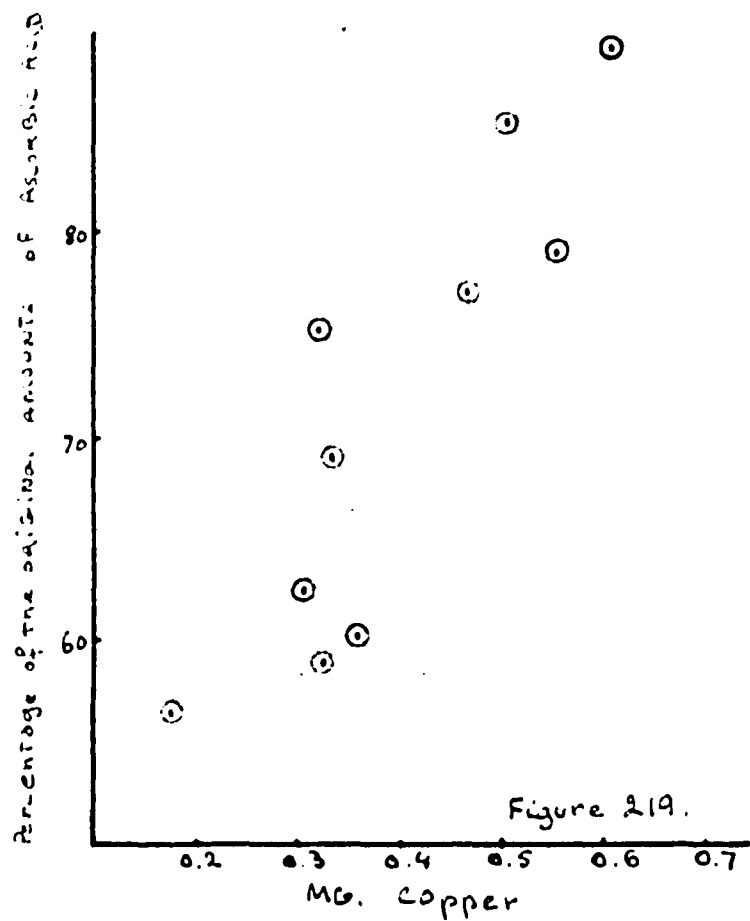


Figure 223. This figure may be found after figure 159.

Figure 224. Percentages of the original amounts of ascorbic acid, 30 hours after harvest, in the tissues from wheat plants grown on nutrient solutions supplied with various amounts of copper, plotted against the amounts of dry matter, expressed in grams per 100 grams of fresh matter

Figure 225. Percentages of the original amounts of ascorbic acid, 14 hours after harvest, in the tissues from wheat plants grown on nutrient solution supplied with various amounts of iron, plotted against the amounts of dry matter, expressed in grams per hundred grams of fresh matter.

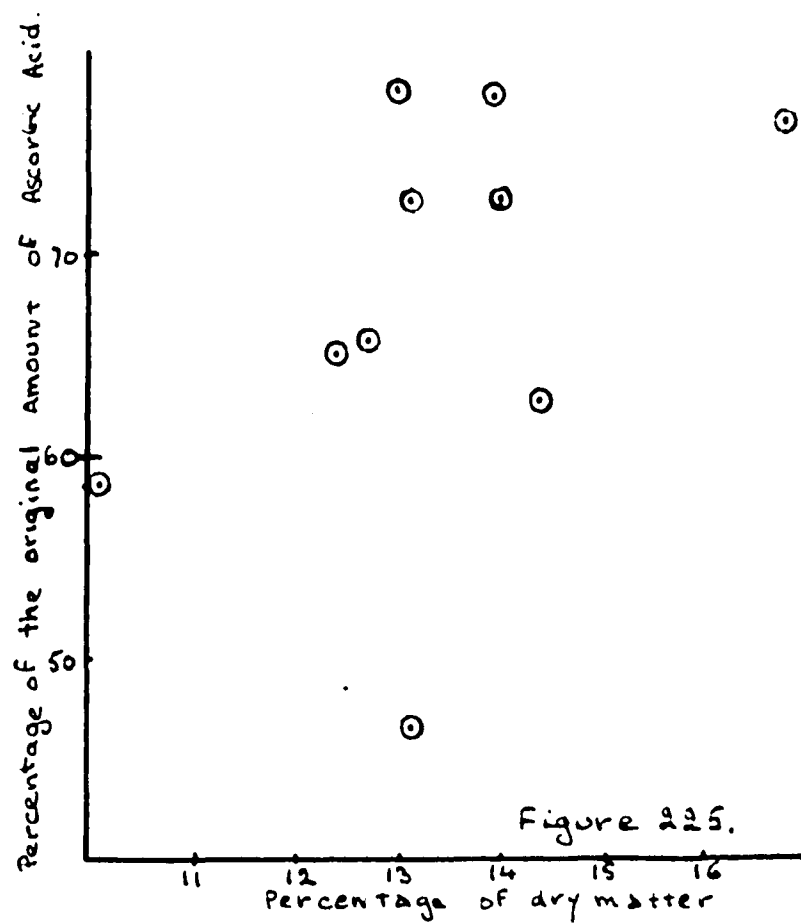
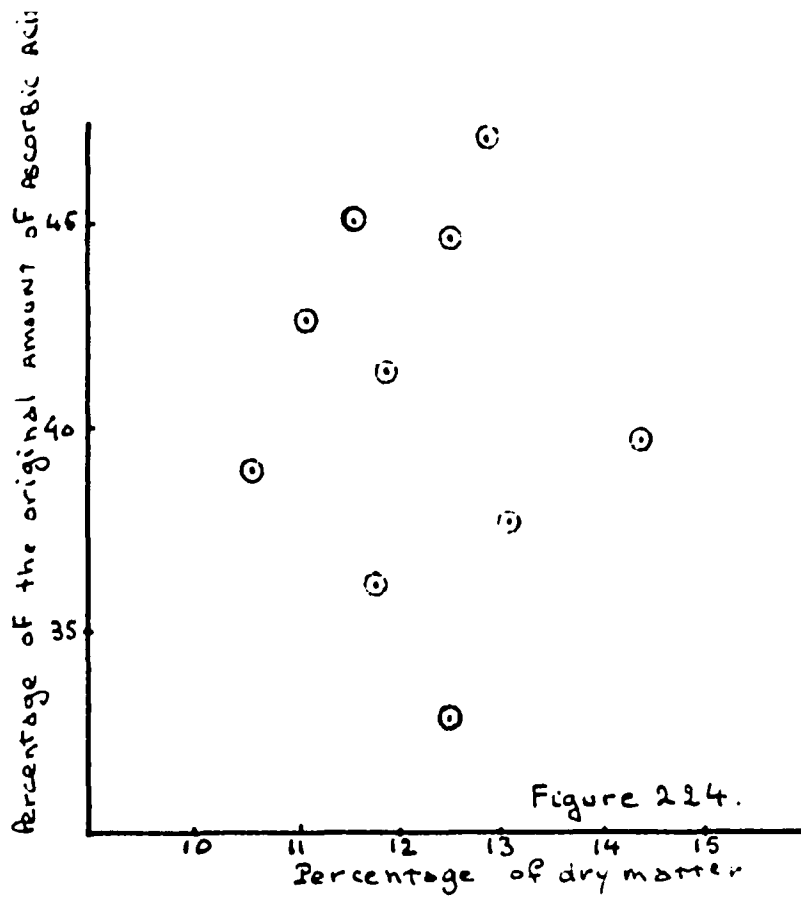




Figure 226. Percentages of the original amounts of water, 13, 30, 52, 100, and 147 hours after harvest, in the tissues from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 227. Percentages of the original amounts of ascorbic acid, 13, 30, 52, 100 and 147 hours after harvest, in the tissues from wheat plants, plotted against the concentration of copper in the nutrient solution, expressed in parts per million.

Figure 228. Percentages of the original amounts of water, 14, 43, 62, 110, and 157 hours after harvest, in the tissues from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 229. Percentages of the original amounts of ascorbic acid, 14, 43, 62, 110, and 157 hours after harvest, in the tissues from wheat plants, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

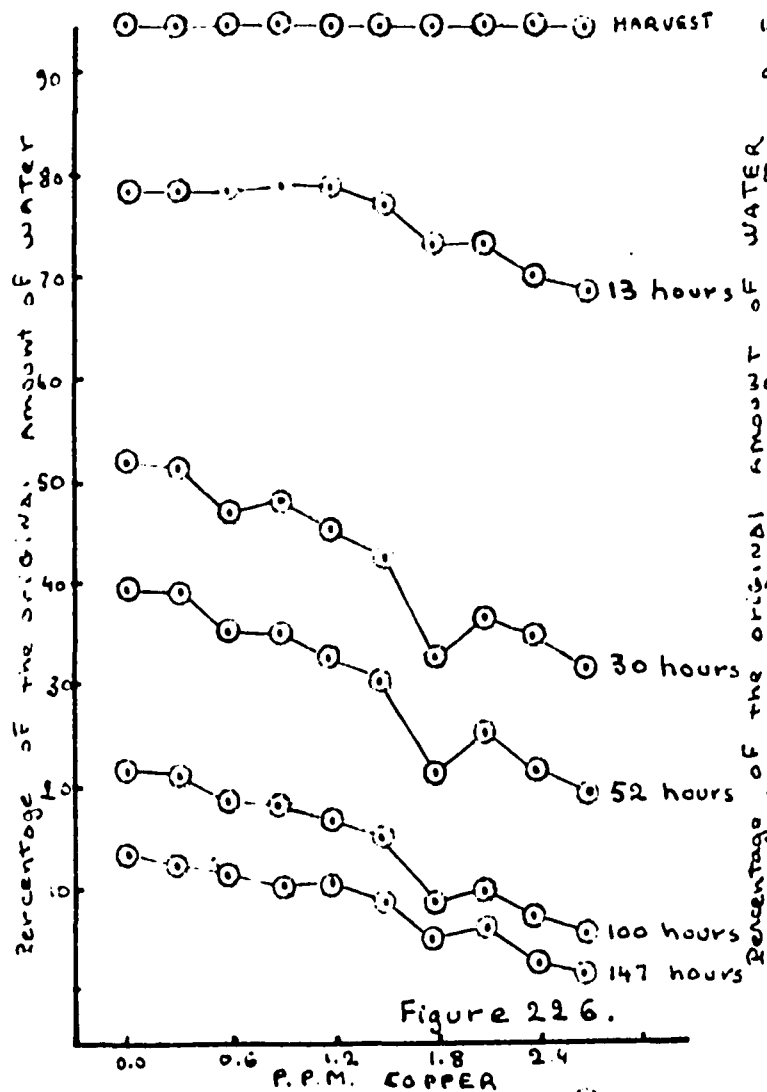


Figure 226.

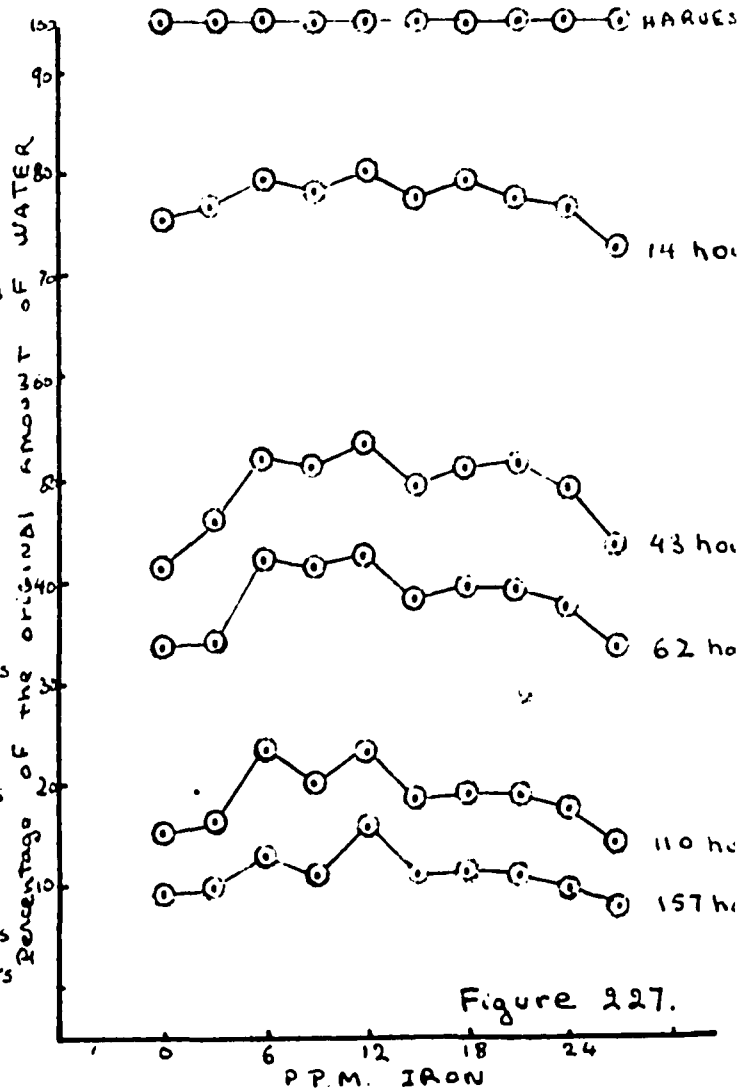


Figure 227.

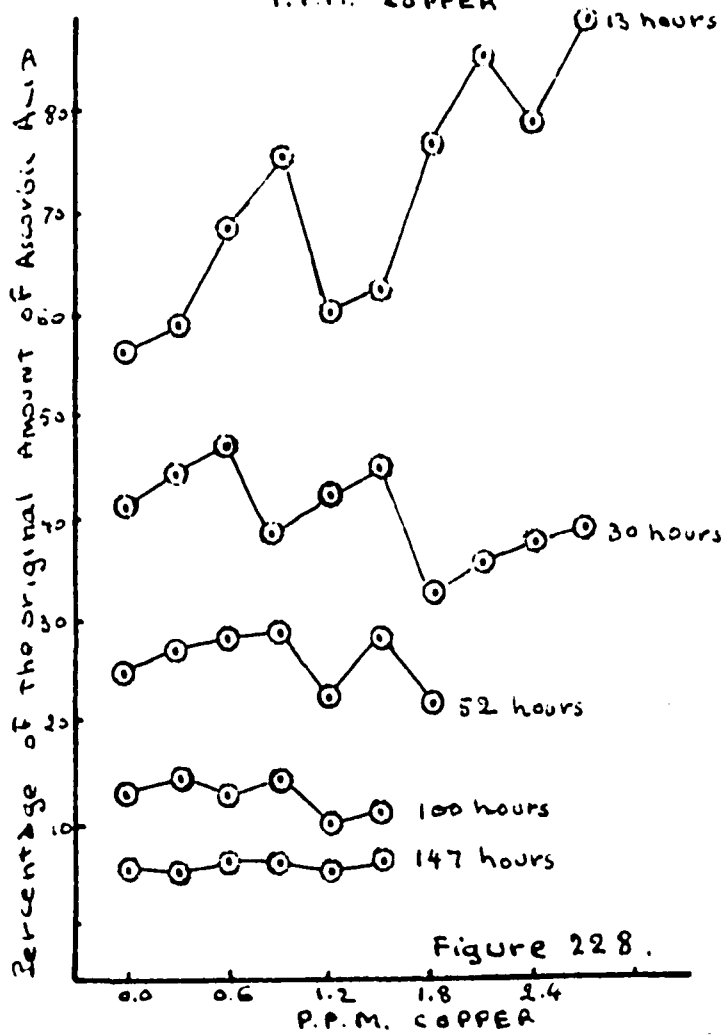


Figure 228.

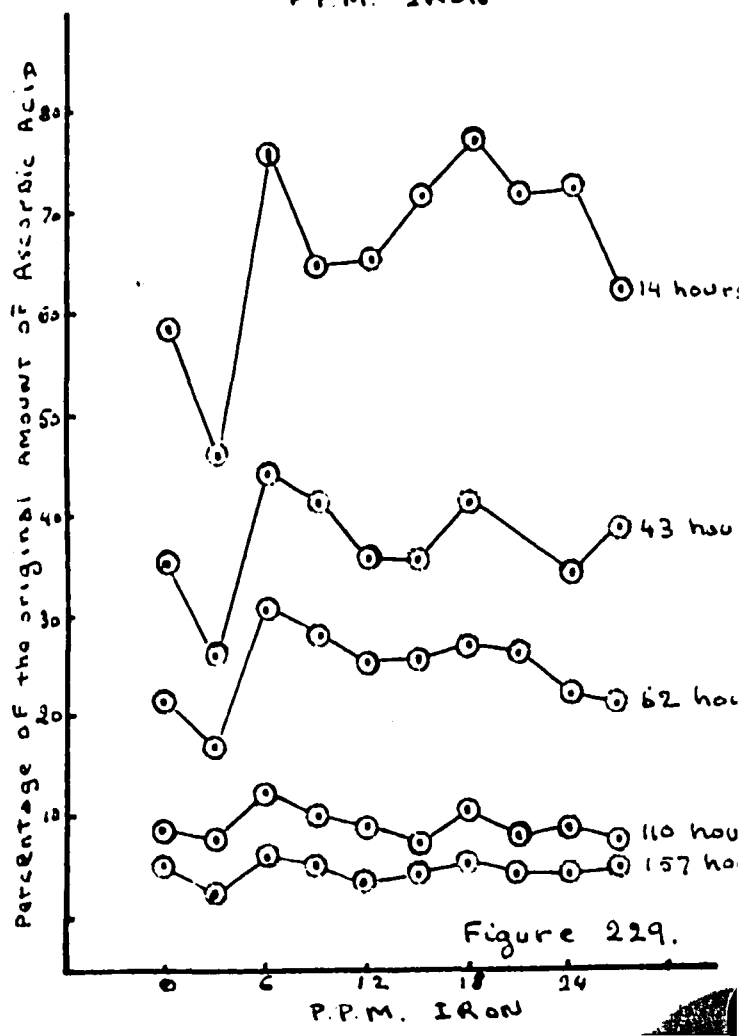
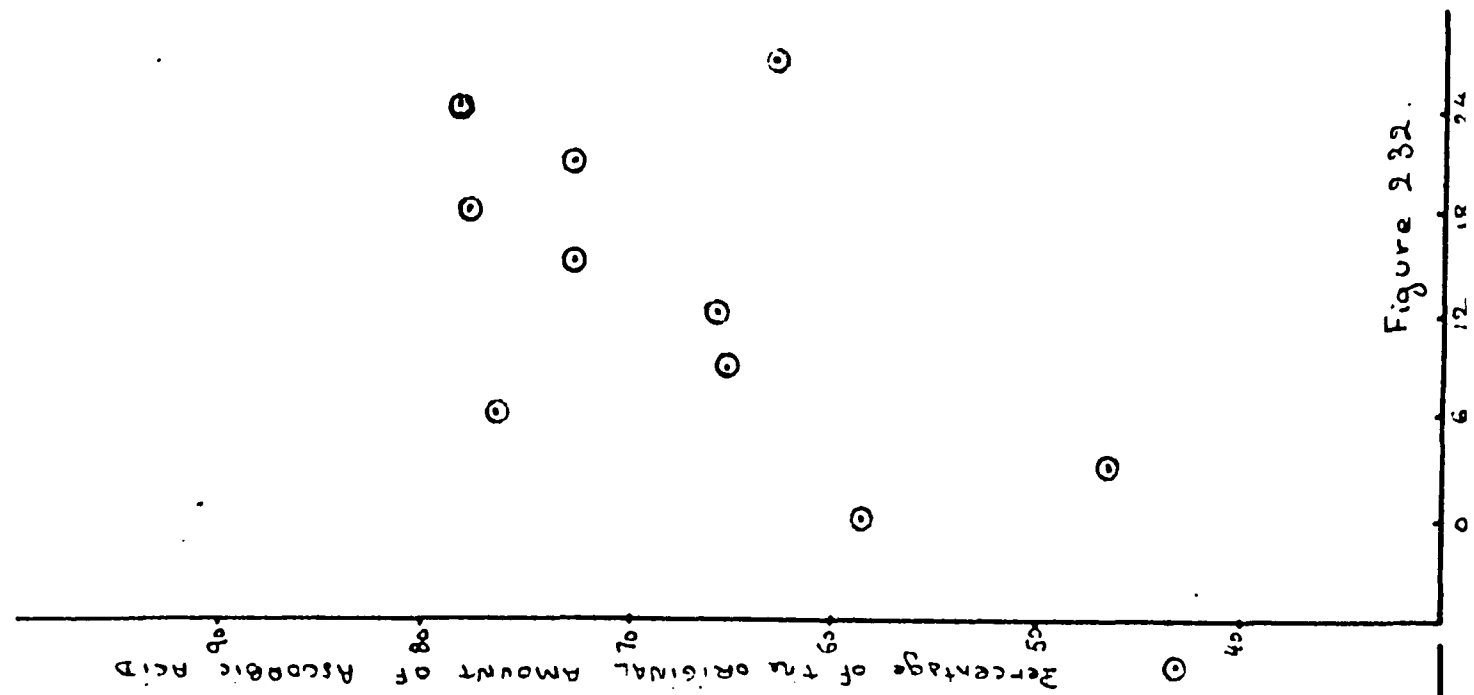
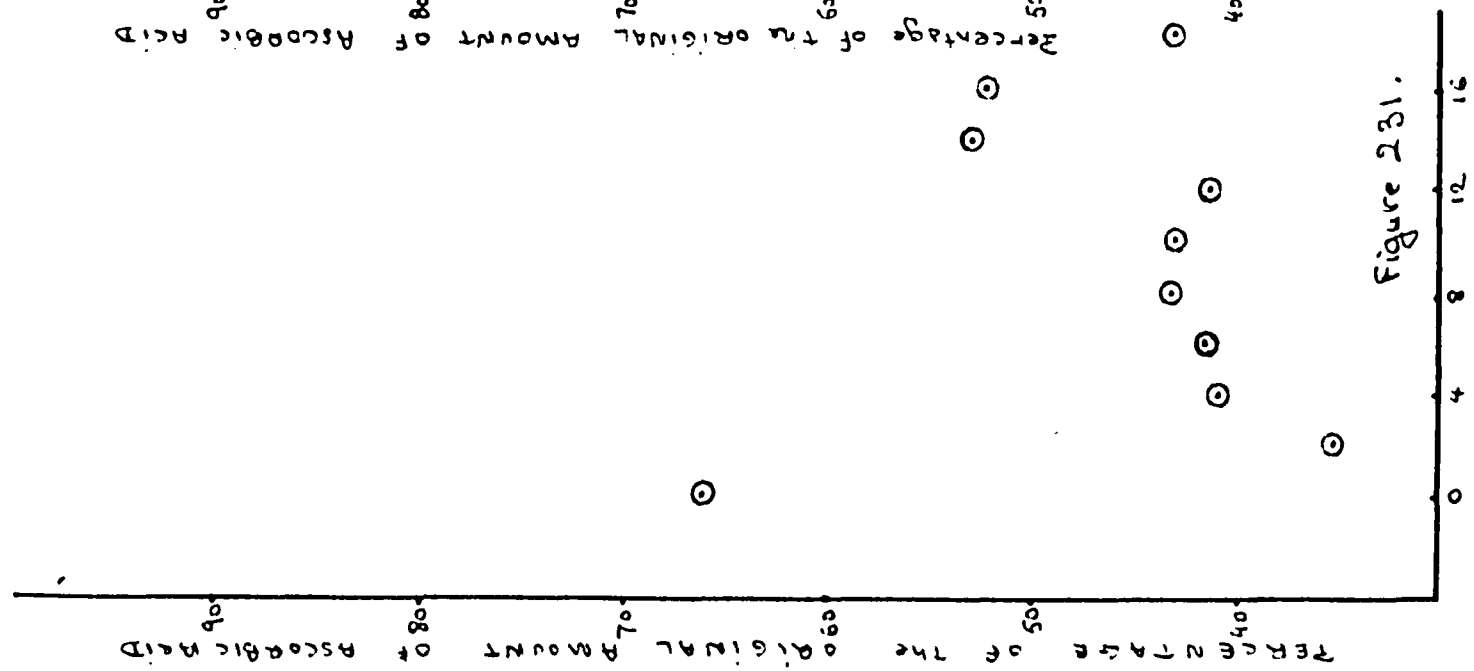
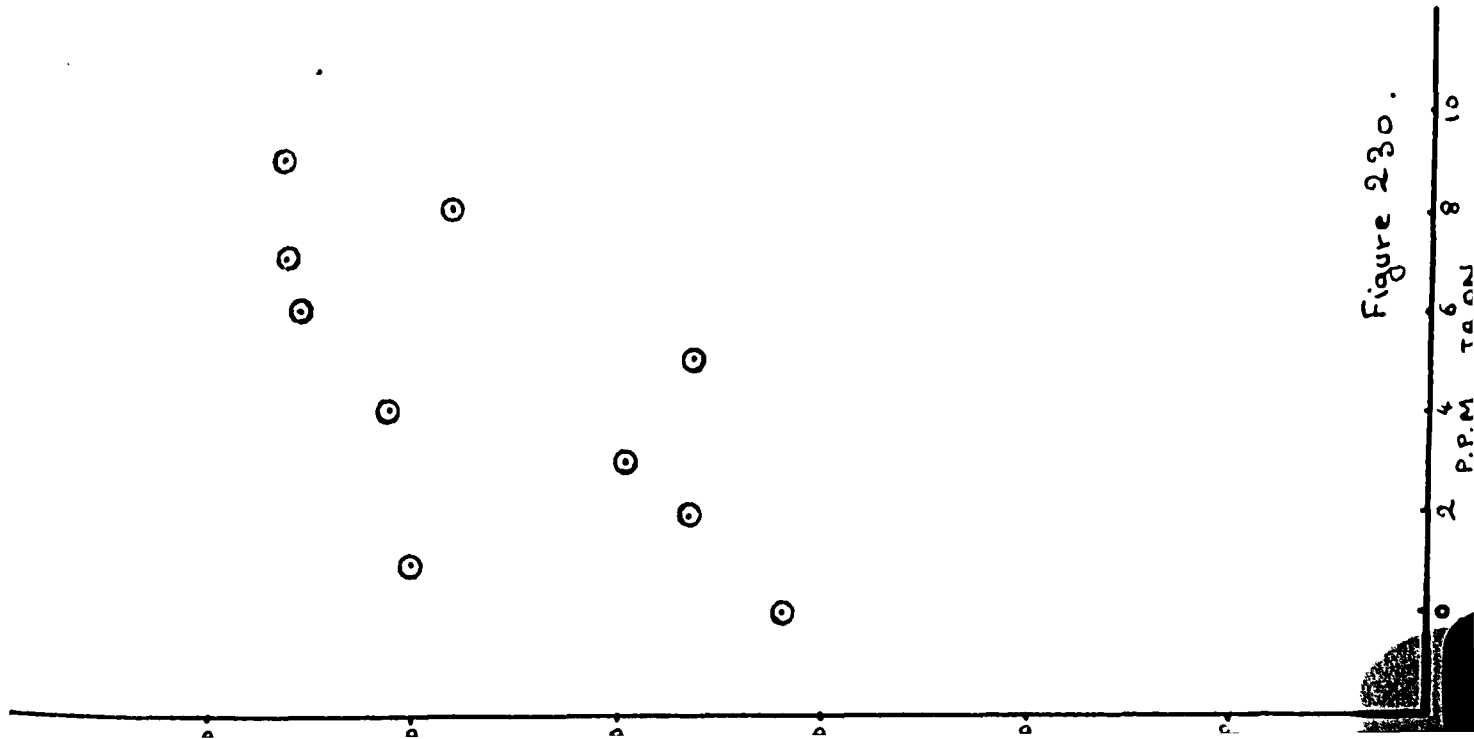


Figure 229.

Figure 230. Percentages of the original amounts of ascorbic acid, just after drying, in the tissues from wheat plants of the first experiment, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 231. Percentages of the original amounts of ascorbic acid, just after drying, in the tissues from wheat plants of the second experiment, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.

Figure 232. Percentages of the original amounts of ascorbic acid, 14 hours after harvest, in the tissues from wheat plants of the third experiment, plotted against the concentration of iron in the nutrient solution, expressed in parts per million.



Experiment 4.

Table 37. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 10 plants.(tops)

| Parts per million<br>of iron in the<br>nutrient solution | Grams of<br>fresh weight | Milligrams of<br>iron | Milligrams of<br>manganese | Milligrams of<br>ascorbic acid | Grams of<br>dry weight |
|----------------------------------------------------------|--------------------------|-----------------------|----------------------------|--------------------------------|------------------------|
| 0.0                                                      | 36.1                     | 0.845                 | 0.811                      | 28.99                          | 4.8                    |
| 0.5                                                      | 39.9                     | 0.936                 | 0.796                      | 26.09                          | 5.2                    |
| 1.0                                                      | 44.4                     | 1.017                 | 0.918                      | 26.11                          | 6.2                    |
| 5.0                                                      | 41.0                     | 0.912                 | 0.784                      | 28.99                          | 5.3                    |
| 10.0                                                     | 42.0                     | 0.952                 | 0.812                      | 26.38                          | 5.6                    |

Experiment 4.

Table 38. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of fresh material from the tops and the roots of the plants.

| PLANTS TOPS                                              |                       |                            |                                |                        | PLANTS ROOTS                   |
|----------------------------------------------------------|-----------------------|----------------------------|--------------------------------|------------------------|--------------------------------|
| Parts per million<br>of iron in the<br>nutrient solution | Milligrams of<br>iron | Milligrams of<br>manganese | Milligrams of<br>ascorbic acid | Grams of<br>dry matter | Milligrams of<br>ascorbic acid |
| 0.0                                                      | 2.46                  | 2.37                       | 80.3                           | 14.0                   | 3.79                           |
| 0.5                                                      | 2.52                  | 2.14                       | 65.4                           | 14.0                   | 2.11                           |
| 1.0                                                      | 2.53                  | 2.28                       | 56.8                           | 15.4                   | 4.80                           |
| 5.0                                                      | 2.55                  | 2.19                       | 70.7                           | 14.8                   | 6.20                           |
| 10.0                                                     | 2.48                  | 2.12                       | 62.8                           | 14.6                   | 3.95                           |

Experiment 4.

Table 39. Experimental results obtained from wheat plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of dry material from the tops and the roots of the plants.

| PLANTS TOPS                                        |                             |                    |                         | PLANTS ROOTS       |                         |
|----------------------------------------------------|-----------------------------|--------------------|-------------------------|--------------------|-------------------------|
| Parts per million of iron in the nutrient solution | Milligrams of ascorbic acid | Milligrams of iron | Milligrams of manganese | Milligrams of iron | Milligrams of manganese |
| 0.0                                                | 573.6                       | 17.6               | 16.9                    | 14.3               | 2.5                     |
| 0.5                                                | 467.1                       | 18.0               | 15.3                    | 45.0               | 10.0                    |
| 1.0                                                | 381.8                       | 16.4               | 14.8                    | 43.5               | 4.5                     |
| 5.0                                                | 477.7                       | 17.2               | 14.8                    | 65.0               | 4.8                     |
| 10.0                                               | 430.1                       | 17.0               | 14.5                    | 100.0              | 5.5                     |

Figure 233. Average amounts of iron, expressed in milligrams per 10 wheat plants, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 234. Average amounts of manganese, expressed in milligrams per 10 wheat plants, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 235. Average amounts of ascorbic acid at harvest, expressed in milligrams per 10 wheat plants, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 236. Average fresh weight, expressed in grams per 10 wheat plants, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.



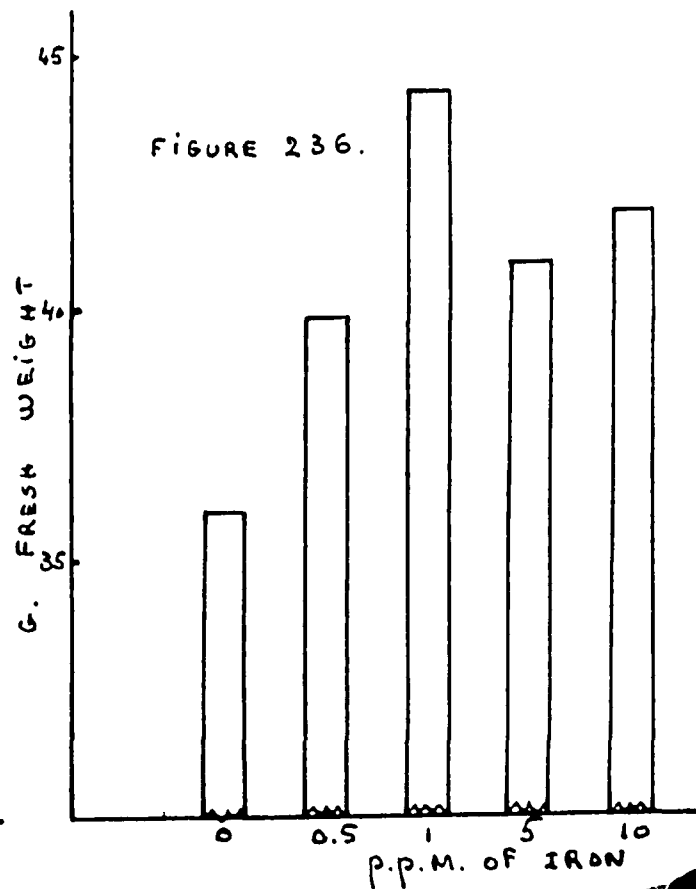
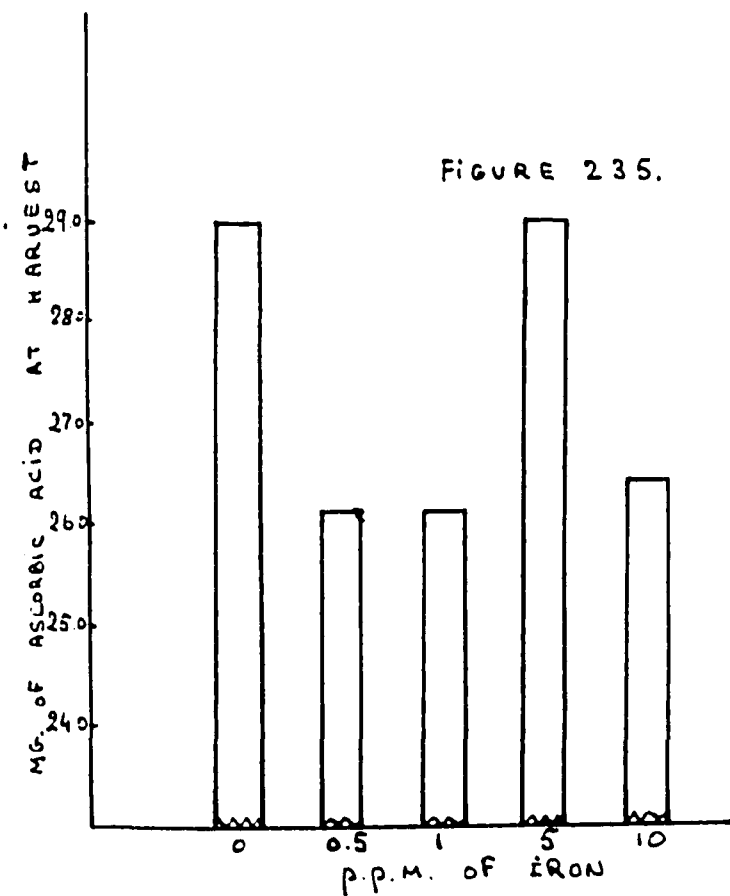
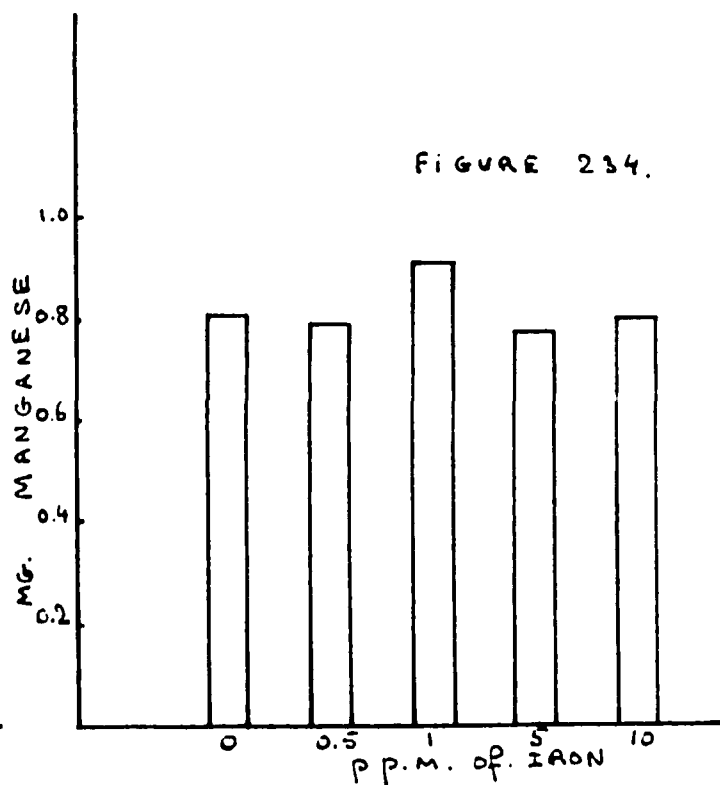
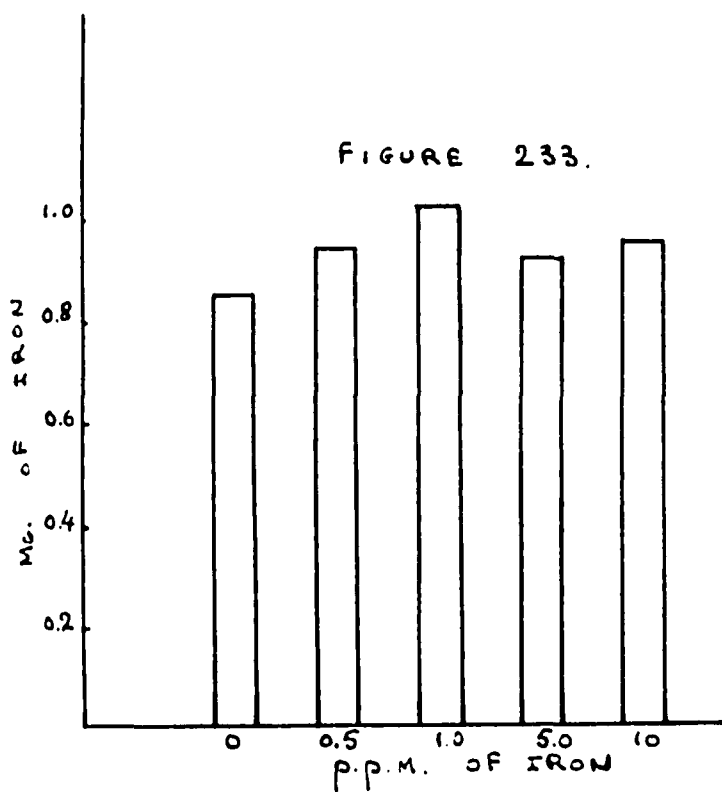


Figure 238. Average amounts of iron, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 239. Average amounts of manganese, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 240. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 241. Average amounts of dry weight, expressed in grams per 100 grams of fresh matter from wheat plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

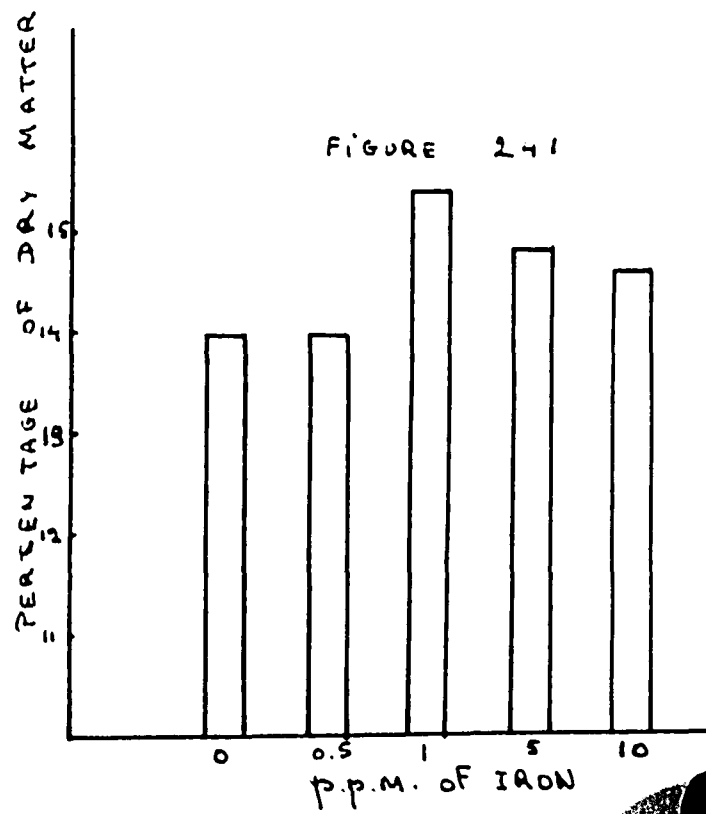
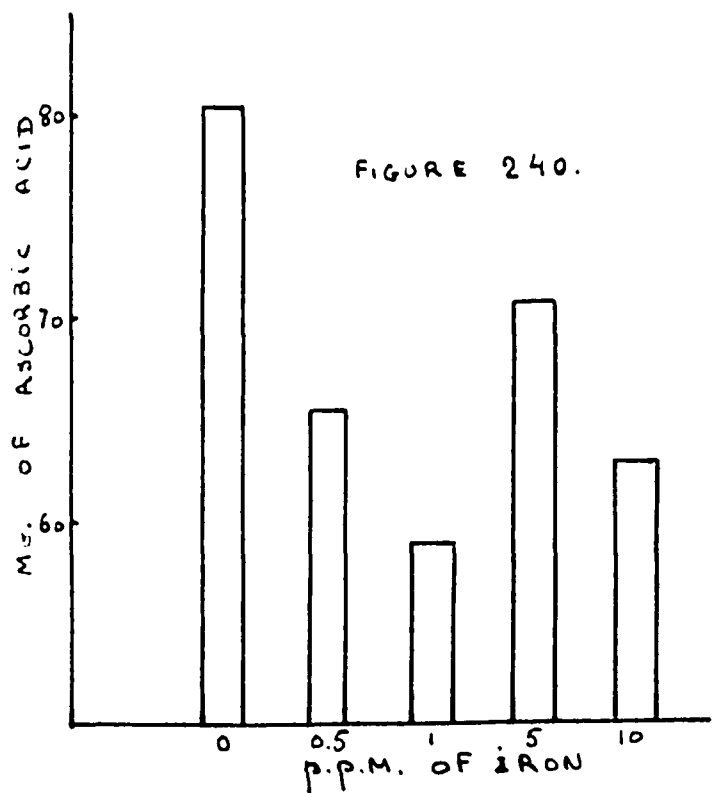
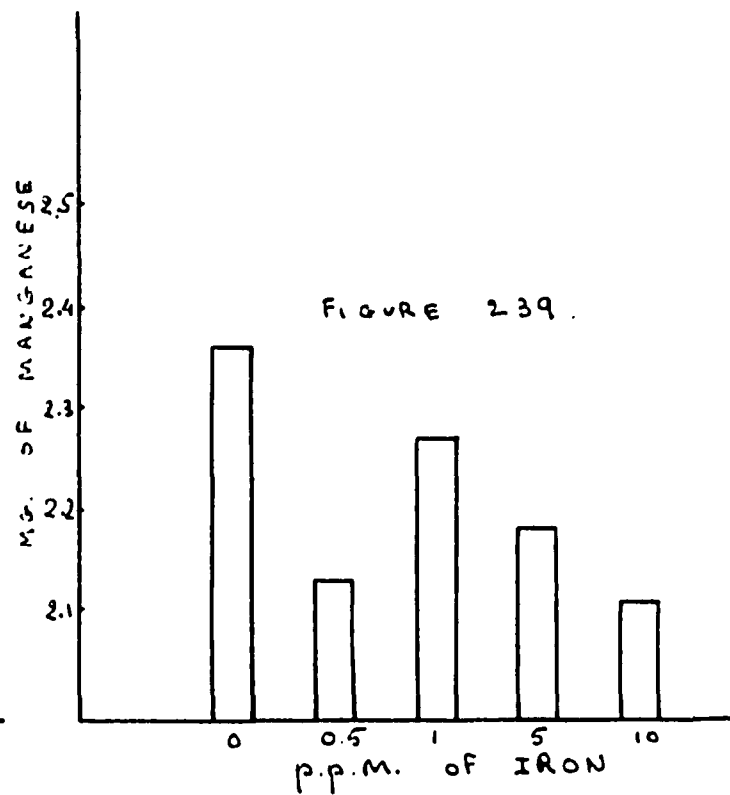
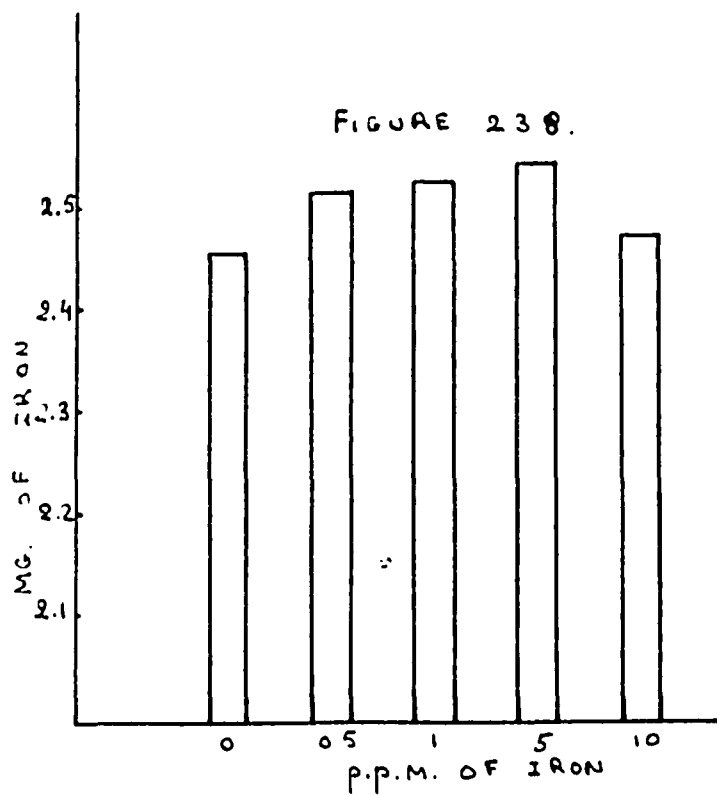


Figure 242. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from wheat roots, grown on nutrient solution supplied with various amounts of iron, expressed in parts per million.

Figure 243. Average amounts of iron, expressed in milligrams per 100 grams of dry material from wheat tops, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 244. Average amounts of manganese, expressed in milligrams per 100 grams of dry material from wheat tops, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 245. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of dr. material from wheat tops, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

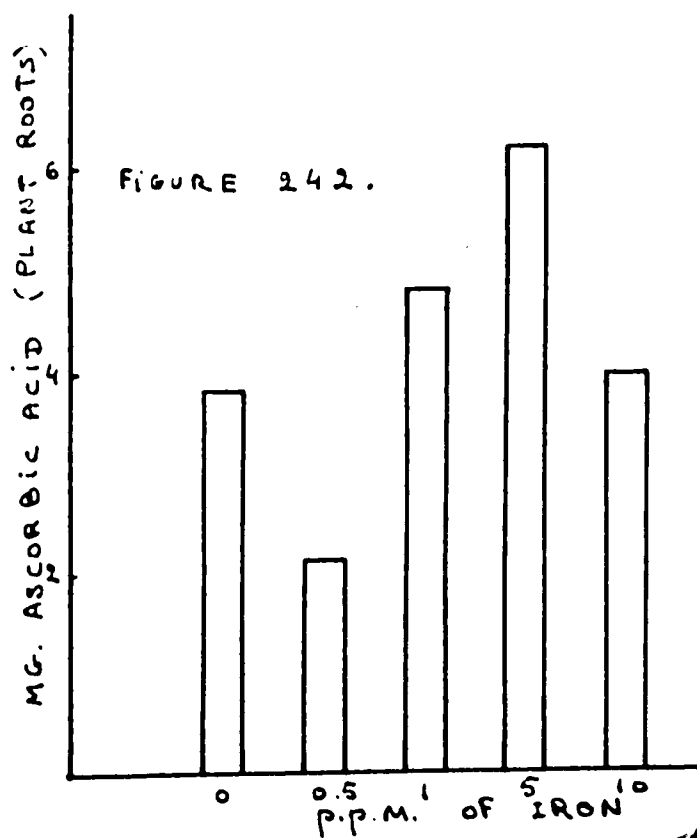
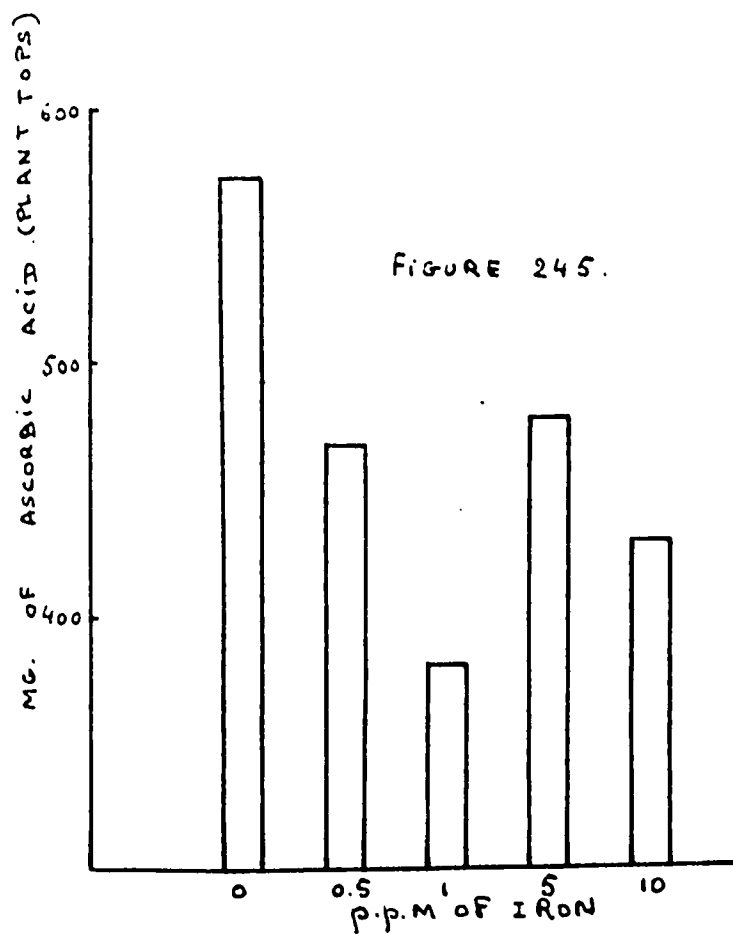
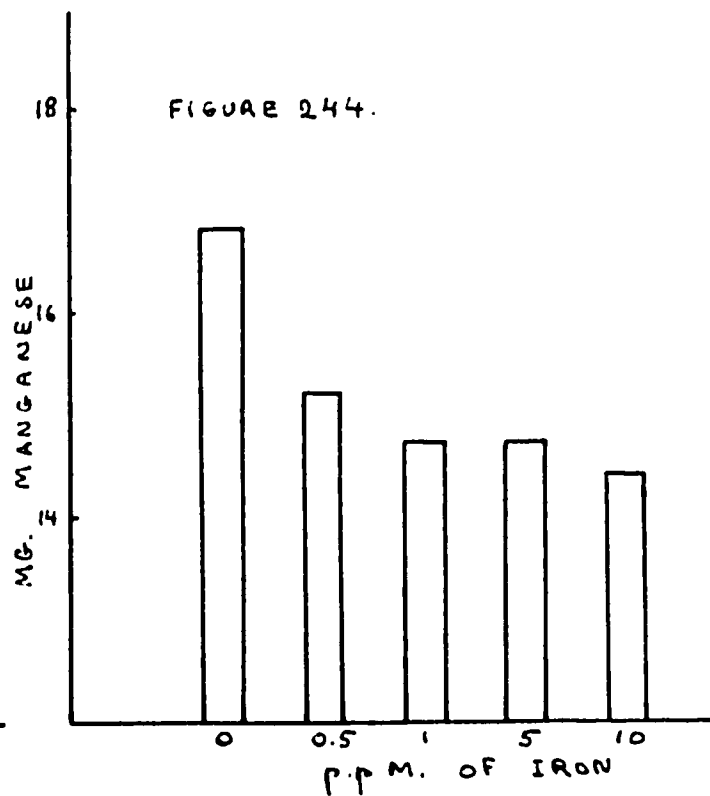
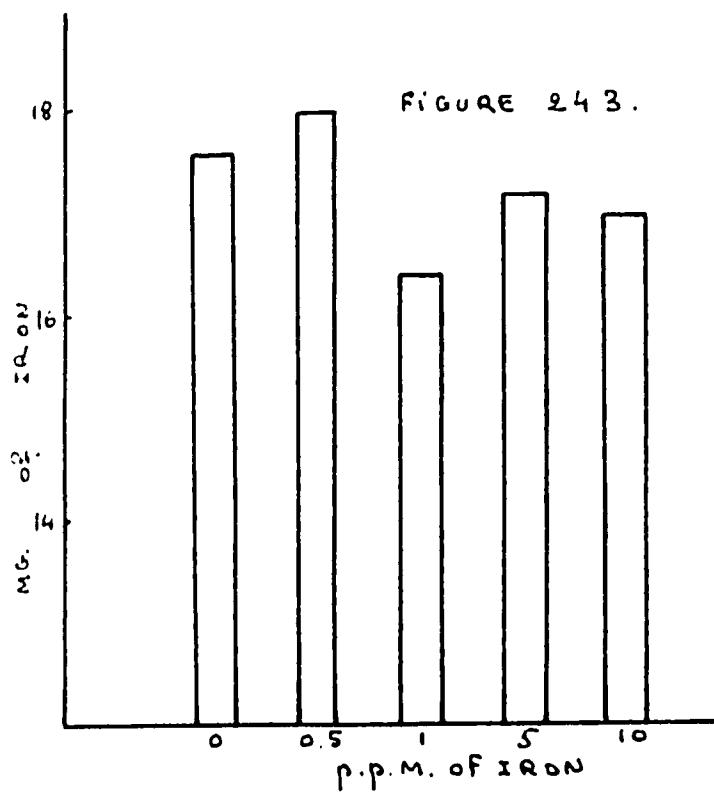
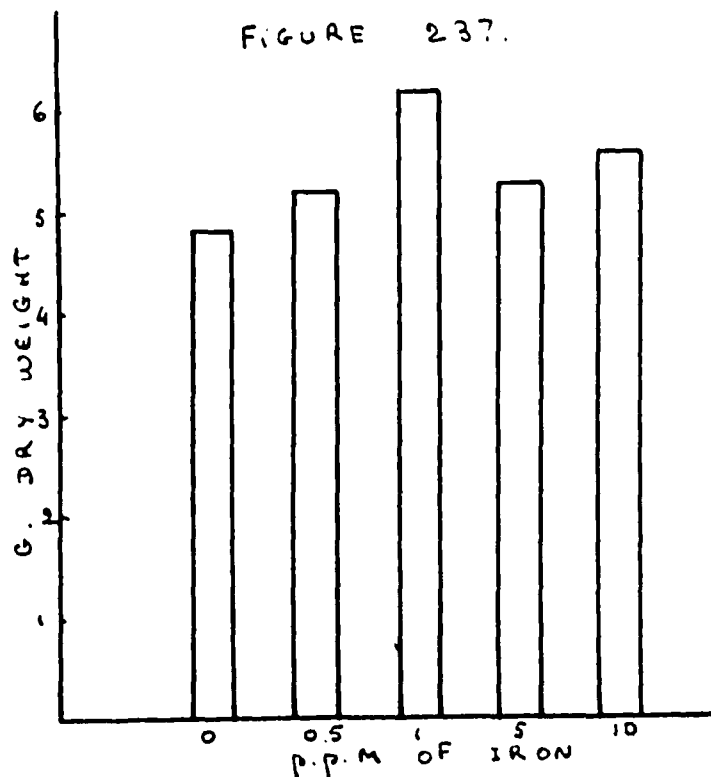
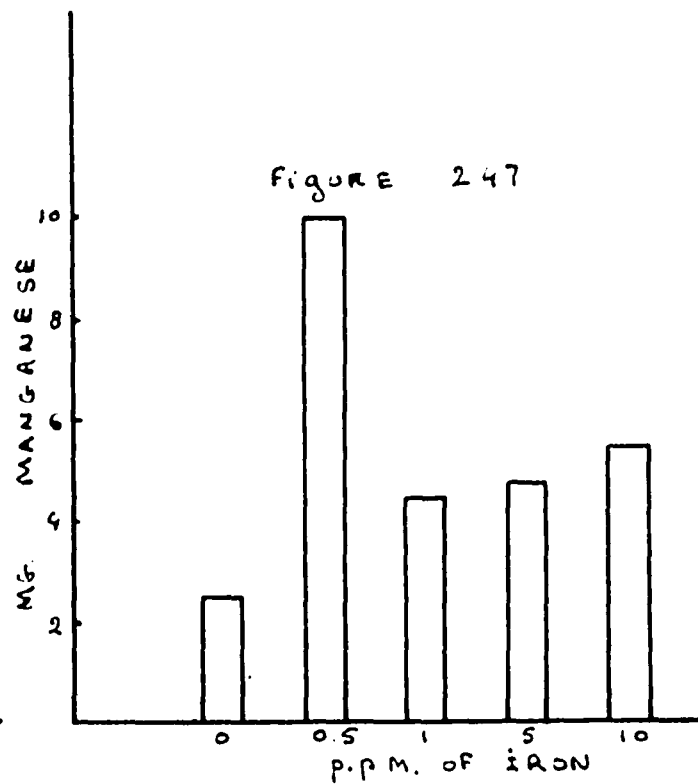
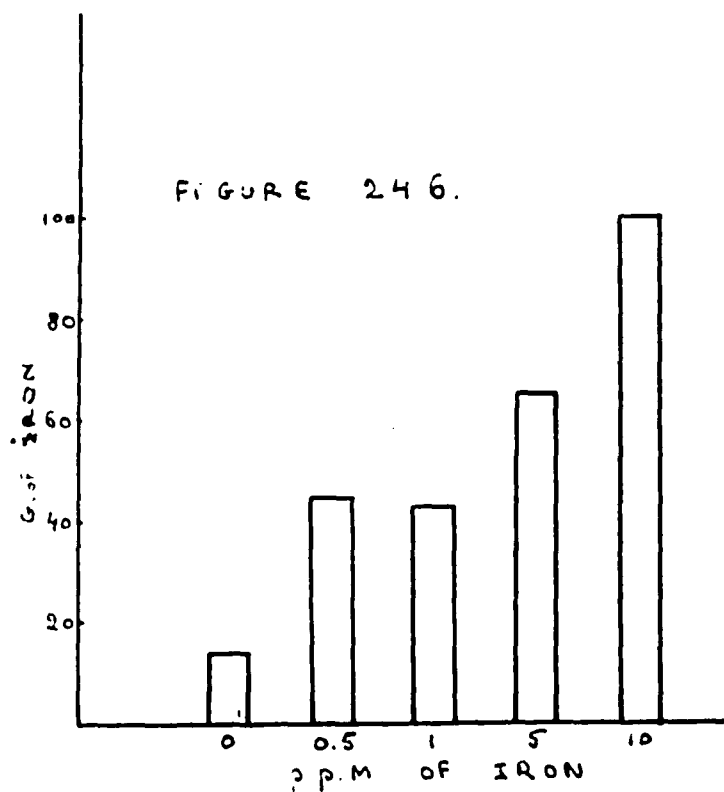


Figure 246. Average amounts of iron, expressed in milligrams per 100 grams of dry material from wheat roots grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 247. Average amounts of manganese, expressed in milligrams per 100 grams of dry material from wheat roots, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 237. Average dry weight, expressed in grams per 10 wheat tops, grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.



Experiment 5.

Table 40. Experimental results obtained from tomato plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of fresh material from the basal and the terminal leaves of the plants.

| Parts per million of iron in the nutrient solution | Grams of dry matter |                 | Milligrams of ascorbic acid |                 | Milligrams of iron |                 | Milligrams of manganese |                 |
|----------------------------------------------------|---------------------|-----------------|-----------------------------|-----------------|--------------------|-----------------|-------------------------|-----------------|
|                                                    | Basal leaves        | Terminal leaves | Basal leaves                | Terminal leaves | Basal leaves       | Terminal leaves | Basal leaves            | Terminal leaves |
| 0.0                                                | 14.0                | 18.0            | 55.2                        | 61.6            | 84.1               | 32.9            | 23.13                   | 12.0            |
| 0.5                                                | 13.0                | 17.0            | 50.1                        | 70.3            | 90.6               | 30.0            | 16.37                   | 9.6             |
| 1.0                                                | 13.3                | 20.0            | 57.7                        | 77.1            | 79.8               | 32.7            | 20.75                   | 10.7            |
| 5.0                                                | 12.4                | 15.3            | 45.8                        | 78.9            | 75.8               | 42.1            | 20.82                   | 13.7            |
| 10.0                                               | 12.7                | 15.3            | 52.8                        | 89.7            | 76.0               | 35.0            | 18.38                   | 16.6            |



Experiment 5

Table 41. Experimental results obtained from tomato plants grown on nutrient solutions varying in concentrations of iron; averages expressed per 100 grams of dry material from the basal and the terminal leaves of the plants.

| Parts per<br>million of<br>iron in the<br>nutrient<br>solution | Milligrams of<br>ascorbic acid |                    | Milligrams of<br>iron |                    | Milligrams of<br>manganese |                    |
|----------------------------------------------------------------|--------------------------------|--------------------|-----------------------|--------------------|----------------------------|--------------------|
|                                                                | Basal<br>leaves                | Terminal<br>leaves | Basal<br>leaves       | Terminal<br>leaves | Basal<br>leaves            | Terminal<br>leaves |
| 0.0                                                            | 394                            | 342                | 11.8                  | 6.0                | 3.24                       | 2.16               |
| 0.5                                                            | 385                            | 414                | 11.8                  | 5.1                | 2.13                       | 1.63               |
| 1.0                                                            | 434                            | 386                | 10.6                  | 6.5                | 2.76                       | 2.13               |
| 5.0                                                            | 369                            | 516                | 9.4                   | 6.4                | 2.58                       | 2.10               |
| 10.0                                                           | 416                            | 586                | 9.6                   | 5.4                | 2.33                       | 2.53               |

Figure 248. Average amounts of iron , expressed in milligrams per hundred grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 249. Average amounts of manganese, expressed in milligrams per hundred grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

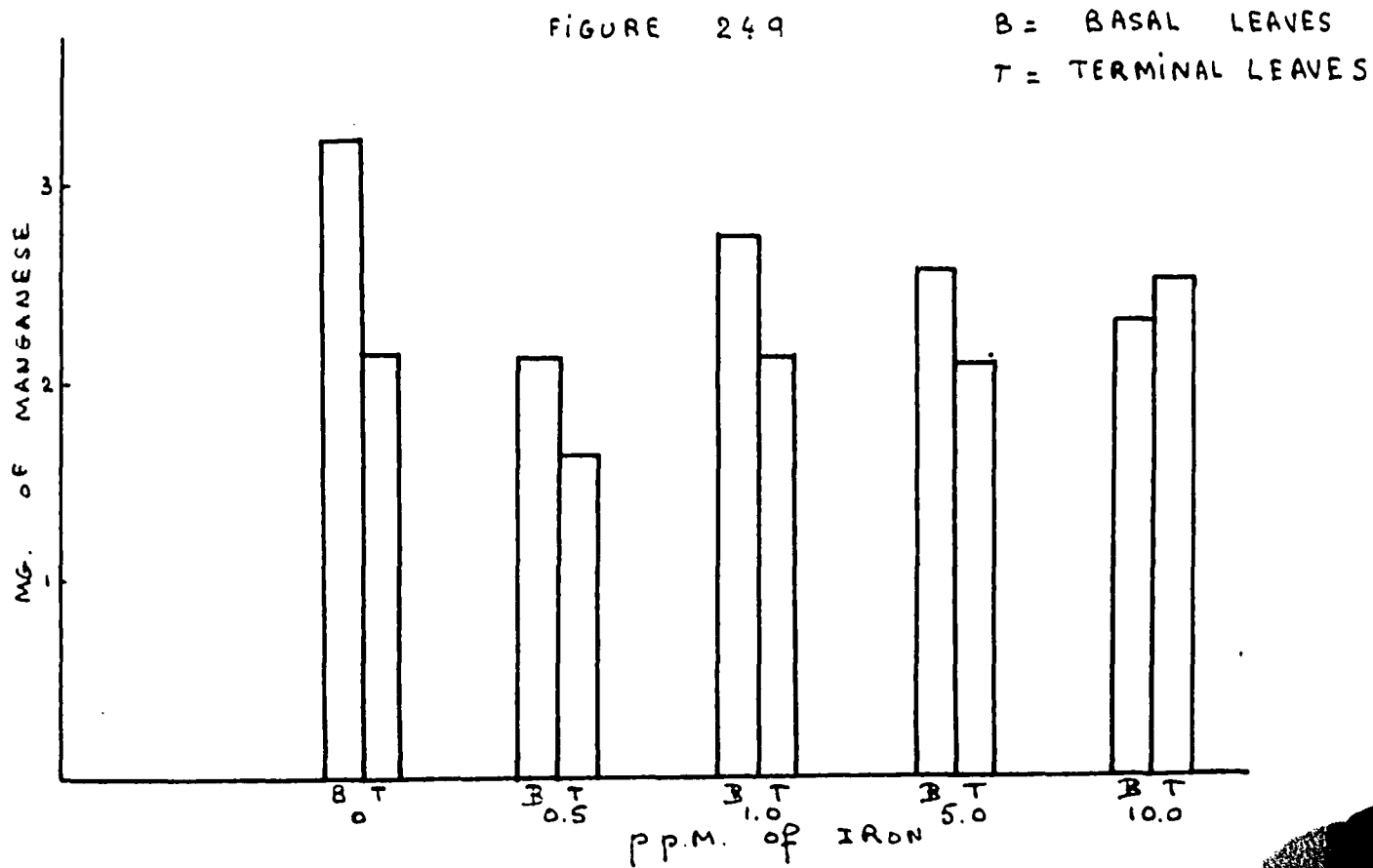
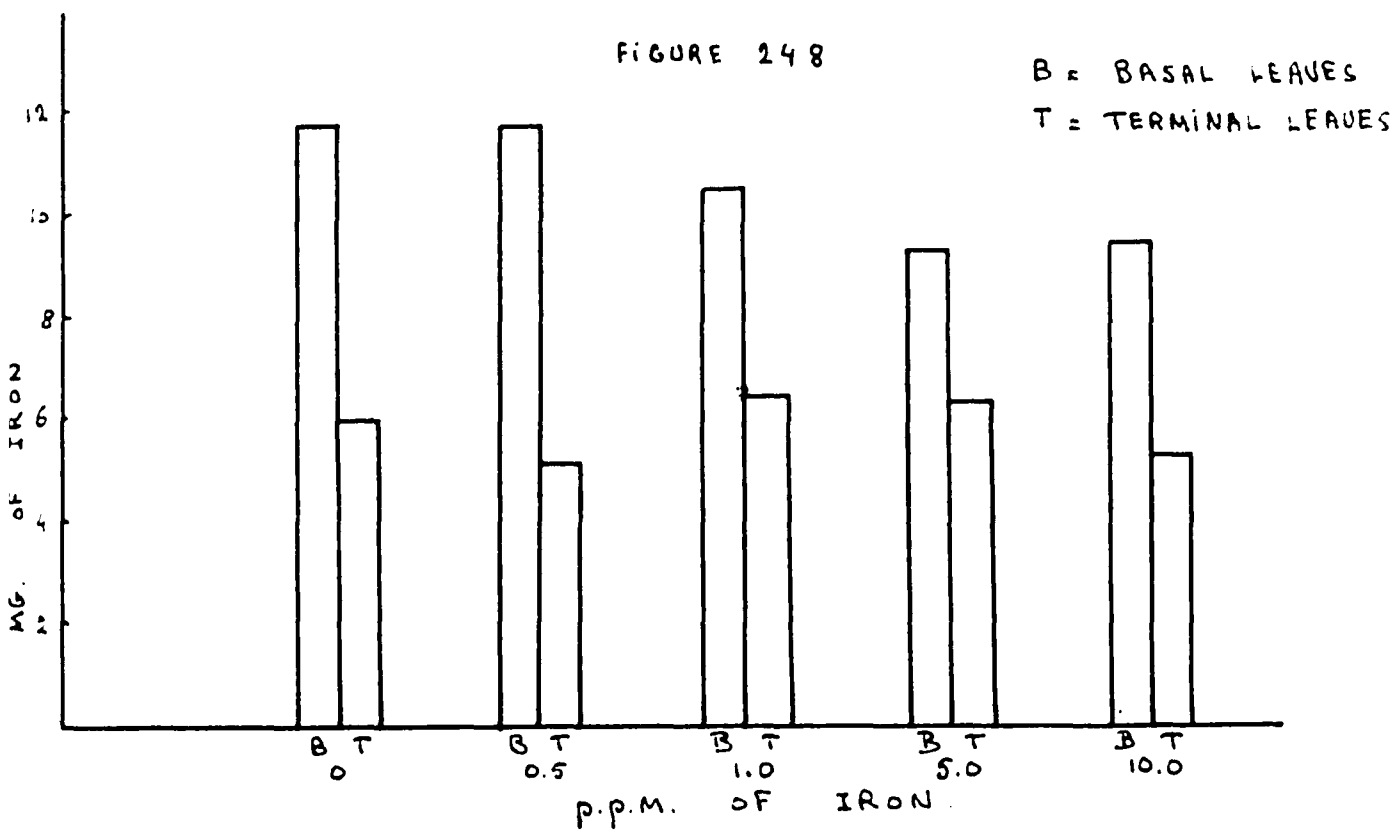


Figure 250. Average amounts of dry matter, expressed in grams per 100 grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 251. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solution supplied with various amounts of iron, expressed in parts per million.

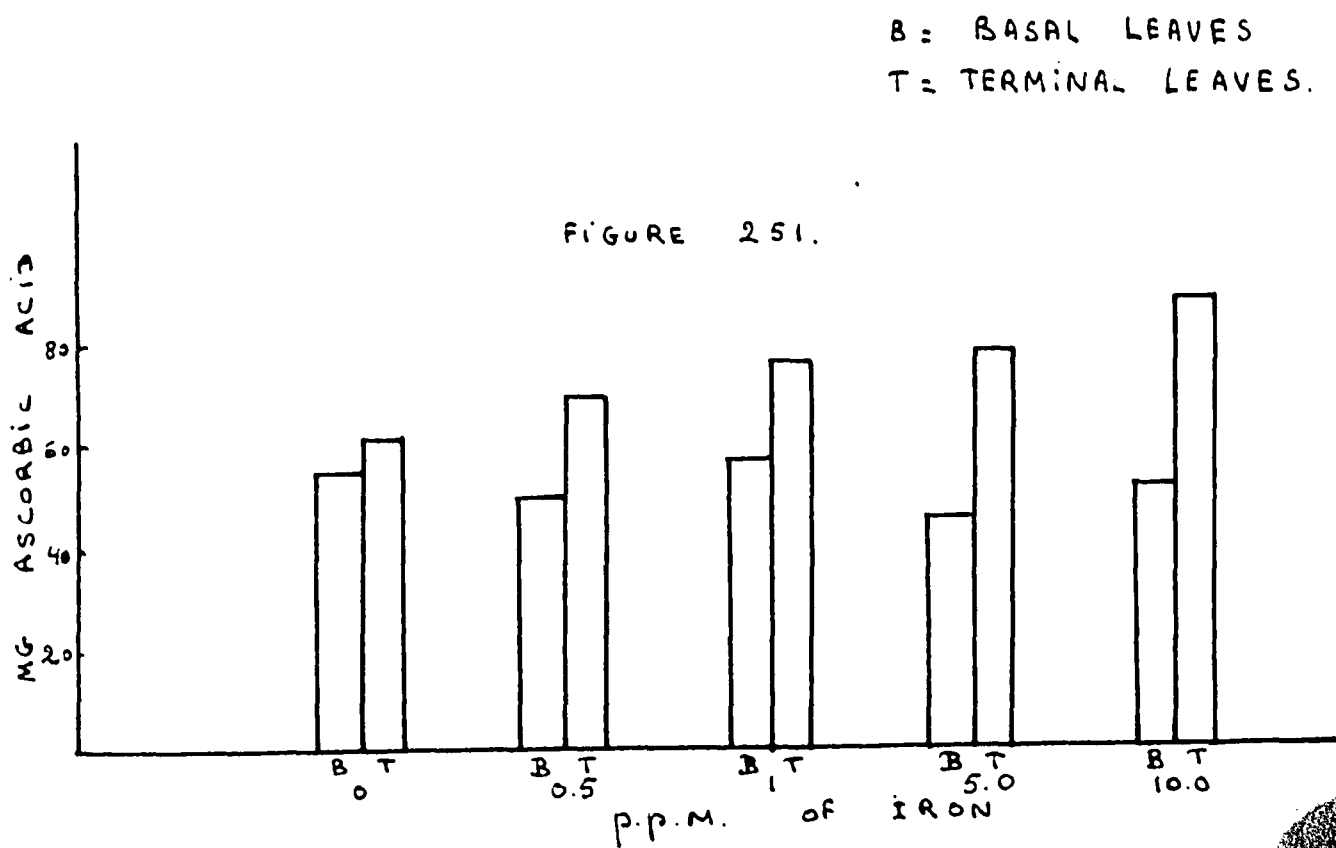
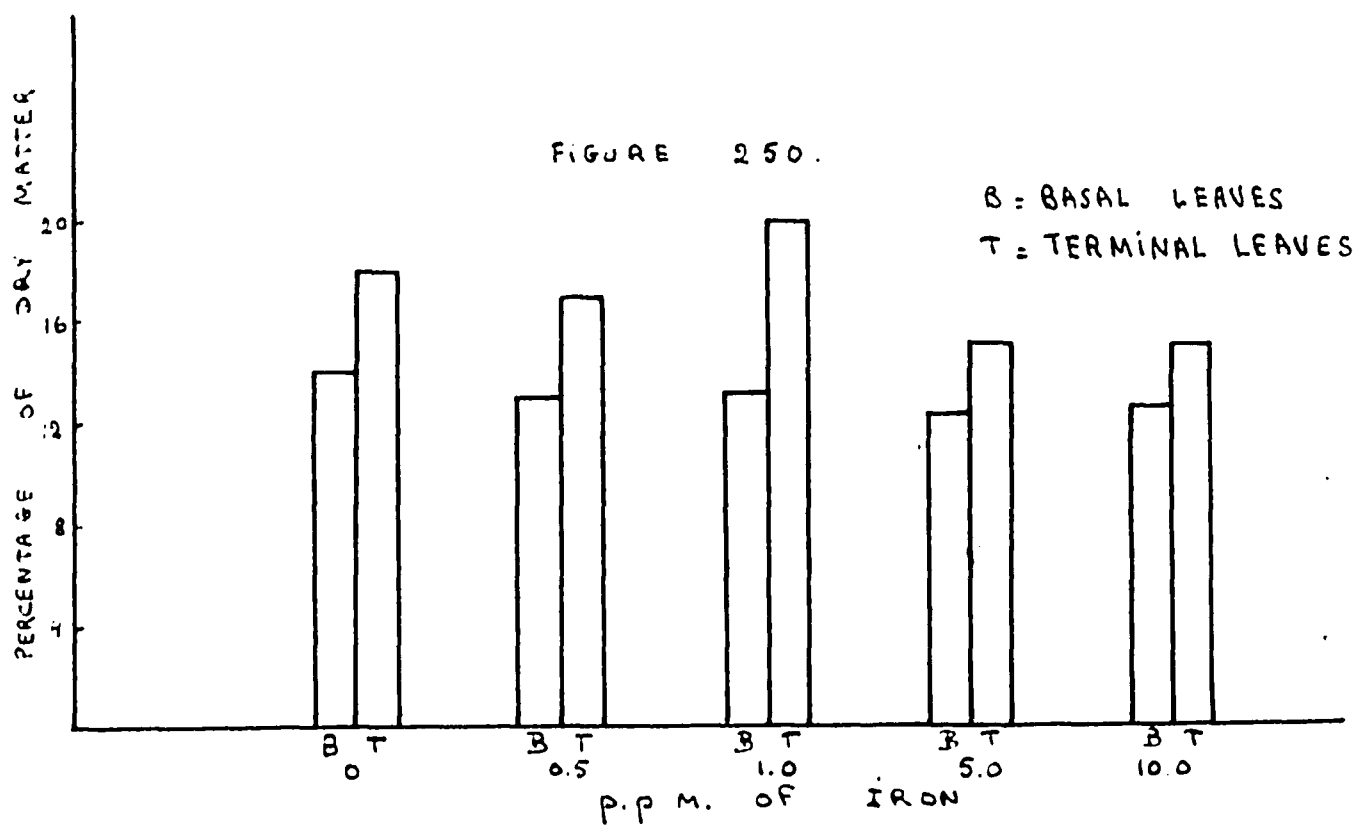


Figure 252. Average amounts of iron, expressed in milligrams per 100 grams of dry matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 253. Average amounts of manganese, expressed in milligrams per 100 grams of dry matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

FIGURE 252.

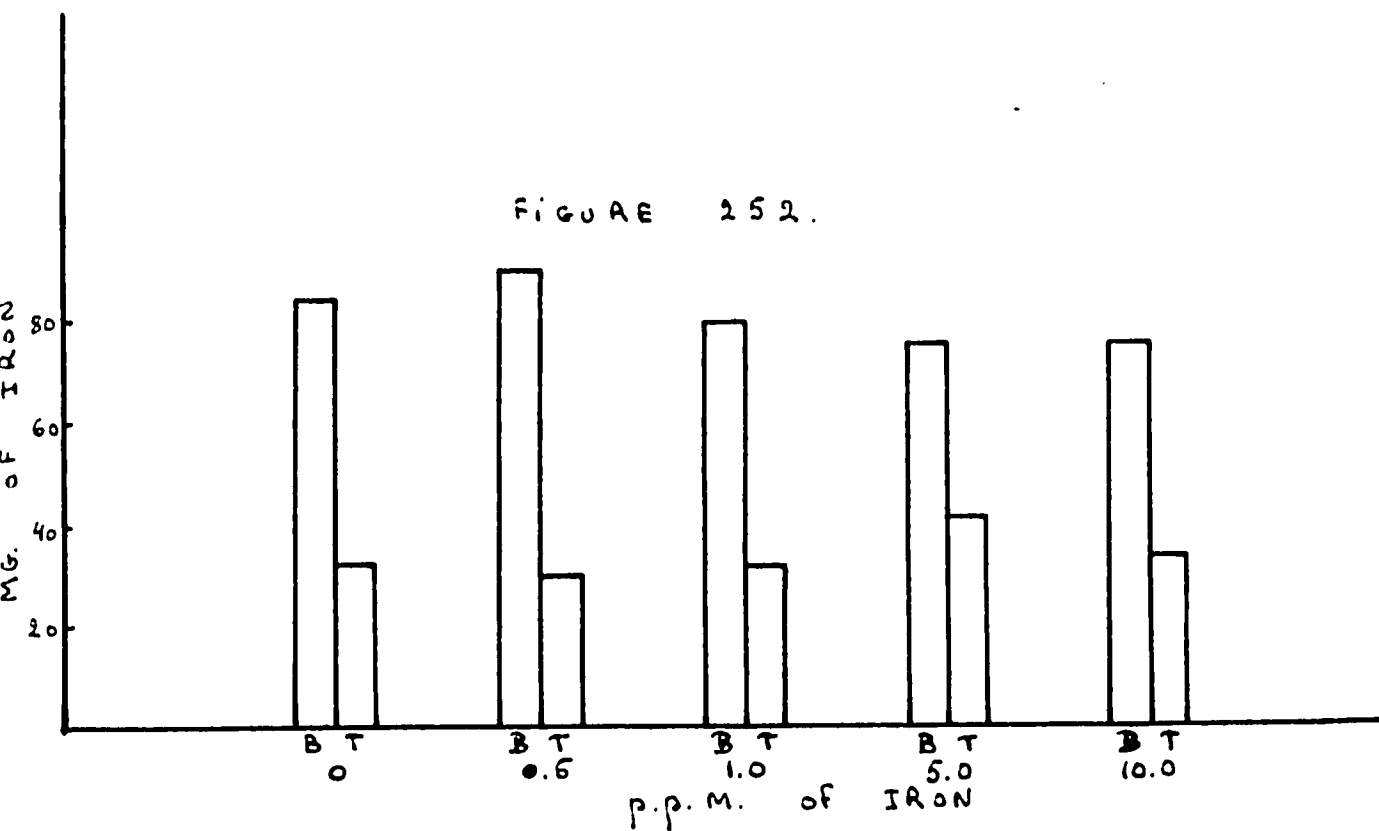


FIGURE 253.

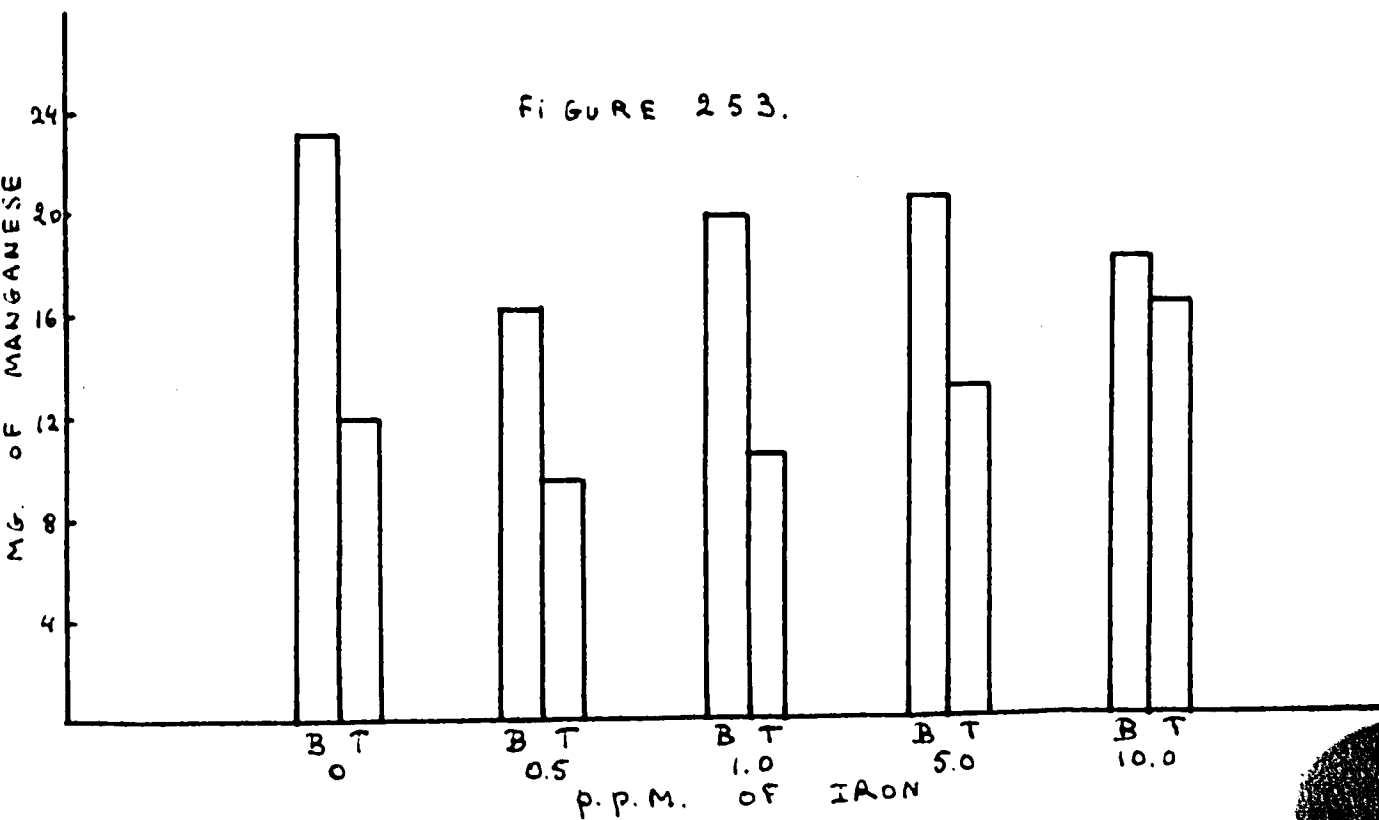


Figure 254. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of dry matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, expressed in parts per million.

Figure 255. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry matter from the basal and the terminal leaves of tomato plants grown on nutrient solution supplied with various amounts of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of dry matter.

Figure 256. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of dry matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amounts of manganese, expressed in milligrams per 100 grams of dry matter.



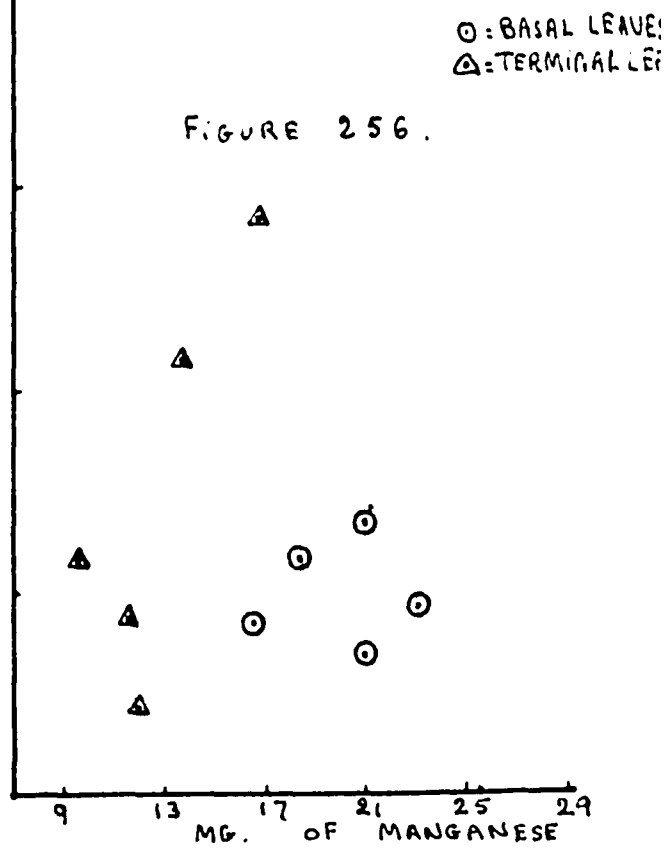
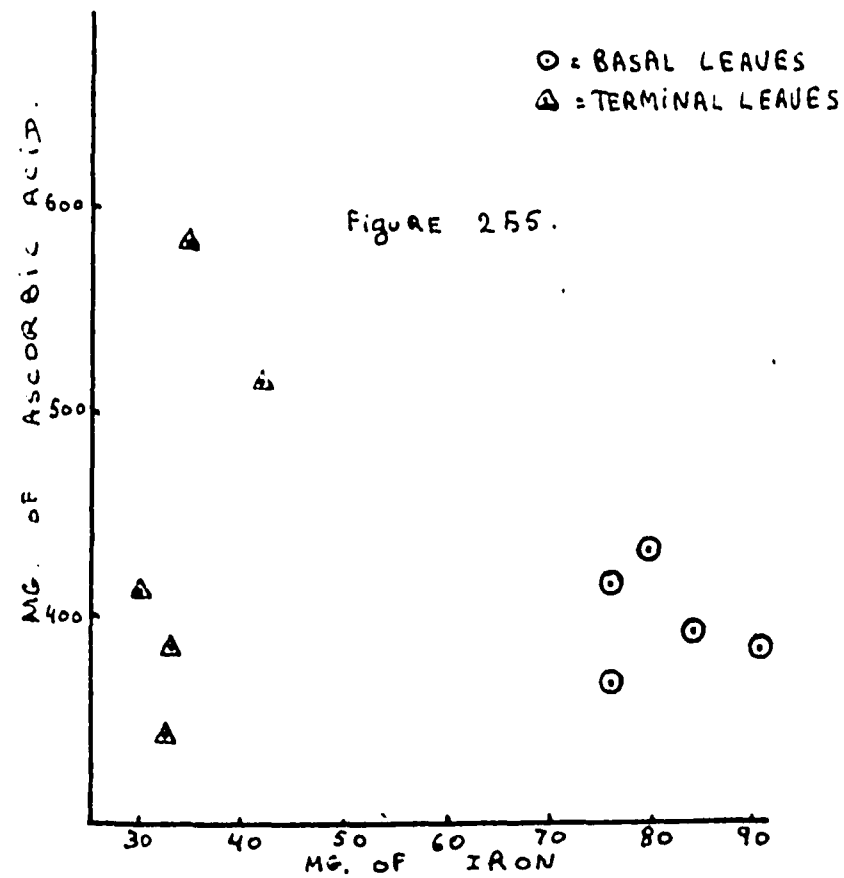
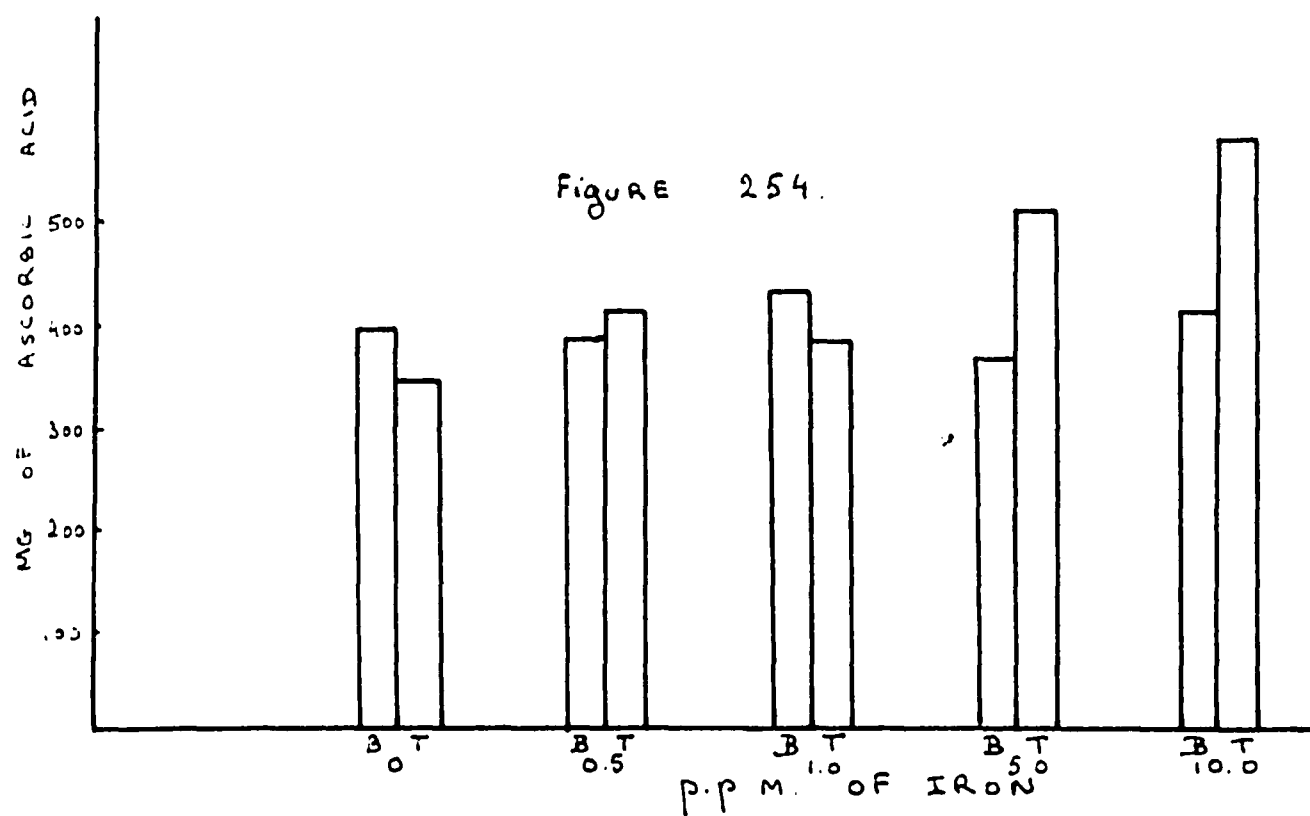
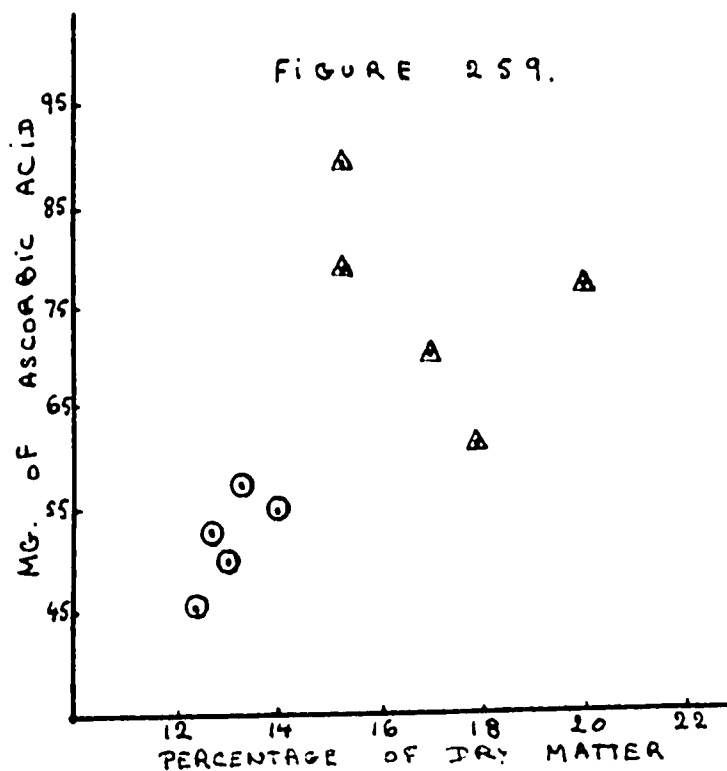
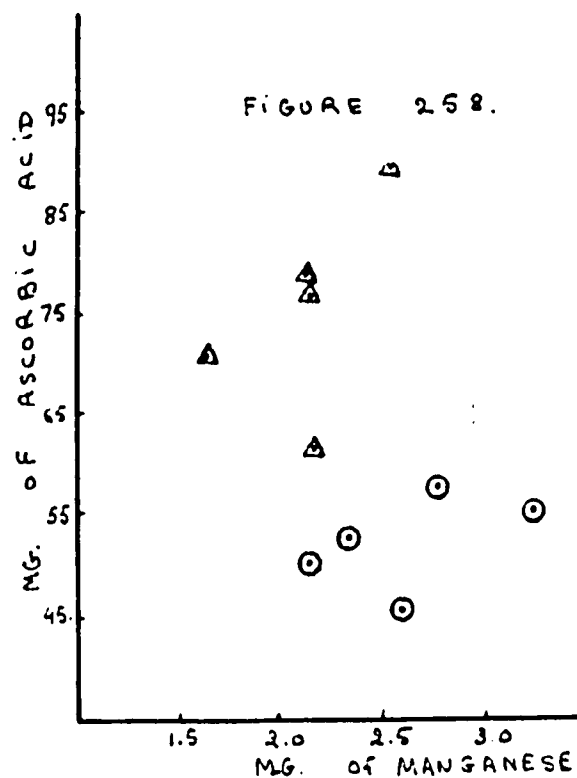
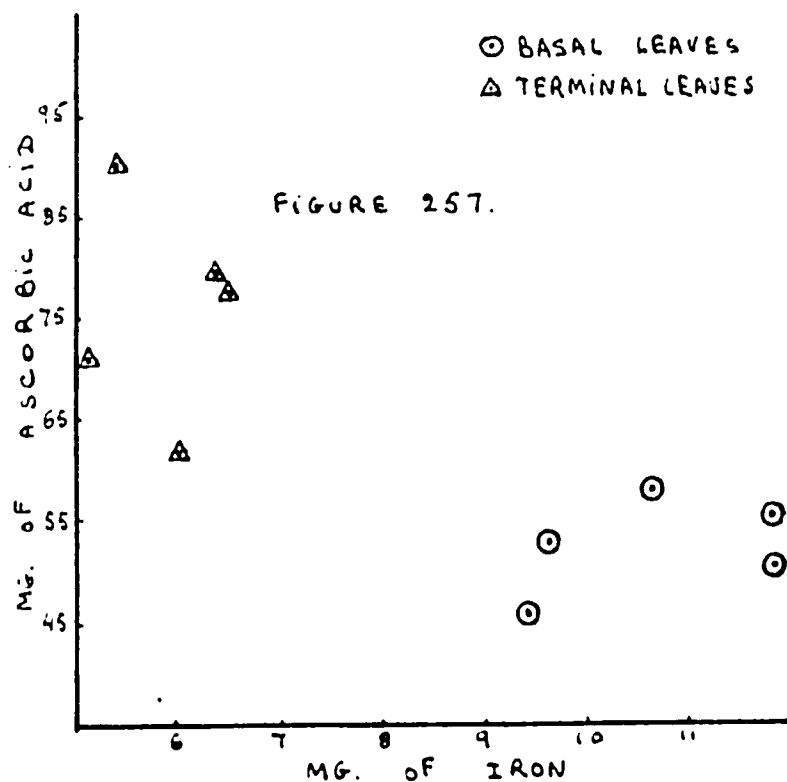


Figure 257. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amounts of iron, expressed in milligrams per 100 grams of fresh matter.

Figure 258. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amounts of manganese, expressed in milligrams per 100 grams of fresh matter.

Figure 259. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the basal and the terminal leaves of tomato plants grown on nutrient solutions supplied with various amounts of iron, plotted against the amounts of dry matter, expressed in grams per 100 grams of fresh matter.



Experiment 6

Table 42. Average amounts of iron in milligrams per 100 grams of fresh matter from tomato plants grown on nutrient solutions containing no copper and 0.04 parts per million of copper and various concentrations of iron at intervals during the growth of the plants.

| Parts per million<br>of copper in the<br>nutrient solution | Parts per million<br>of iron in the<br>nutrient solution | November 25, 1950<br>58 days old | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------|----------------------------------------------------------|----------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                            |                                                          |                                  | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                       | 0.0                                                      | -----                            | -----                            | 1.000        | 1.234                          | 1.048        |
| 0.000                                                      | 0.5                                                      | 0.855                            | 1.347                            | 1.092        | 0.926                          | 0.917        |
| 0.00                                                       | 1.0                                                      | 0.685                            | 1.163                            | 1.032        | 1.092                          | 1.300        |
| 0.00                                                       | 5.0                                                      | 0.992                            | 1.172                            | 1.155        | 1.227                          | 1.169        |
| 0.00                                                       | 10.0                                                     | 2.322                            | 2.002                            | 1.254        | 1.391                          | 1.196        |
| 0.04                                                       | 0.0                                                      | 0.924                            | 1.272                            | 1.291        | 0.985                          | 1.079        |
| 0.04                                                       | 0.5                                                      | 0.537                            | 1.380                            | 1.010        | 1.075                          | 1.096        |
| 0.04                                                       | 1.0                                                      | 0.813                            | 1.452                            | 0.974        | 1.051                          | 1.197        |
| 0.04                                                       | 5.0                                                      | 0.655                            | 1.328                            | 0.747        | 1.607                          | 1.487        |
| 0.04                                                       | 10.0                                                     | 1.663                            | 1.253                            | 0.762        | 0.855                          | 1.284        |

Experiment 6.

Table 43. Average amounts of manganese in milligrams per 100 grams of fresh matter from tomato plants grown on nutrient solutions containing no copper and 0.04 parts per million of copper and various concentrations of iron at interval during the growth of the plants.

| Parts per million<br>of copper in the<br>nutrient solution | Parts per million<br>of iron in the<br>nutrient solution | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------|----------------------------------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                            |                                                          | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                       | 0.0                                                      | -----                            | 1.000        | 0.552                          | 1.361        |
| 0.00                                                       | 0.5                                                      | 0.770                            | 1.027        | 0.647                          | 1.288        |
| 0.00                                                       | 1.0                                                      | 0.454                            | 0.969        | 0.459                          | 1.146        |
| 0.00                                                       | 5.0                                                      | 0.861                            | 0.713        | 0.361                          | 1.049        |
| 0.00                                                       | 10.0                                                     | 0.477                            | 0.845        | 0.336                          | 0.929        |
| 0.04                                                       | 0.0                                                      | 1.284                            | 1.157        | 0.760                          | 1.798        |
| 0.04                                                       | 0.5                                                      | 0.799                            | 1.043        | 0.597                          | 1.070        |
| 0.04                                                       | 1.0                                                      | 0.687                            | 0.743        | 0.678                          | 1.410        |
| 0.04                                                       | 5.0                                                      | 0.683                            | 0.476        | 0.470                          | 0.878        |
| 0.04                                                       | 10.0                                                     | 0.730                            | 0.762        | 0.392                          | 0.827        |

# Experiment 6

Table 44. Average amounts of ascorbic acid in milligrams per 100 grams of fresh matter from tomato plants grown on nutrient solutions containing no copper and 0.04 parts per million of copper and various concentrations of iron at intervals during the growth of the plants.

| Parts per<br>million of<br>copper in the<br>nutrient<br>solution | Parts per<br>million of<br>iron in the<br>nutrient<br>solution | November 25, 1950<br>58 days old | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------------|----------------------------------------------------------------|----------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                                  |                                                                |                                  | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                             | 0.0                                                            | 30.1                             | 36.7                             | 8.8          | 25.1                           | 14.1         |
| 0.00                                                             | 0.5                                                            | 29.9                             | 30.2                             | 7.6          | 21.2                           | 11.7         |
| 0.000                                                            | 1.0                                                            | 22.8                             | 32.7                             | 9.9          | 25.3                           | 10.3         |
| 0.00                                                             | 5.0                                                            | 26.5                             | 27.0                             | 8.8          | 27.7                           | 10.9         |
| 0.00                                                             | 10.0                                                           | 26.7                             | 30.9                             | 9.1          | 29.9                           | 11.7         |
| 0.04                                                             | 0.0                                                            | 29.9                             | 27.6                             | 9.5          | 24.2                           | 13.0         |
| 0.04                                                             | 0.5                                                            | 32.0                             | 28.6                             | 8.9          | 31.0                           | 10.0         |
| 0.04                                                             | 1.0                                                            | 27.4                             | 27.0                             | 7.7          | 24.6                           | 9.7          |
| 0.04                                                             | 5.0                                                            | 24.0                             | 35.4                             | 10.3         | 32.7                           | 11.0         |
| 0.04                                                             | 10.0                                                           | 27.8                             | 37.2                             | 12.6         | 21.3                           | 11.0         |

Experiment 6.

Table 45. Average dry weights, in grams per 100 grams of fresh matter from tomato plants grown on nutrient solutions containing no copper and 0.04 parts per million of copper and various concentrations of iron at intervals during the growth of the plants.

| Parts per million of copper in the nutrient solution | Parts per million of iron in the nutrient solution | November 25, 1950<br>58 days old | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------|----------------------------------------------------|----------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                      |                                                    |                                  | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                 | 0.0                                                | 5.95                             | 6.76                             | 6.68         | 8.02                           | 8.22         |
| 0.00                                                 | 0.5                                                | 5.89                             | 7.33                             | 6.64         | 7.72                           | 7.41         |
| 0.00                                                 | 1.0                                                | 6.08                             | 7.31                             | 6.64         | 7.80                           | 7.70         |
| 0.00                                                 | 5.0                                                | 6.15                             | 7.02                             | 6.80         | 7.85                           | 7.48         |
| 0.00                                                 | 10.0                                               | 6.30                             | 7.06                             | 6.66         | 7.89                           | 7.65         |
| 0.04                                                 | 0.0                                                | 7.18                             | 7.70                             | 6.94         | 8.21                           | 7.99         |
| 0.04                                                 | 0.5                                                | 7.09                             | 7.30                             | 6.85         | 7.96                           | 7.16         |
| 0.04                                                 | 1.0                                                | 7.08                             | 7.37                             | 6.61         | 7.74                           | 7.72         |
| 0.04                                                 | 5.0                                                | 6.91                             | 7.66                             | 6.97         | 7.84                           | 8.07         |
| 0.04                                                 | 10.0                                               | 6.85                             | 7.68                             | 6.79         | 8.04                           | 7.88         |

Experiment 6.

Table 46. Average amounts of iron expressed in milligrams per 100 grams of dry matter from tomato plants grown on nutrient solutions supplied with no copper and 0.04 parts per million of copper and various concentrations of iron, at intervals during the growth of the plants.

| Parts per<br>million of<br>copper in the<br>nutrient<br>solution | Parts per<br>million of<br>iron in the<br>nutrient<br>solution | November 25, 1950<br>58 days old | Decembre 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------------|----------------------------------------------------------------|----------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                                  |                                                                |                                  | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                             | 0.0                                                            | -----                            | 14.97                            | -----        | 12.75                          | 15.38        |
| 0.00                                                             | 0.5                                                            | 14.52                            | 16.44                            | 18.37        | 12.38                          | 12.00        |
| 0.00                                                             | 1.0                                                            | 11.26                            | 15.54                            | 15.91        | 16.88                          | 14.00        |
| 0.00                                                             | 5.0                                                            | 16.13                            | 16.98                            | 16.69        | 15.63                          | 15.63        |
| 0.00                                                             | 10.0                                                           | 36.89                            | 18.83                            | 28.36        | 15.63                          | 17.63        |
| 0.04                                                             | 0.0                                                            | 12.87                            | 18.60                            | 16.52        | 13.50                          | 12.00        |
| 0.04                                                             | 0.5                                                            | 7.57                             | 14.74                            | 18.90        | 15.25                          | 13.50        |
| 0.04                                                             | 1.0                                                            | 11.48                            | 14.72                            | 19.70        | 15.50                          | 13.58        |
| 0.04                                                             | 5.0                                                            | 9.48                             | 10.71                            | 17.34        | 18.43                          | 20.50        |
| 0.04                                                             | 10.0                                                           | 24.28                            | 11.22                            | 16.32        | 16.30                          | 10.63        |



Experiment 6

Table 47. Average amounts of manganese expressed in milligrams per 100 grams of dry matter from tomato plants grown on nutrient solutions supplied with no copper and 0.04 parts per million of copper and various concentrations of iron, at intervals during the growth of the plants.

| Parts per million<br>of copper in the<br>nutrient solution | Parts per million<br>of iron in the<br>nutrient solution | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------|----------------------------------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                            |                                                          | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                       | 0.0                                                      | 14.97                            | -----        | 16.56                          | 6.38         |
| 0.00                                                       | 0.5                                                      | 15.46                            | 10.50        | 17.38                          | 8.38         |
| 0.00                                                       | 1.0                                                      | 14.60                            | 6.21         | 14.88                          | 5.88         |
| 0.00                                                       | 5.0                                                      | 10.49                            | 12.26        | 14.02                          | 4.60         |
| 0.00                                                       | 10.0                                                     | 12.68                            | 6.75         | 12.14                          | 4.26         |
| 0.04                                                       | 0.0                                                      | 16.67                            | 17.90        | 22.50                          | 9.26         |
| 0.04                                                       | 0.5                                                      | 15.23                            | 10.94        | 14.88                          | 7.50         |
| 0.04                                                       | 1.0                                                      | 11.24                            | 9.32         | 18.26                          | 8.76         |
| 0.04                                                       | 5.0                                                      | 8.96                             | 8.92         | 10.88                          | 6.00         |
| 0.04                                                       | 10.0                                                     | 11.22                            | 9.50         | 10.50                          | 4.88         |

Experiment 6

Table 48. Average amounts of ascorbic acid expressed in milligrams per 100 grams of dry matter from tomato plants grown on nutrient solutions supplied with no copper and 0.04 parts per million of copper and various concentrations of iron, at intervals during the growth of the plants.

| Parts per<br>million of<br>copper in the<br>nutrient<br>solution | Parts per<br>million of<br>iron in the<br>nutrient<br>solution | November 25, 1950<br>58 days old | December 15, 1950<br>78 days old |              | January 3, 1950<br>97 days old |              |
|------------------------------------------------------------------|----------------------------------------------------------------|----------------------------------|----------------------------------|--------------|--------------------------------|--------------|
|                                                                  |                                                                |                                  | Terminal leaves                  | Basal leaves | Terminal leaves                | Basal leaves |
| 0.00                                                             | 0.0                                                            | 506                              | 541                              | 132          | 313                            | 172          |
| 0.00                                                             | 0.5                                                            | 508                              | 412                              | 114          | 275                            | 158          |
| 0.00                                                             | 1.0                                                            | 375                              | 447                              | 149          | 324                            | 134          |
| 0.00                                                             | 5.0                                                            | 431                              | 385                              | 129          | 353                            | 146          |
| 0.00                                                             | 10.0                                                           | 424                              | 438                              | 137          | 479                            | 153          |
| 0.04                                                             | 0.0                                                            | 416                              | 358                              | 137          | 295                            | 163          |
| 0.04                                                             | 0.5                                                            | 451                              | 392                              | 130          | 389                            | 139          |
| 0.04                                                             | 1.0                                                            | 387                              | 366                              | 117          | 318                            | 126          |
| 0.04                                                             | 5.0                                                            | 347                              | 462                              | 148          | 417                            | 136          |
| 0.04                                                             | 10.0                                                           | 406                              | 484                              | 186          | 265                            | 140          |

Figure 260 to figure 265 included. Average amounts of dry matter, expressed in grams per hundred grams of fresh matter from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for each harvest: 58, 78, and 97 days after seeding. The shaded portions correspond to the increment of one copper series over the other.

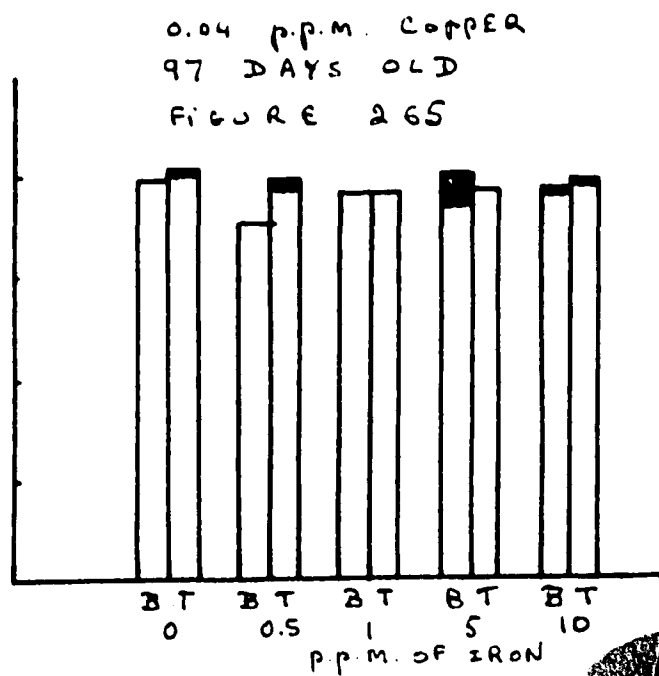
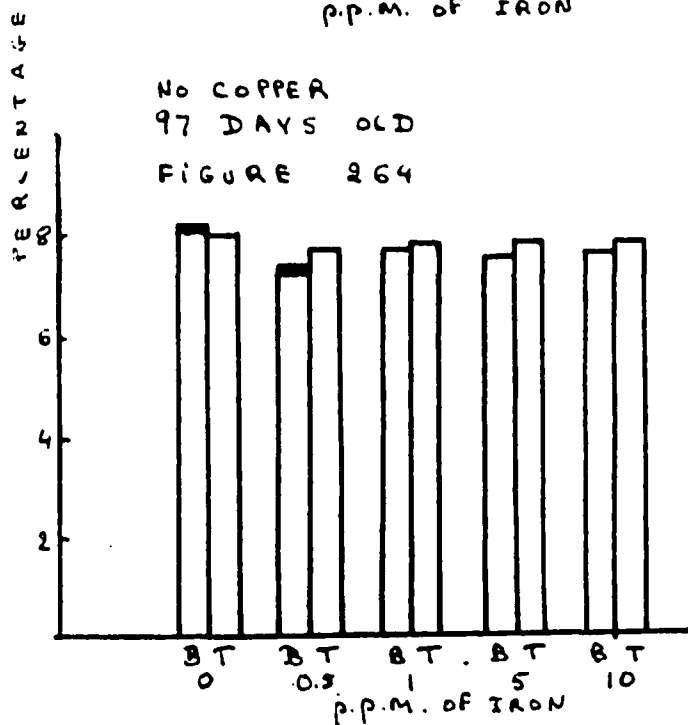
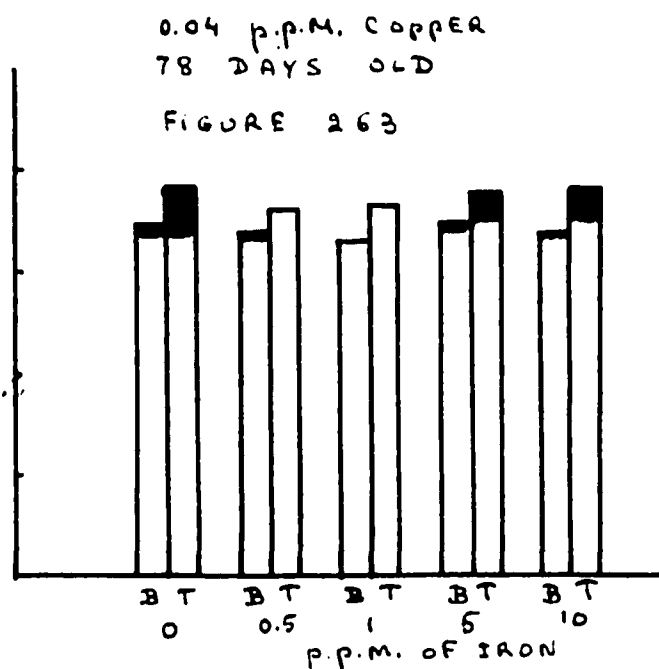
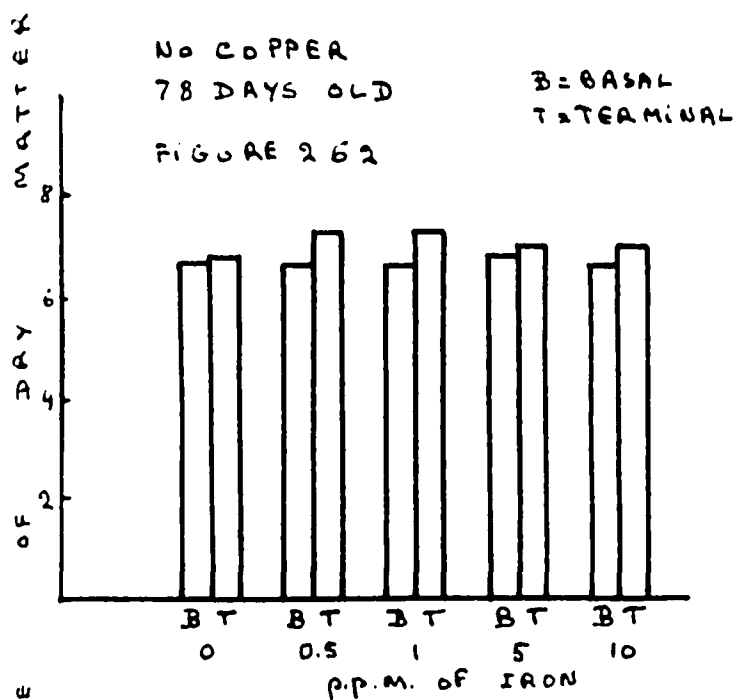
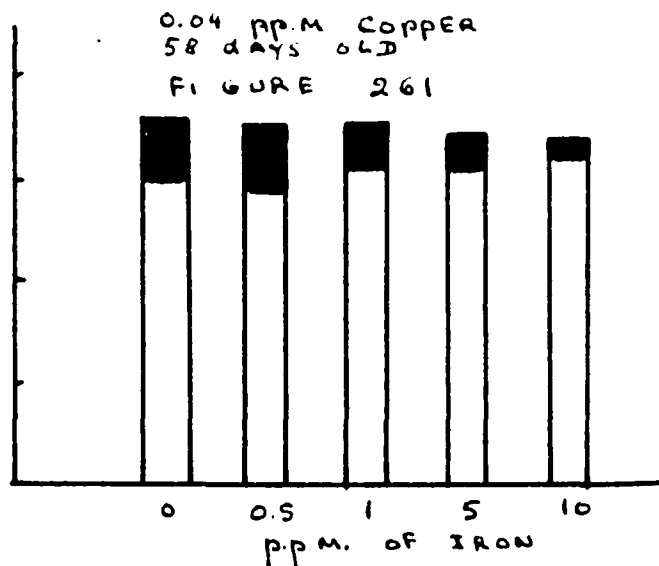
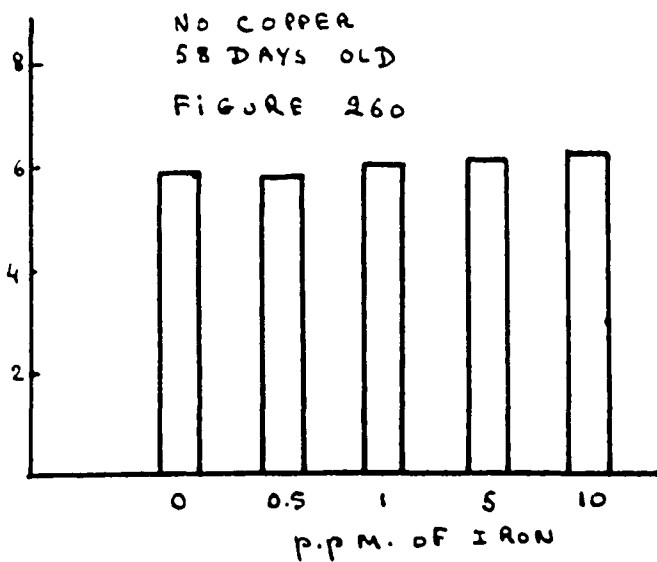


Figure 266 to figure 271 included. Average amounts of iron, expressed in milligrams per 100 grams of fresh matter from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for each harvest: 58, 76 and 97 days after seeding. The shaded portions correspond to the increment of one copper series over the other.

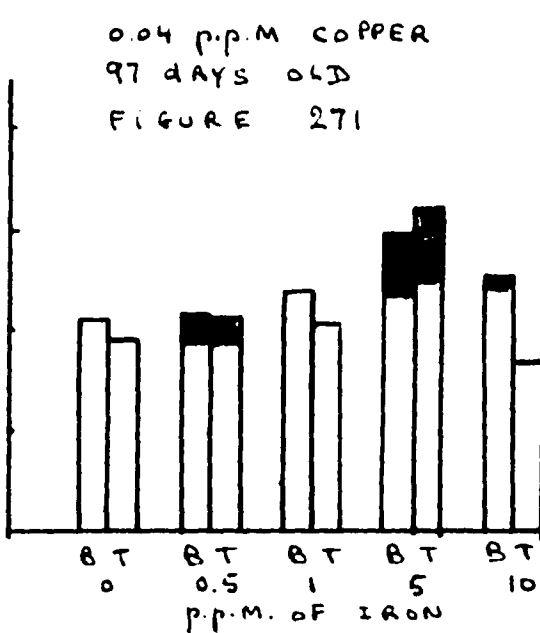
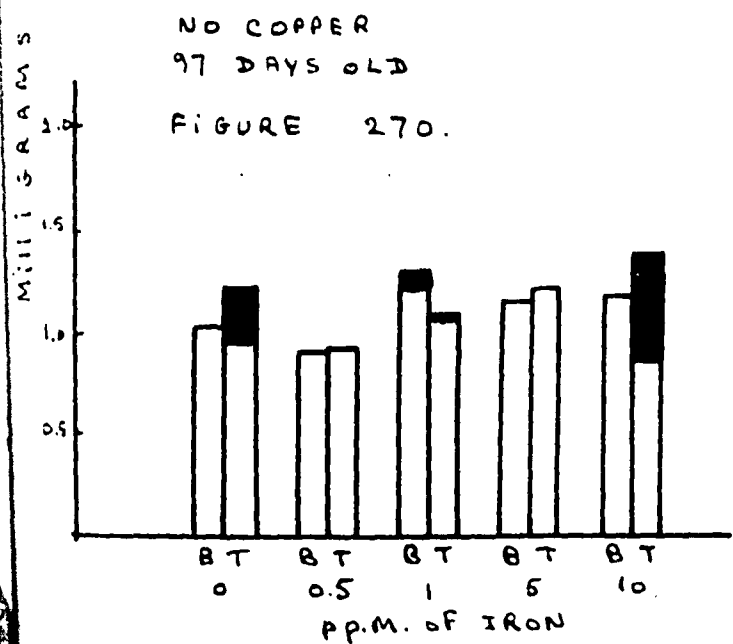
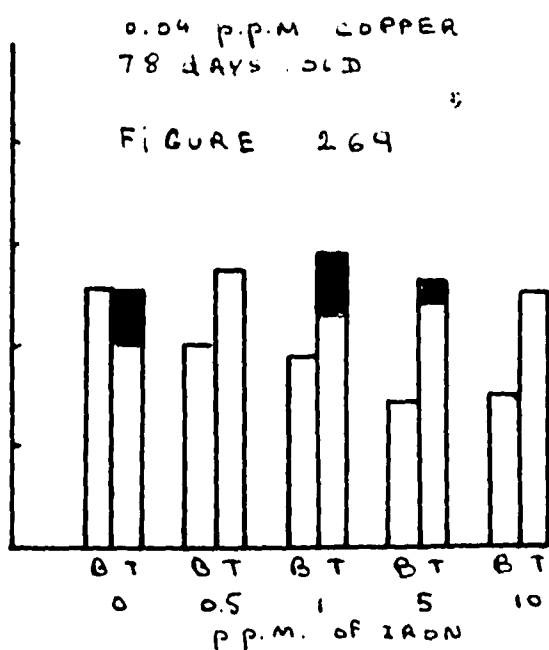
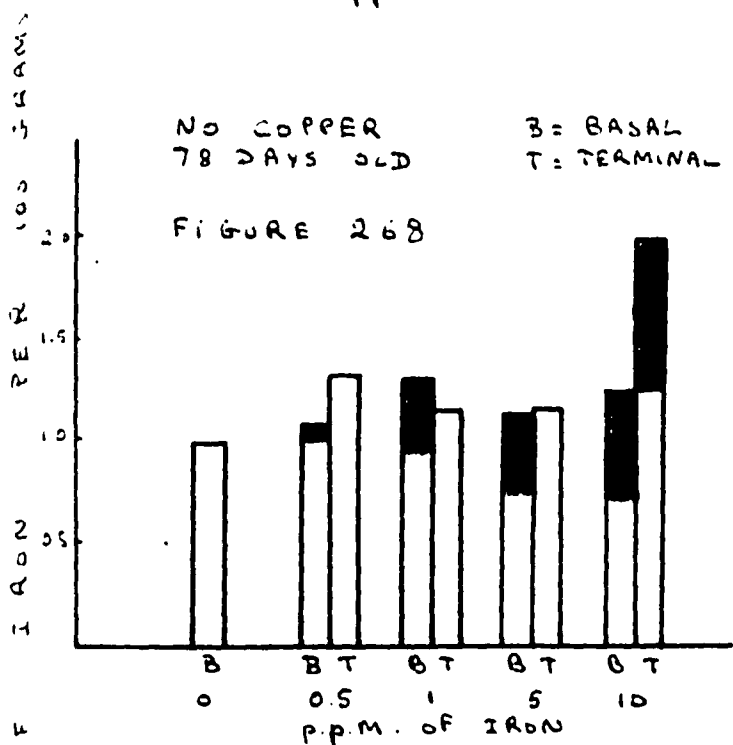
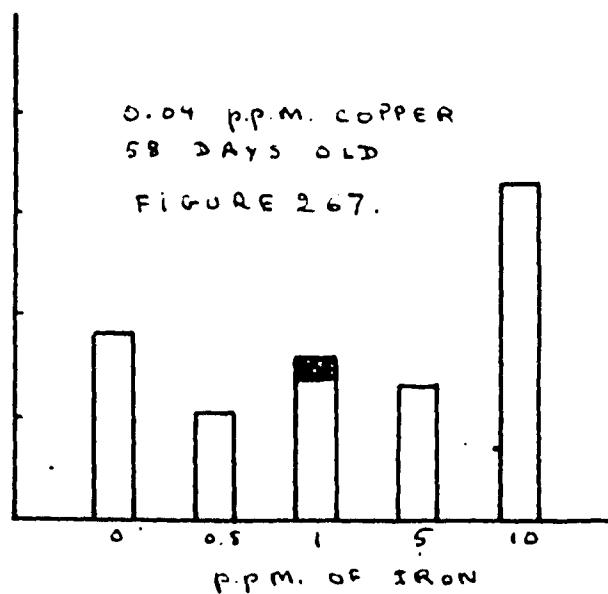
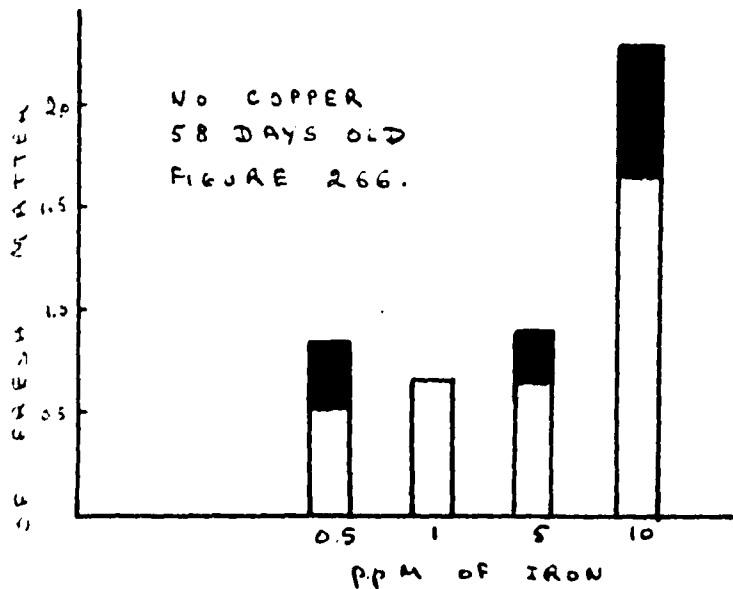


Figure 272 to figure 277 included. Average amounts of iron, expressed in milligrams per 100 grams of oven dry tissue from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for each harvest: 58, 78, and 97 days after seeding. the shaded portions correspond to the increments of one copper series over the other.

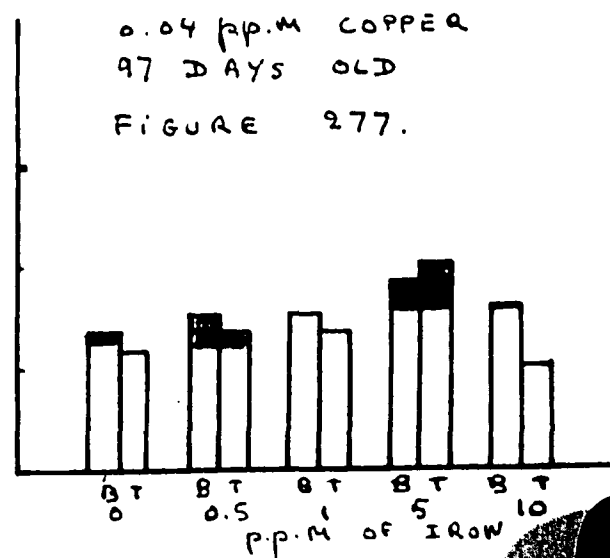
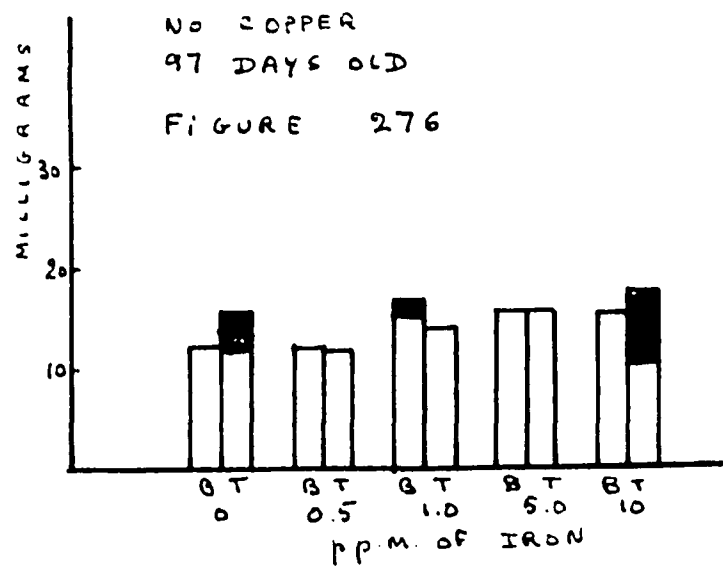
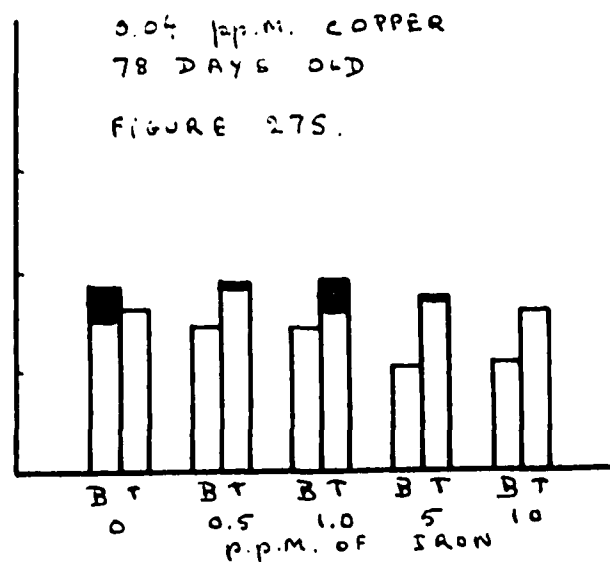
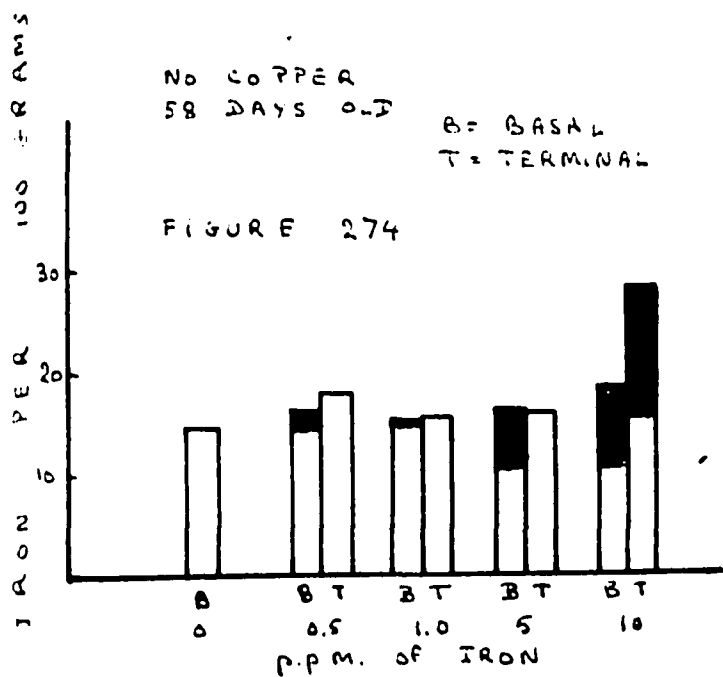
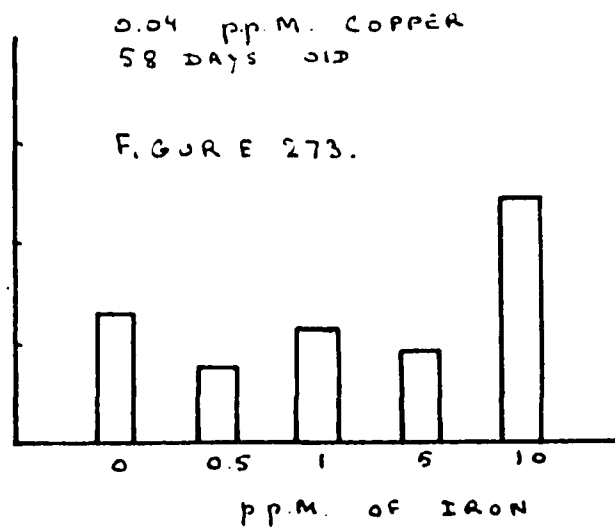
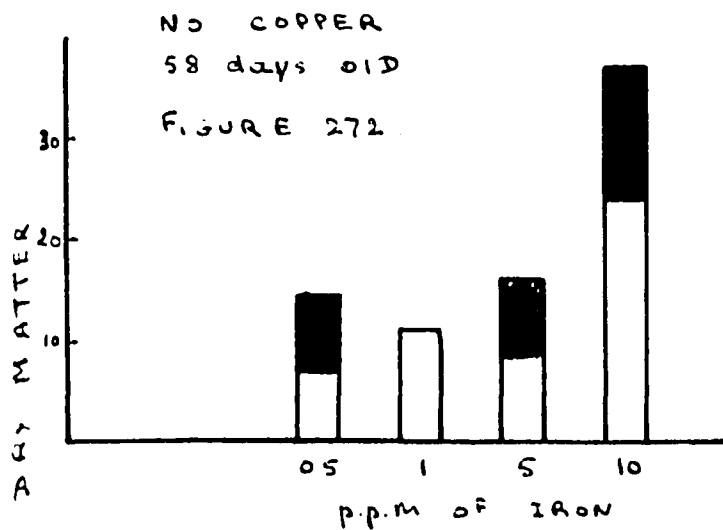
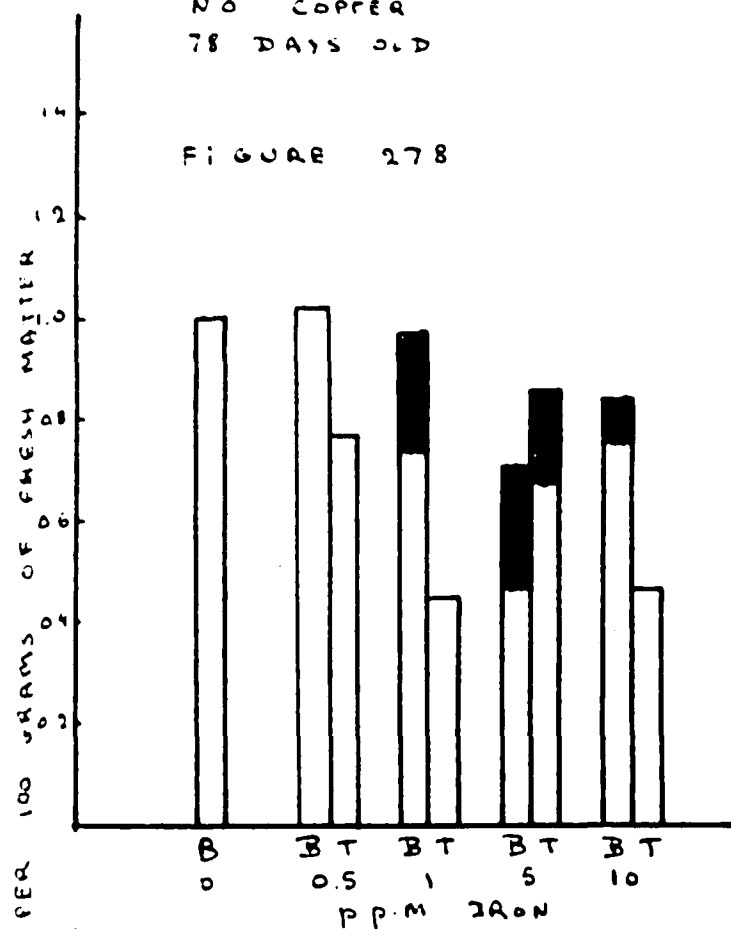




Figure 278 to figure 281 included. Average amounts of manganese, expressed in milligrams per 100 grams of fresh matter from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for the two last harvest: 78 and 97 days after seeding. The shaded portions correspond to the increments of one copper series over the other.

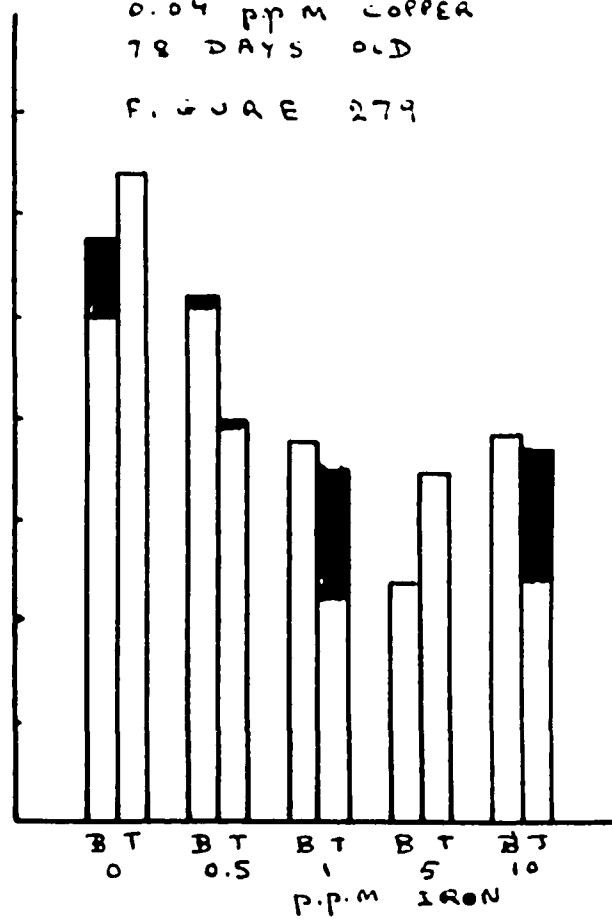
NO COPPER  
78 DAYS OLD

FIGURE 278



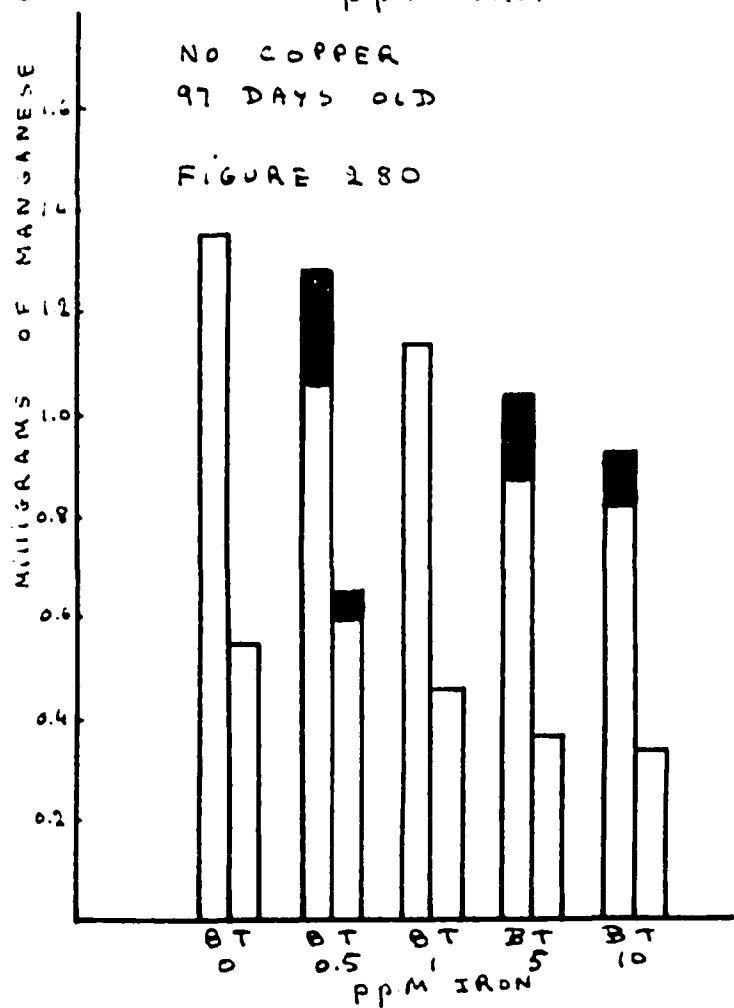
0.04 ppm COPPER  
78 DAYS OLD

FIGURE 279



NO COPPER  
97 DAYS OLD

FIGURE 280



0.04 ppm COPPER  
97 DAYS OLD

FIGURE 281

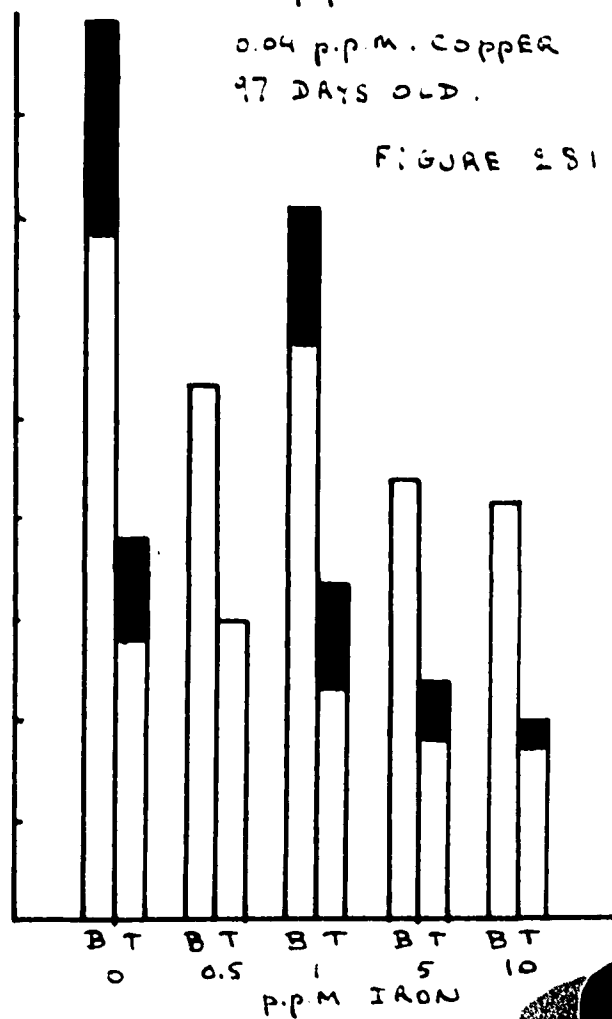
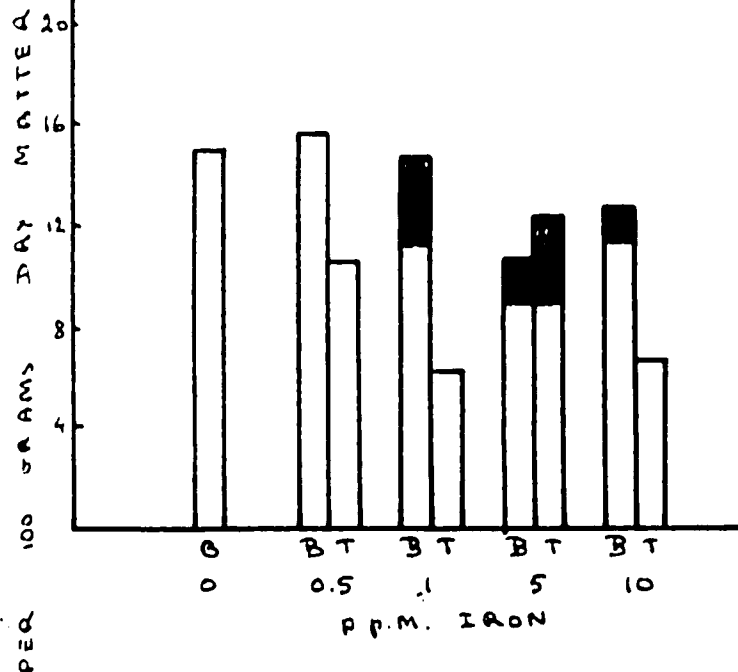


Figure 282 to figure 285 included. Average amounts of manganese, expressed in milligrams per 100 grams of oven dry tissue from tomato plants grown on nutrient solution supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for the two last harvests: 78 and 97 days after seeding. The shaded portions correspond to the increments of one copper treatment over the other.

NO COPPER

78 DAYS OLD

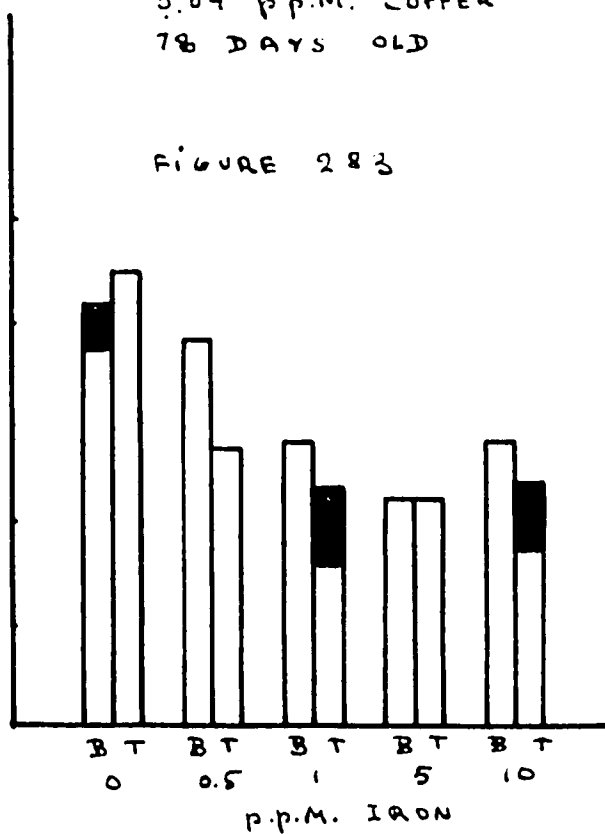
FIGURE 282



0.04 P.P.M. COPPER

78 DAYS OLD

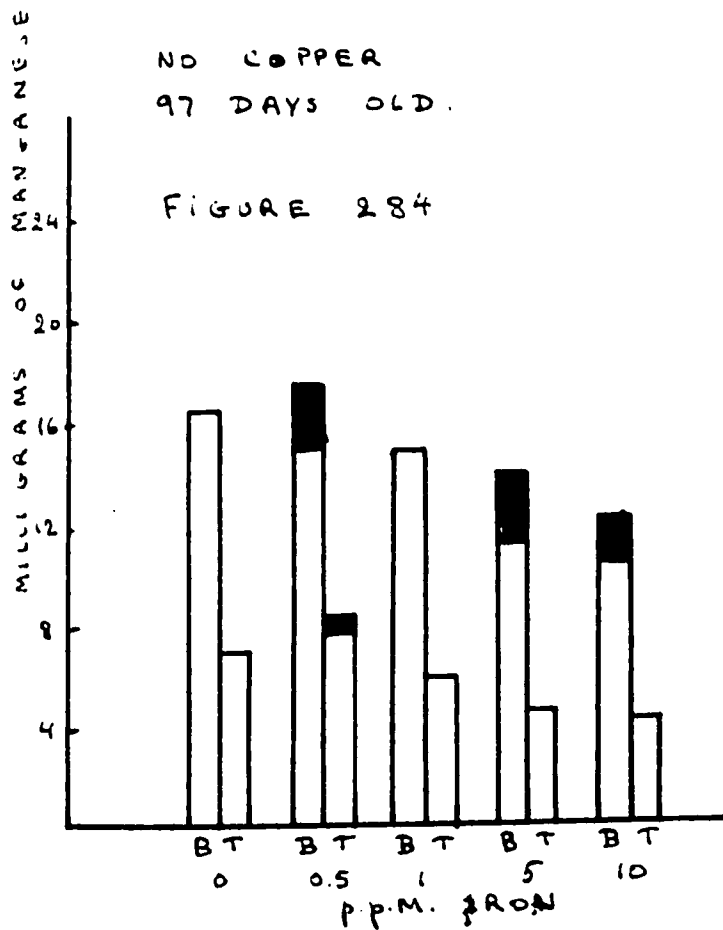
FIGURE 283



NO COPPER

97 DAYS OLD

FIGURE 284



0.04 P.P.M. COPPER

97 DAYS OLD

FIGURE 285

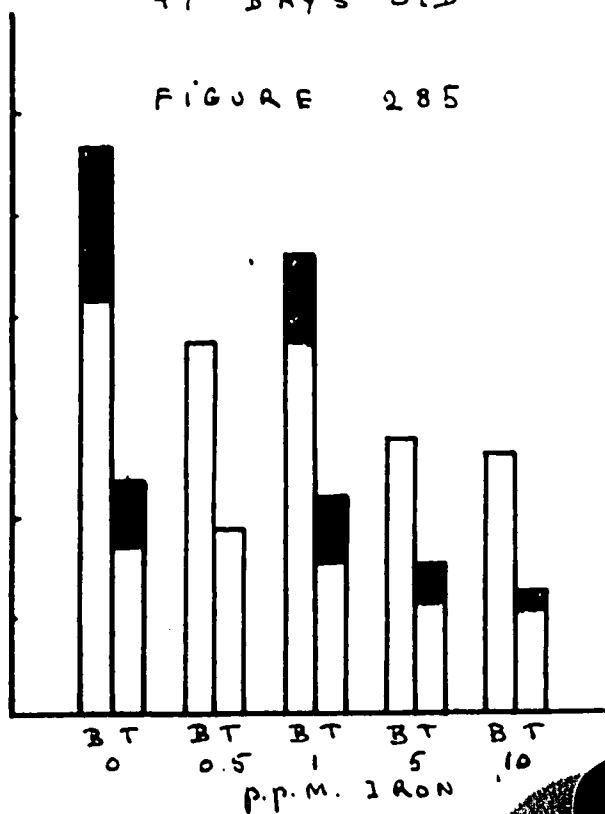
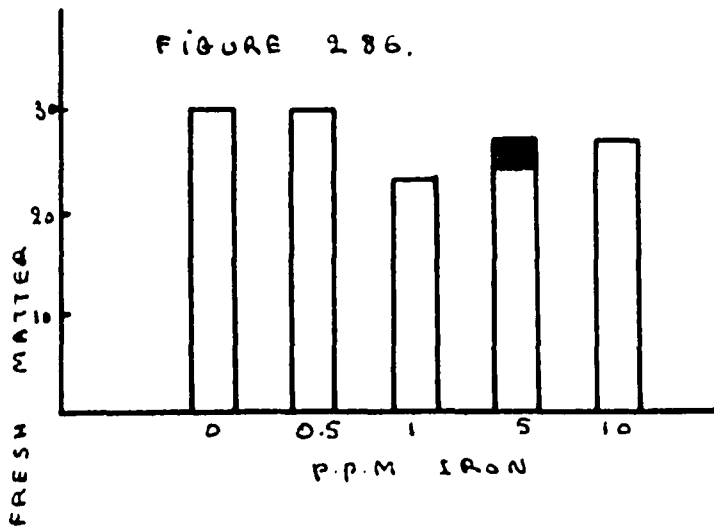


Figure 286 to figure 291 included. Average amounts of ascorbic acid expressed in milligrams per hundred grams of fresh matter from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for each harvest: 58, 78 and 97 days after seeding. The shaded portions correspond to the increment of one copper series over the other.

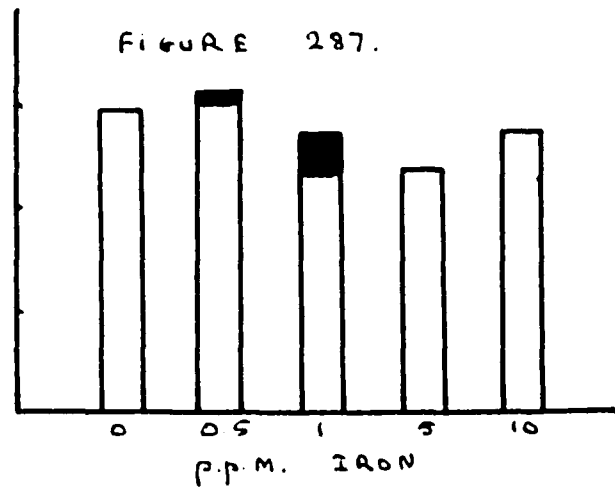
NO COPPER  
58 DAYS OLD

FIGURE 286.



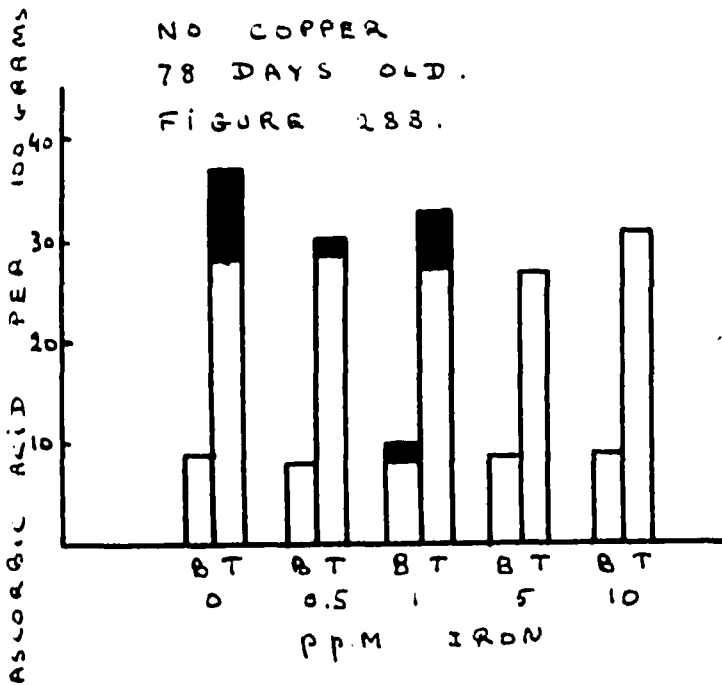
0.04 p.p.m. COPPER  
58 DAYS OLD

FIGURE 287.



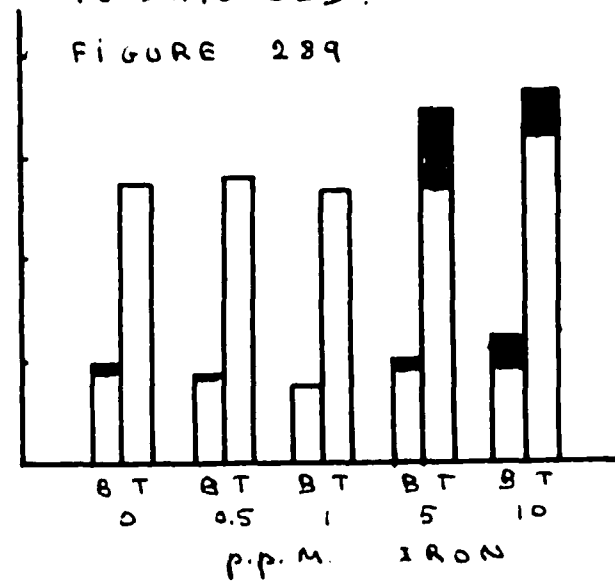
NO COPPER  
78 DAYS OLD.

FIGURE 288.



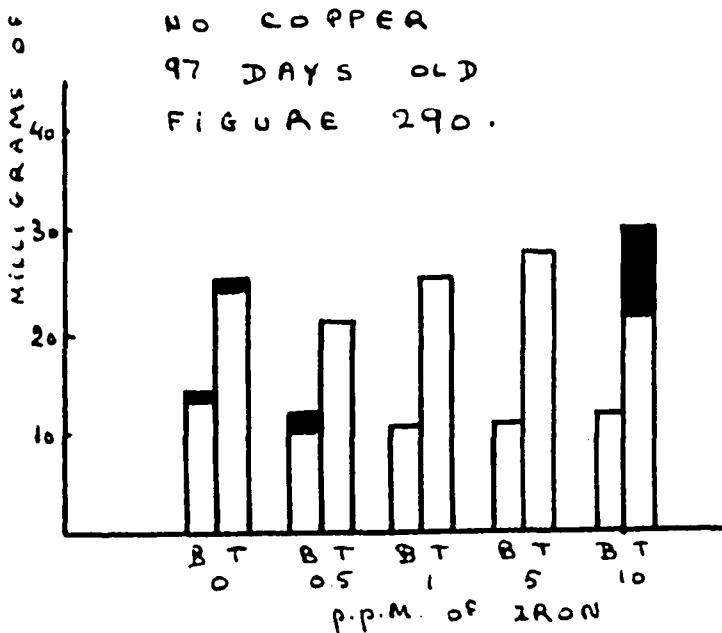
0.04 p.p.m. COPPER  
78 DAYS OLD.

FIGURE 289



NO COPPER  
97 DAYS OLD

FIGURE 290.



0.04 p.p.m. COPPER  
97 DAYS OLD.

FIGURE 291.

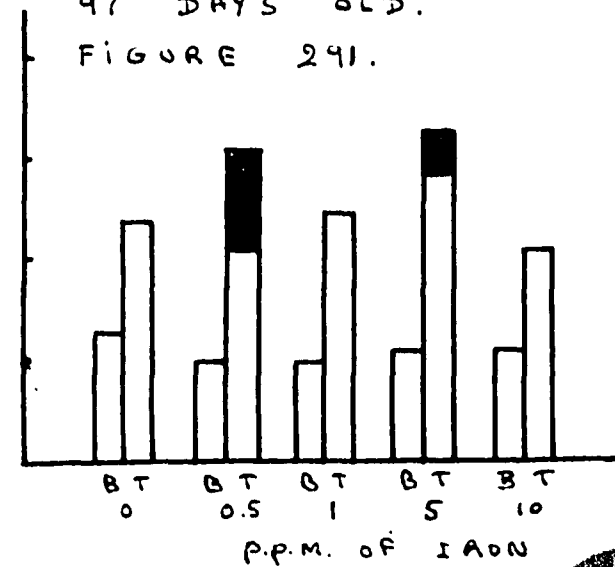
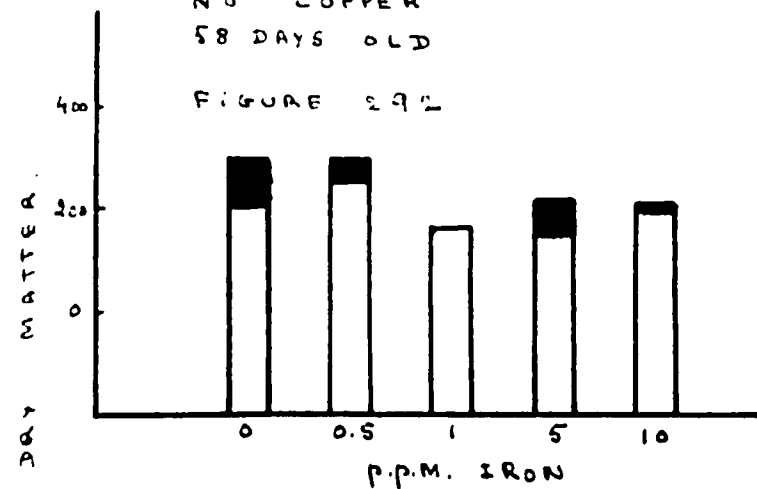


Figure 292 to figure 297 included. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of oven dry tissue from tomato plants grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper. The values for the basal and the terminal leaves are represented by bar graphs for each harvest: 58, 78 and 97 days after seeding. The shaded portions correspond to the increment of one copper series over the other.

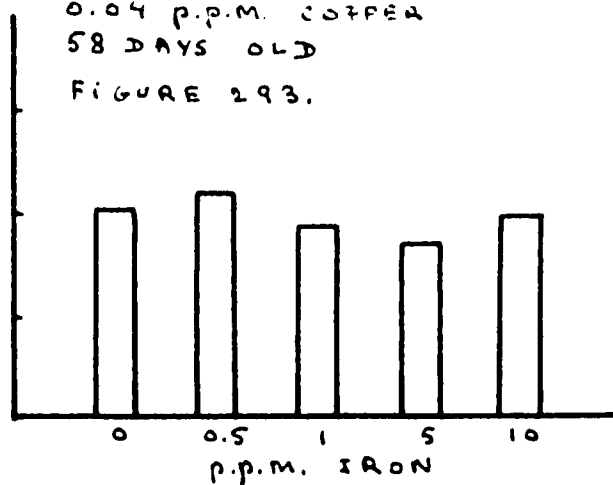
NO COPPER  
58 DAYS OLD

FIGURE 292



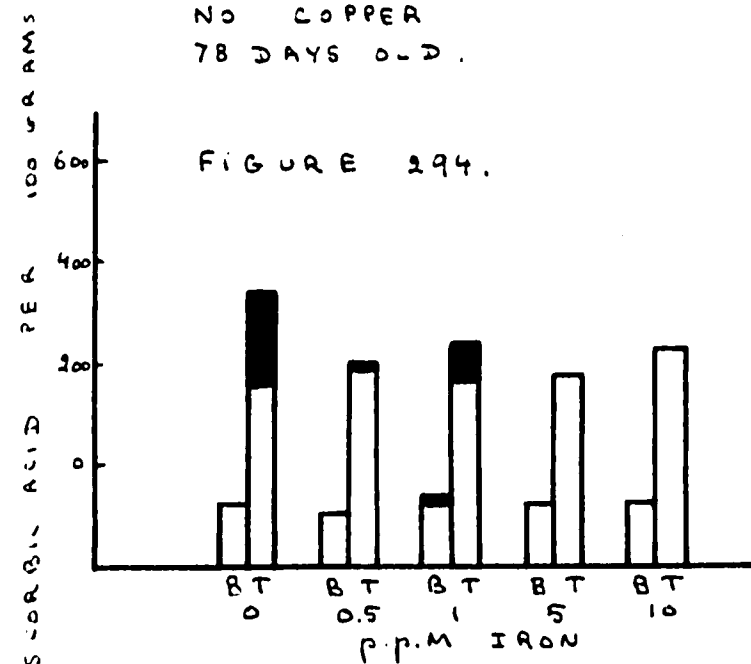
0.04 p.p.m. COPPER  
58 DAYS OLD

FIGURE 293.



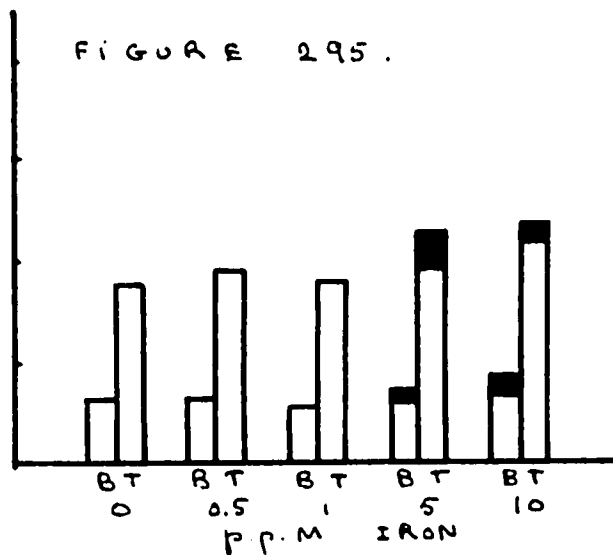
NO COPPER  
78 DAYS OLD.

FIGURE 294.



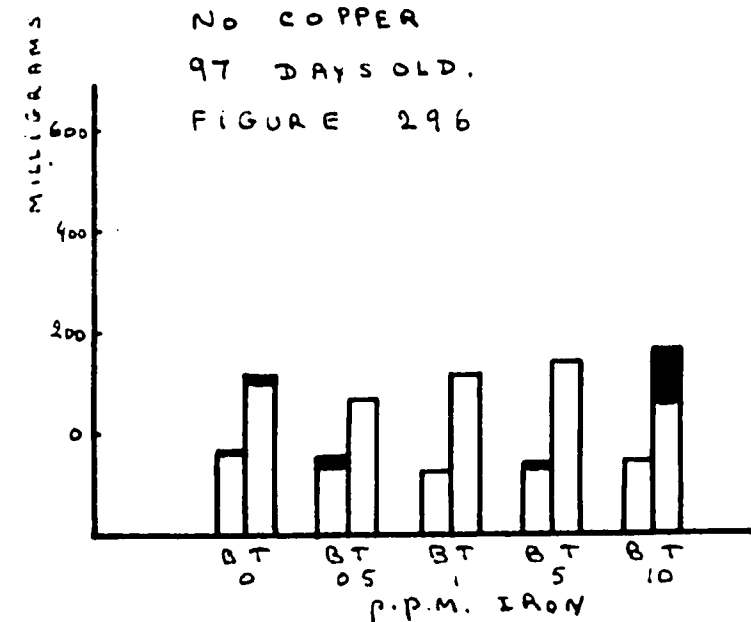
0.04 p.p.m. COPPER  
78 DAYS OLD

FIGURE 295.



NO COPPER  
97 DAYS OLD.

FIGURE 296



0.04 p.p.m. COPPER  
97 DAYS OLD

FIGURE 297

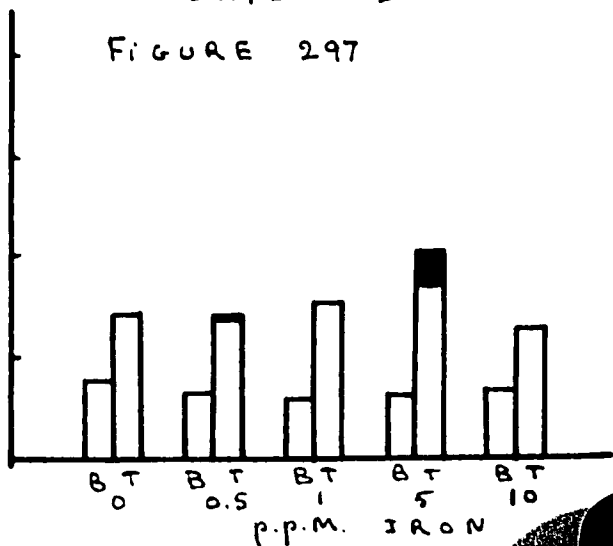




Figure 298. Average amounts of ascorbic acid at harvest, expressed in milligrams per hundred grams of fresh matter from tomato plants, 58 days old grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper, plotted against the amounts of iron, expressed in milligrams per hundred grams of the corresponding fresh plant material.

Figure 299. Same as figure 298, for the 78 days old plants.

Figure 301. Same as figure 298, for the 97 days old plants.

Figure 300. Average amounts of ascorbic acid at harvest, expressed in milligrams per hundred grams of fresh matter from tomato plants, 78 days old grown on various levels of iron in the nutrient solution without and with 0.04 parts per million of copper, plotted against the amounts of manganese, expressed in milligrams per 100 grams of the corresponding fresh plant material.

Figure 302. Same as figure 300, for the 97 days old plants.

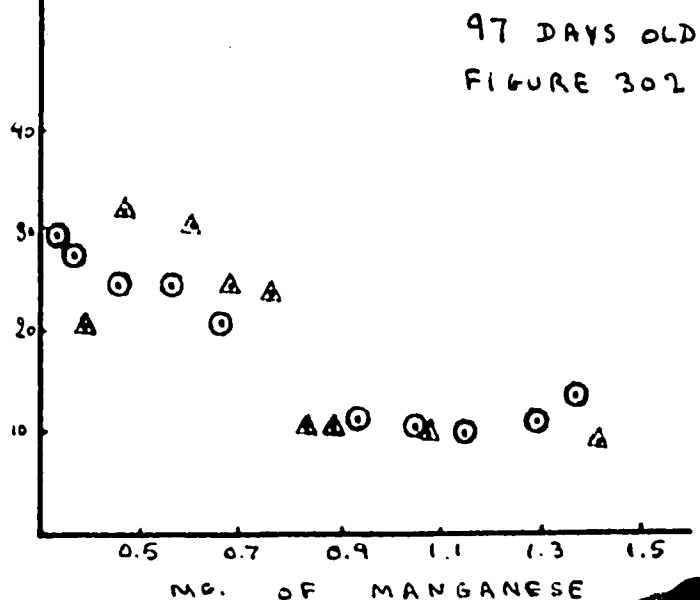
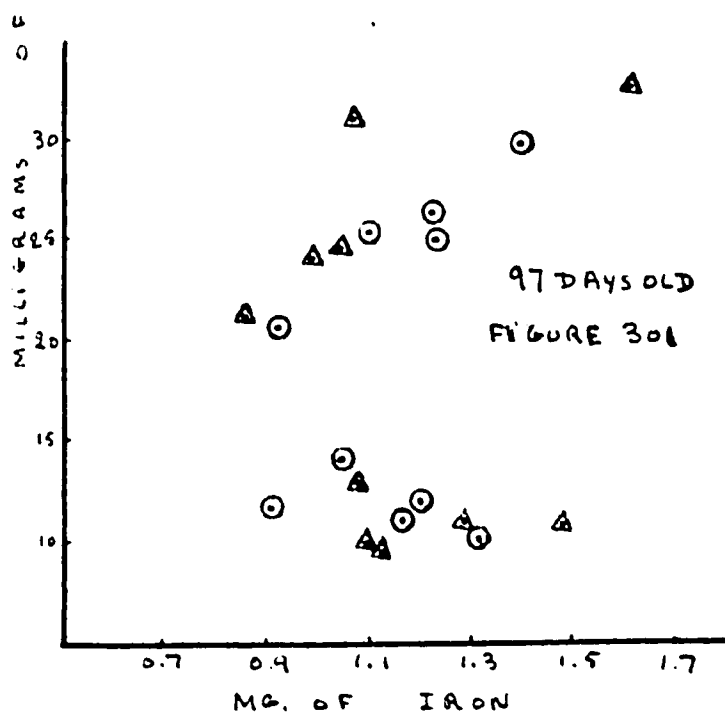
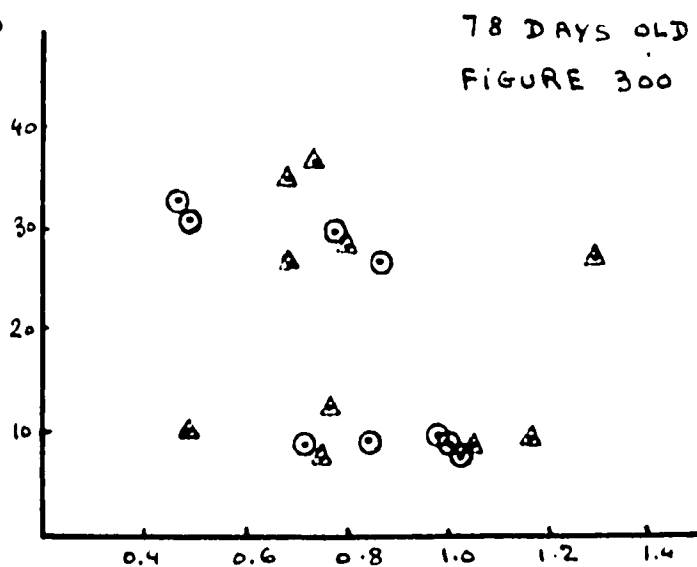
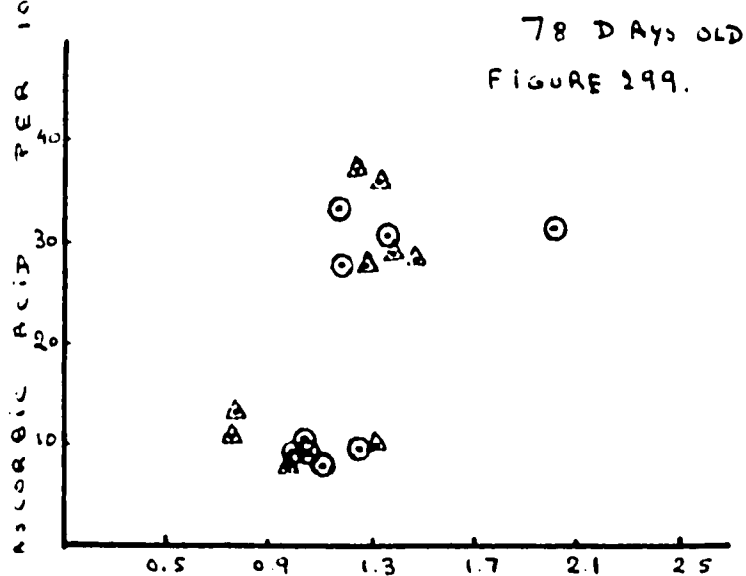
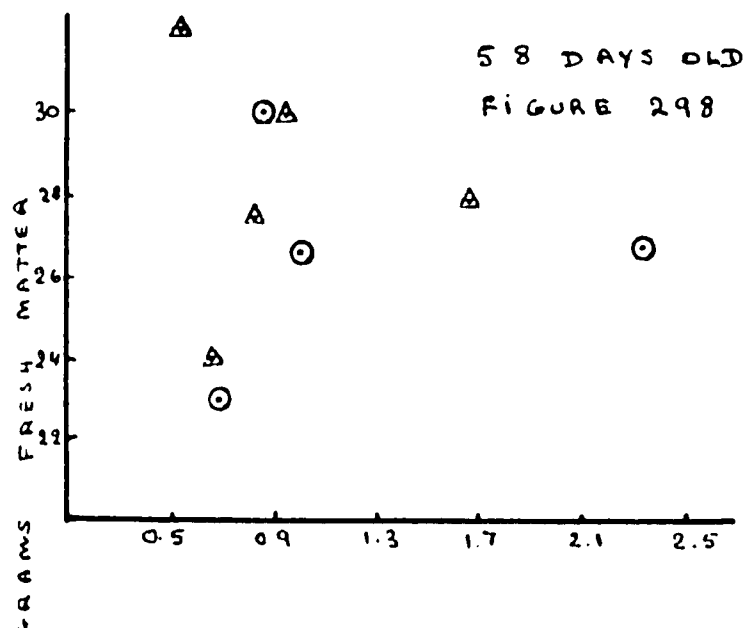
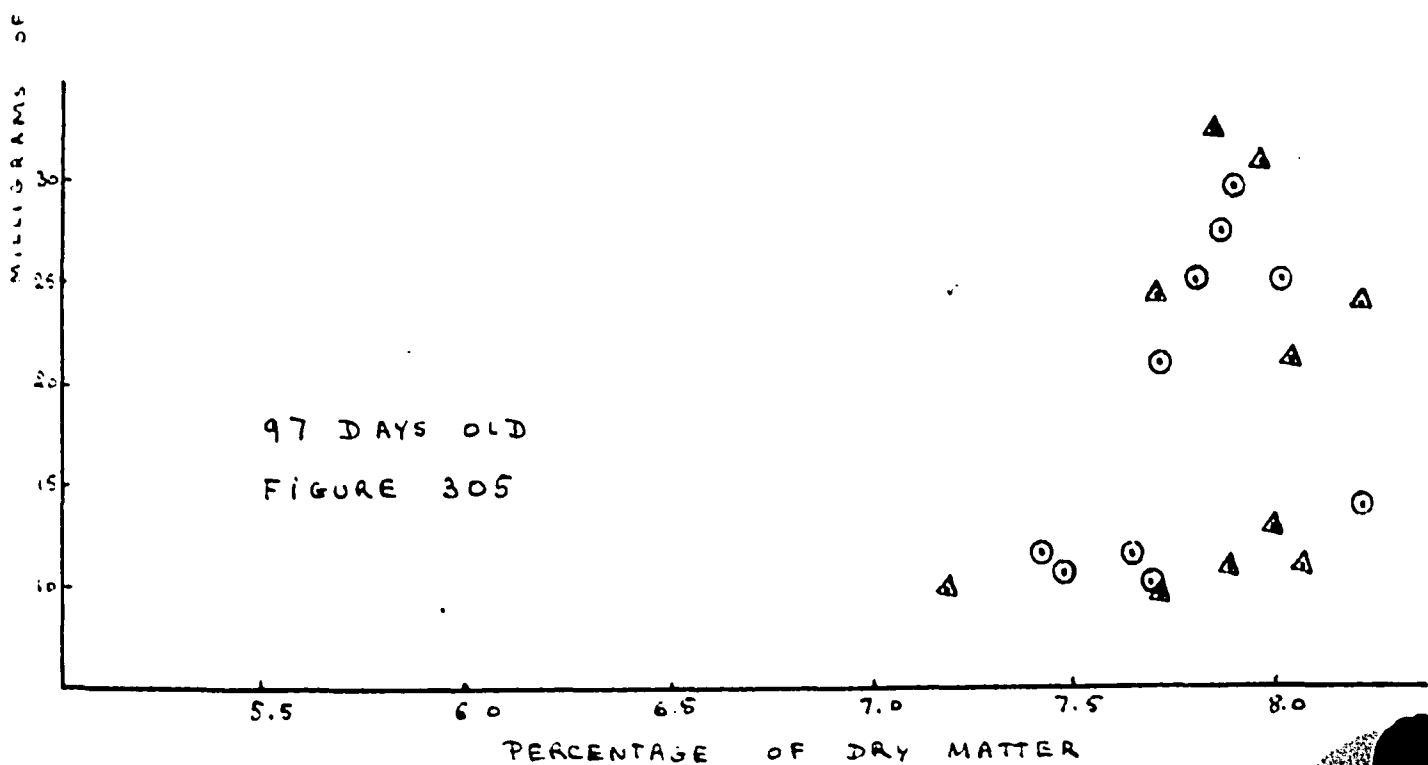
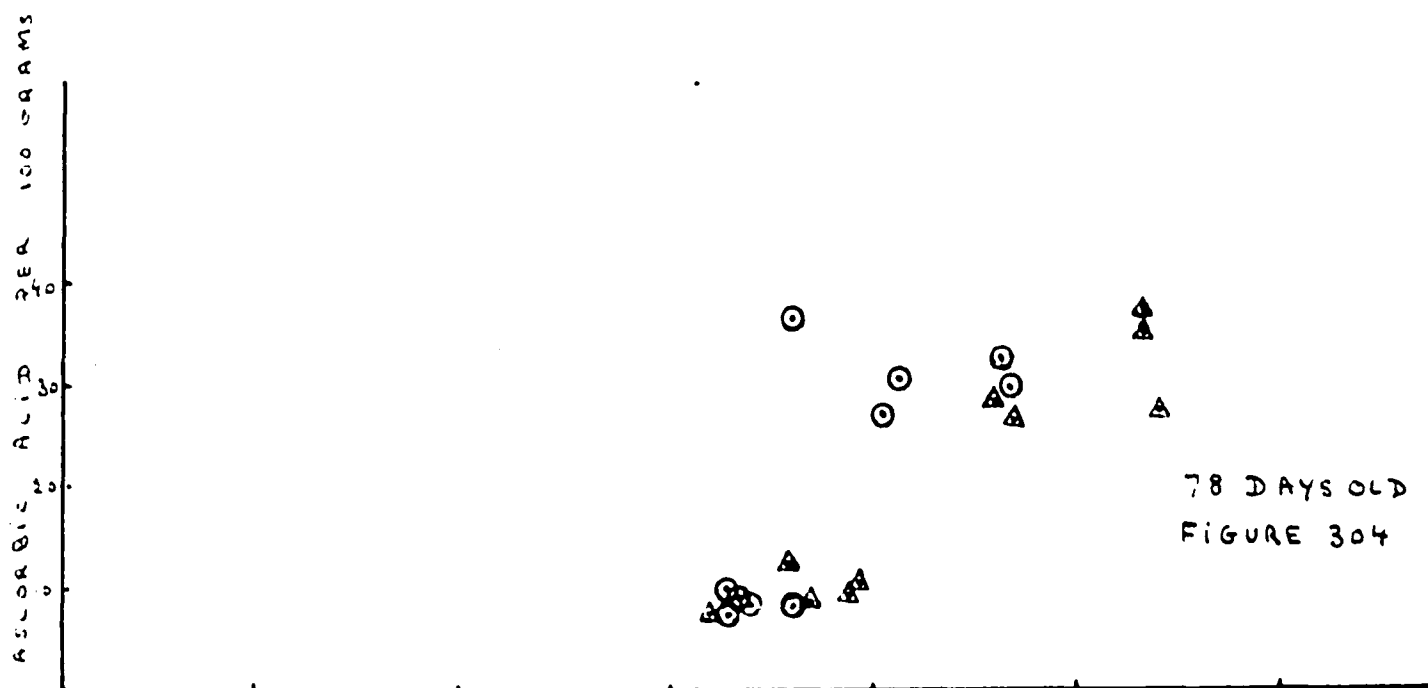
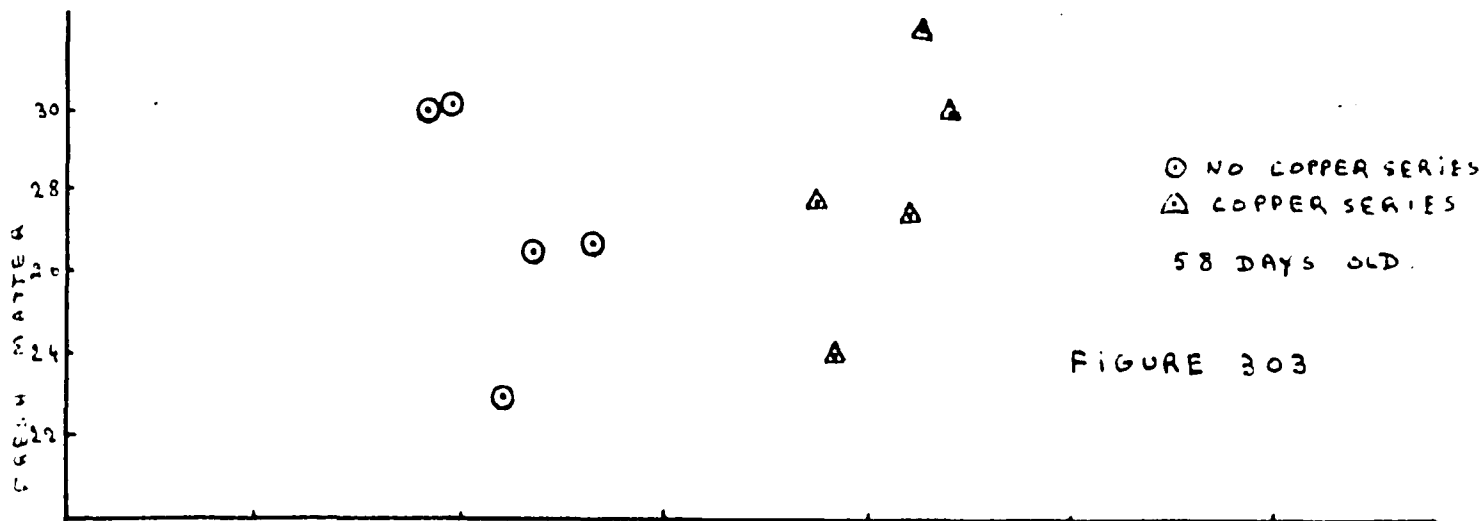


Figure 303. Average amounts of ascorbic acid at harvest, expressed in milligrams per hundred grams of fresh matter from tomato plants, 58 days old, grown on nutrient solutions supplied with various amounts of iron and without or with 0.04 parts per million of copper, plotted against the amounts of dry matter, expressed in grams per hundred grams of the corresponding plant material.

Figure 304. Same as figure 303, for the 78 days old plants.

Figure 305. Same as figure 303, for the 97 days old plants.



Experiment 7

Table 49. Experimental results obtained from the various leaves and parts of stem of a tobacco plant grown on soil; values expressed per 100 grams of fresh material; the total fresh weight of each is expressed in grams.

| Distance from<br>the leaf to<br>the soil in<br>centimeters | Grams of the<br>total fresh<br>of each leaf | Grams of<br>dry matter | Grams of<br>water | Milligrams of<br>ascorbic acid<br>at harvest | Milligrams of<br>iron | Milligrams of<br>manganese |
|------------------------------------------------------------|---------------------------------------------|------------------------|-------------------|----------------------------------------------|-----------------------|----------------------------|
| 9                                                          | 10.5                                        | 6.49                   | 93.51             | 14.8                                         | 2.603                 | -----                      |
| 14                                                         | 12.6                                        | 7.09                   | 92.91             | 18.1                                         | 1.330                 | 0.151                      |
| 18                                                         | 16.1                                        | 7.89                   | 92.11             | 18.5                                         | 1.422                 | 0.108                      |
| 23                                                         | 18.1                                        | 8.10                   | 91.90             | 24.2                                         | 0.822                 | 0.301                      |
| 29                                                         | 15.2                                        | 8.47                   | 91.53             | 24.3                                         | 0.713                 | 0.212                      |
| 34                                                         | 13.3                                        | 9.11                   | 90.89             | 28.1                                         | 0.817                 | 0.215                      |
| 39                                                         | 10.5                                        | 10.30                  | 89.70             | 27.1                                         | 1.480                 | 0.300                      |
| 43 (2 leaves)                                              | 14.8                                        | 10.95                  | 89.05             | 25.7                                         | 1.403                 | 0.411                      |
| 51 (6 leaves)                                              | 12.3                                        | 10.20                  | 89.80             | 28.6                                         | 1.596                 | 0.235                      |
| 62 (14 leaves)                                             | 8.6                                         | 10.91                  | 89.09             | 22.9                                         | 1.617                 | 0.412                      |
| Stem (upper 19 cm.)                                        |                                             | 6.85                   | 93.15             | 16.0                                         | 0.238                 | 0.000                      |
| Stem (bottom 43 cm.)                                       |                                             | 9.17                   | 90.83             | 9.6                                          | 0.083                 | 0.028                      |

Experiment 7.

Preliminary experiment

Table 50. Experimental results obtained from the various leaves and parts of stem of a tobacco plant grown on soil; values expressed per 100 grams of dry plant material.

| Distance from<br>the leaf to<br>the soil in<br>centimeters | Milligrams of<br>iron | Milligrams of<br>manganese | Grams of<br>fresh matter |
|------------------------------------------------------------|-----------------------|----------------------------|--------------------------|
| 9                                                          | 40.10                 | ----                       | 1540.8                   |
| 14                                                         | 18.76                 | 2.13                       | 1410.4                   |
| 18                                                         | 18.02                 | 1.37                       | 1267.4                   |
| 23                                                         | 10.15                 | 3.72                       | 1234.6                   |
| 29                                                         | 8.42                  | 2.50                       | 1180.6                   |
| 34                                                         | 8.97                  | 2.36                       | 1097.7                   |
| 39                                                         | 14.37                 | 2.91                       | 970.9                    |
| 43                                                         | 12.82                 | 3.75                       | 913.2                    |
| 51                                                         | 15.65                 | 2.30                       | 980.4                    |
| 62                                                         | 14.83                 | 3.78                       | 916.6                    |
| Stem (upper 19 cm.)                                        | 3.48                  | 0.00                       | 1459.9                   |
| Stem (bottom 43 cm.)                                       | 0.91                  | 0.31                       | 1090.5                   |

Experiment 7

Table 51. Experimental results obtained from terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and supplied with no copper and with 0.08 parts per million of copper. Averages expressed per 100 grams of fresh plant material.

| Culture                   | kind of leaves | Milligrams of iron              |                       | Milligrams of manganese         |                       | Milligrams of dry matter        |                       |
|---------------------------|----------------|---------------------------------|-----------------------|---------------------------------|-----------------------|---------------------------------|-----------------------|
|                           |                | Series with 0.08 ppm. of copper | Series with no copper | Series with 0.08 ppm. of copper | Series with no copper | Series with 0.08 ppm. of copper | Series with no copper |
| Iron-Frit                 | Terminal       | 3.24                            | 4.30                  | 0.97                            | 1.13                  | 11.16                           | 10.2                  |
| Iron-Frit                 | Median         | 4.80                            | 5.46                  | 0.94                            | 0.96                  | 7.42                            | 8.4                   |
| Iron-Frit                 | Basal          | 3.72                            | 3.87                  | 0.80                            | 0.68                  | 6.90                            | 7.1                   |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 4.04                            | 4.78                  | 1.09                            | 0.97                  | 10.50                           | 10.0                  |
|                           | Median         | 4.55                            | 7.32                  | 0.81                            | 0.88                  | 8.15                            | 8.1                   |
|                           | Basal          | 3.70                            | 5.47                  | 0.66                            | 0.97                  | 6.73                            | 6.5                   |
| NO-iron                   | Terminal       | 1.64                            | 2.53                  | 0.96                            | 0.84                  | 8.19                            | 9.8                   |
| NO-iron                   | Median         | 2.82                            | 4.42                  | 1.37                            | 0.88                  | 7.04                            | 8.1                   |
| No-iron                   | Basal          | 3.00                            | 4.58                  | 1.83                            | 0.91                  | 6.52                            | 5.9                   |

Experiment 7.

Table 52. Amounts of ascorbic acid at harvest and at various times during drying, in the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and not supplied with copper. Averages expressed in milligrams per 100 grams of fresh matter.

| Culture                   | Kind of leaves | At harvest | 24 hours after harvest | 48 hours after harvest | 72 hours after harvest | 120 hours after harvest | 144 hours after harvest |
|---------------------------|----------------|------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Iron-Frit                 | Terminal       | 80.4       | 45.4                   | 34.9                   | 19.8                   | 10.8                    | 6.1                     |
| Iron-Frit                 | Median         | 57.1       | 47.6                   | 13.0                   | 12.8                   | 6.9                     | 5.1                     |
| Iron-Frit                 | Basal          | 25.4       | 17.9                   | 12.3                   | 5.8                    | 1.3                     | 1.6                     |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 83.4       | 43.0                   | 27.7                   | 23.5                   | 7.8                     | 5.4                     |
|                           | Median         | 25.7       | 27.8                   | 25.6                   | 18.1                   | 5.4                     | 3.3                     |
|                           | Basal          | 21.0       | 21.9                   | 11.1                   | 4.1                    | 1.6                     | 0.7                     |
| No-iron                   | Terminal       | 70.2       | 52.4                   | 27.7                   | 21.6                   | 8.9                     | ---                     |
| NO-iron                   | Median         | 37.4       | 33.0                   | 17.0                   | 14.7                   | 7.0                     | 7.1                     |
| No-iron                   | Basal          | 13.8       | 16.3                   | 6.5                    | 5.9                    | 1.3                     | 1.4                     |



Table 53. Amounts of ascorbic acid at harvest and at various time during drying, in the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and supplied with 0.08 parts per million of copper. Averages expressed in milligrams per 100 grams of fresh matter.

| Culture                   | Kind of leaves | At harvest | 24 hours after harvest | 48 hours after harvest | 96 hours after harvest | 120 hours after harvest | 192 hours after harvest |
|---------------------------|----------------|------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Iron-Frit                 | Terminal       | 72.0       | 52.6                   | 47.3                   | 14.6                   | 14.1                    | 2.9                     |
| Iron-Frit                 | Median         | 48.4       | 34.2                   | 47.1                   | 14.8                   | 13.9                    | 3.3                     |
| Iron-Frit                 | Basal          | 23.0       | 21.3                   | 19.8                   | 6.9                    | 4.8                     | 0.9                     |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 59.7       | 57.5                   | 38.2                   | 16.4                   | 11.3                    | 5.0                     |
|                           | Median         | 34.5       | 31.2                   | 29.2                   | 13.1                   | 12.1                    | 1.7                     |
|                           | Basal          | 10.8       | 17.4                   | 10.7                   | 5.8                    | 2.4                     | 0.2                     |
| No-iron                   | Terminal       | 47.5       | 26.6                   | 25.7                   | 6.0                    | 5.7                     | 0.0                     |
| No-iron                   | Median         | 34.9       | 20.2                   | 14.6                   | 4.3                    | 4.6                     | ---                     |
| NO-iron                   | Basal          | 17.9       | 14.6                   | 9.7                    | ---                    | 0.1                     | ---                     |

Experiment 7.

Table 54. Experimental results obtained from terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and supplied with no copper and with 0.08 parts per million of copper. Averages expressed per 100 grams of dry matter.

| Culture                   | Kind of leaves | Milligrams of iron              |                       | Milligrams of manganese         |                       |
|---------------------------|----------------|---------------------------------|-----------------------|---------------------------------|-----------------------|
|                           |                | Series with 0.08 ppm. of copper | Series with no copper | Series with 0.08 ppm. of copper | Series with no copper |
| Iron-frit                 | Terminal       | 29.0                            | 42.0                  | 8.8                             | 11.0                  |
| Iron-frit                 | Median         | 51.0                            | 61.1                  | 10.0                            | 10.8                  |
| Iron-frit                 | Basal          | 50.1                            | 54.6                  | 10.8                            | 9.6                   |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 38.5                            | 47.8                  | 10.4                            | 9.7                   |
|                           | Median         | 55.8                            | 90.1                  | 9.9                             | 10.8                  |
|                           | Basal          | 64.5                            | 84.8                  | 11.5                            | 24.4                  |
| No-iron                   | Terminal       | 20.0                            | 25.8                  | 11.7                            | 8.6                   |
| No-iron                   | Median         | 40.0                            | 54.5                  | 19.5                            | 10.8                  |
| No-iron                   | Basal          | 46.0                            | 77.5                  | 28.0                            | 15.3                  |

Experiment 7

Table 55. Percentages of the original amounts of ascorbic acid found in the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and not supplied with copper.

| Culture                   | Kind of leaves | At harvest | 24 hours after harvest | 48 hours after harvest | 72 hours after harvest | 120 hours after harvest | 144 hours after harvest |
|---------------------------|----------------|------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Iron-frit                 | Terminal       | 100        | 56.5                   | 43.4                   | 24.6                   | 13.4                    | 7.6                     |
| Iron-frit                 | Median         | 100        | 83.3                   | 22.8                   | 22.4                   | 12.1                    | 8.9                     |
| Iron-frit                 | Basal          | 100        | 70.5                   | 48.4                   | 22.8                   | 5.1                     | 6.3                     |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 100        | 51.6                   | 33.2                   | 28.2                   | 9.4                     | 6.5                     |
|                           | Median         | 100        | 108.2                  | 99.6                   | 70.4                   | 21.0                    | 12.8                    |
|                           | Basal          | 100        | 104.3                  | 52.9                   | 19.5                   | 7.6                     | 3.3                     |
| NO-iron                   | Terminal       | 100        | 74.6                   | 39.5                   | 30.8                   | 12.7                    | ---                     |
| No-iron                   | Median         | 100        | 88.2                   | 45.5                   | 39.3                   | 18.7                    | 19.0                    |
| No-iron                   | Basal          | 100        | 118.1                  | 47.1                   | 42.8                   | 9.4                     | 10.1                    |

Experiment 7

Table 56. Percentages of the original amounts of ascorbic acid found in the terminal, median, and basal leaves of tobacco plants grown on various cultures differing in iron availability and supplied with 0.08 parts per million of copper.

| Culture                   | Kind of leaves | At harvest | 24 hours after harvest | 48 hours after harvest | 96 hours after harvest | 120 hours after harvest | 192 hours after harvest |
|---------------------------|----------------|------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Iron-frit                 | Terminal       | 100        | 73.0                   | 65.7                   | 20.3                   | 19.6                    | 4.0                     |
| Iron-frit                 | Median         | 100        | 70.7                   | 97.3                   | 30.6                   | 28.7                    | 6.8                     |
| Iron-frit                 | Basal          | 100        | 92.6                   | 86.1                   | 30.0                   | 20.9                    | 3.9                     |
| Gravel-<br>4 ppm.<br>iron | Terminal       | 100        | 96.3                   | 64.0                   | 27.5                   | 13.9                    | 8.4                     |
|                           | Median         | 100        | 90.4                   | 84.6                   | 38.0                   | 35.1                    | 4.9                     |
|                           | Basal          | 100        | 161.1                  | 99.0                   | 53.7                   | 22.2                    | 1.9                     |
| No-iron                   | Terminal       | 100        | 56.0                   | 54.1                   | 12.6                   | 12.0                    | 0.0                     |
| No-iron                   | Median         | 100        | 57.9                   | 41.8                   | 12.3                   | 13.2                    | ----                    |
| No-iron                   | Basal          | 100        | 81.6                   | 54.2                   | ----                   | 0.5                     | ----                    |

Experiment 7.

Table 57. Differences between the percentages of the original amounts of ascorbic acid found in the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability and supplied with no copper, and between those supplied with 0.08 parts per million of copper, at various time of drying.

| Culture                  | Kind of leaves | At harvest | 24 hours after harvest | 48 hours after harvest | 120 hours after harvest |
|--------------------------|----------------|------------|------------------------|------------------------|-------------------------|
| Iron-frit                | Terminal       | 0          | - 16.5                 | - 22.3                 | - 6.2                   |
| Iron-frit                | Median         | 0          | + 12.6                 | - 74.5                 | - 16.4                  |
| Iron-frit                | Basal          | 0          | - 22.1                 | - 37.7                 | - 15.8                  |
| Gravel<br>4 ppm.<br>iron | Terminal       | 0          | - 44.7                 | - 30.8                 | - 9.5                   |
|                          | Median         | 0          | + 17.8                 | + 15.0                 | - 14.1                  |
|                          | Basal          | 0          | - 56.8                 | - 46.1                 | - 14.6                  |
| No-iron                  | Terminal       | 0          | + 18.6                 | - 14.6                 | + 0.7                   |
| No-iron                  | Median         | 0          | + 30.3                 | + 3.7                  | + 5.5                   |
| No-iron                  | Basal          | 0          | + 36.5                 | - 7.1                  | + 8.9                   |

Figure 306. Average amounts of iron, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plants grown on soil.

Figure 307. Average amounts of manganese, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plant grown on soil.

Figure 308. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plant grown on soil.

FIGURE 306.

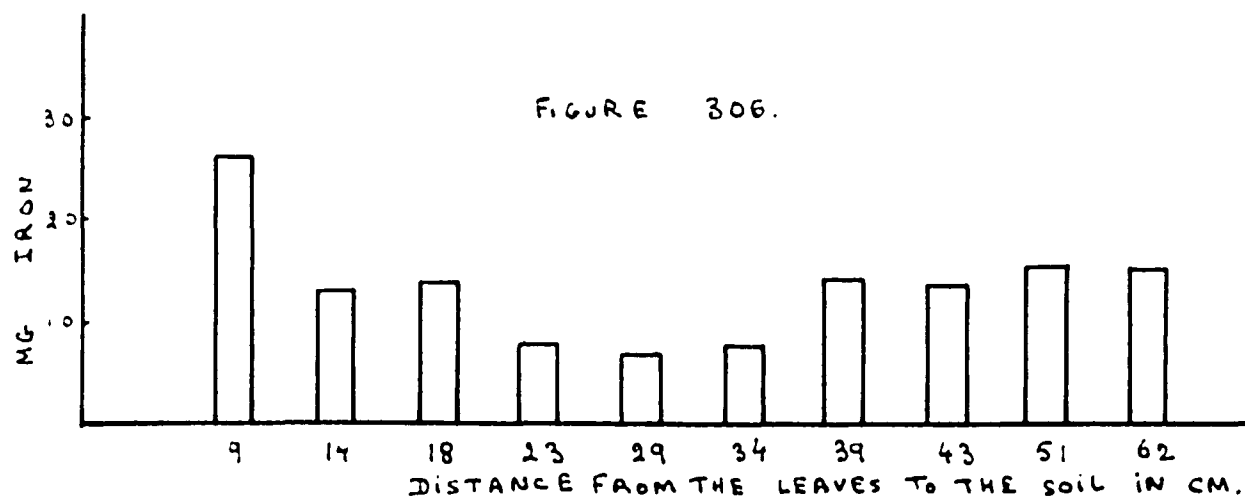


FIGURE 307.

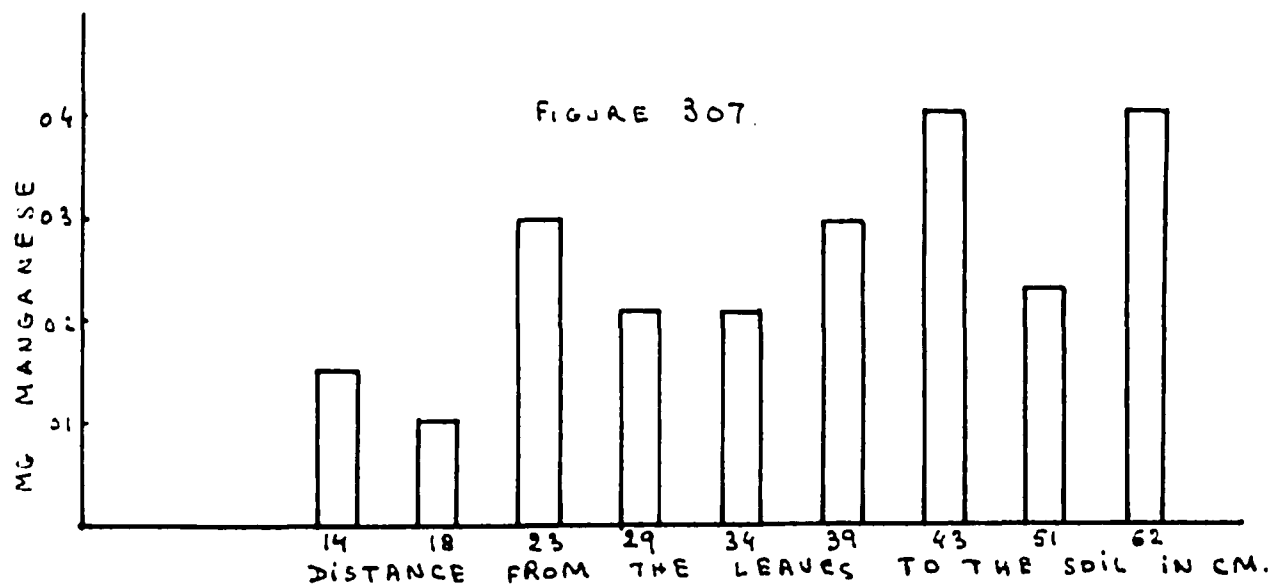


FIGURE 308.

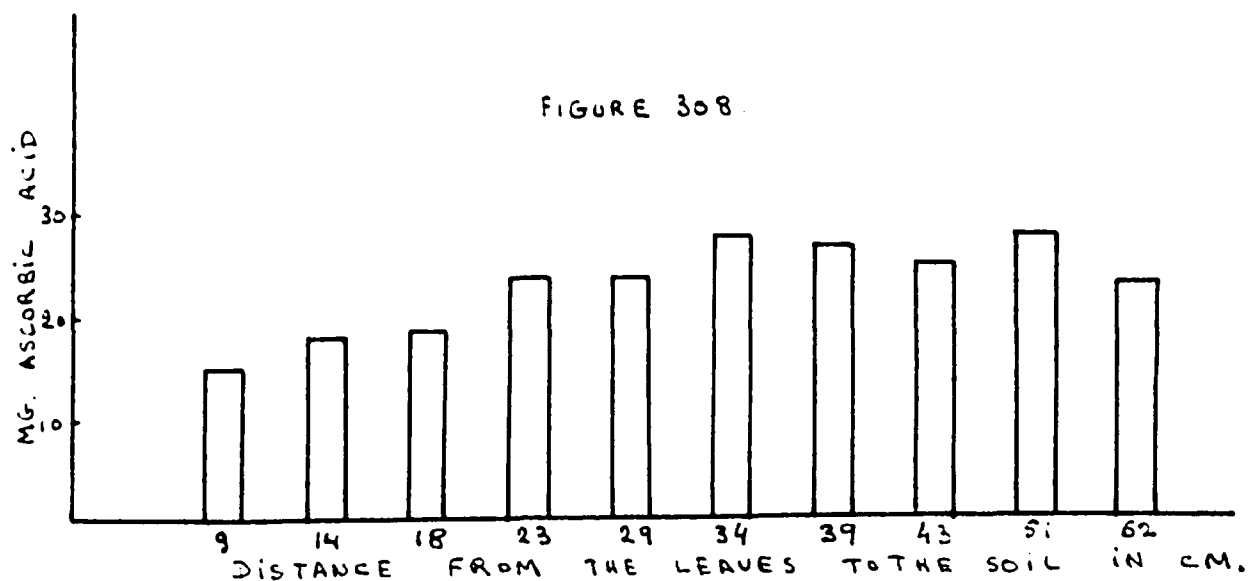


Figure 309. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plant grown on soil, plotted against the amounts of manganese, expressed in milligrams per hundred grams of the corresponding fresh plant material.

Figure 310. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plant grown on soil, plotted against the amounts of iron, expressed in milligrams per hundred grams of the corresponding plant material.

Figure 311. Average amounts of ascorbic acid, expressed in milligrams per hundred grams of fresh matter from the various leaves of a tobacco plant grown on soil, plotted against the amounts of dry matter, expressed in grams per hundred grams of the corresponding fresh plant material.



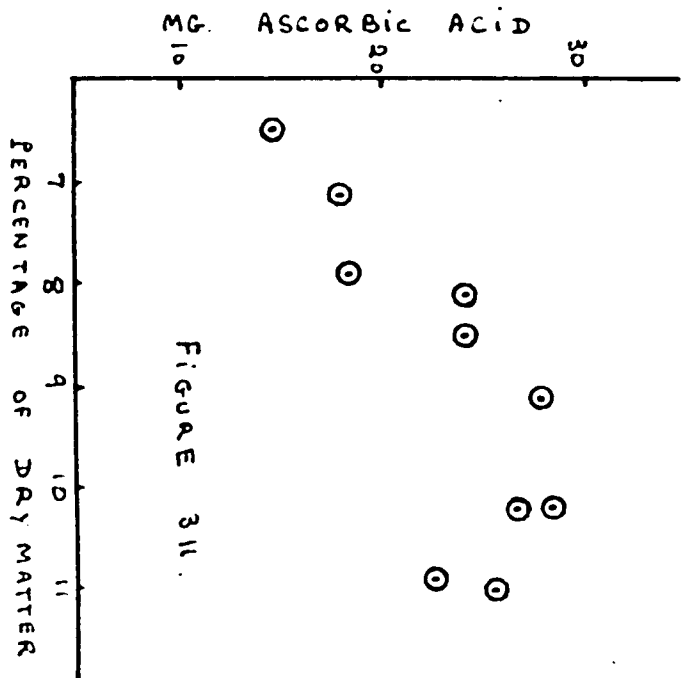
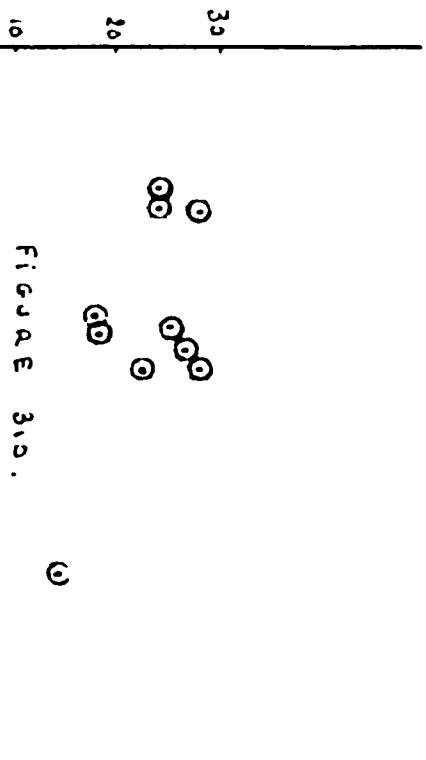
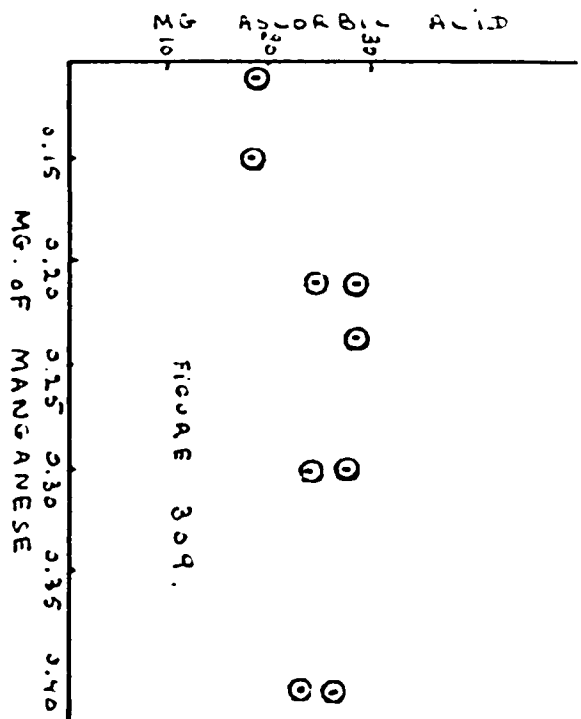


Figure 312. Average amounts of manganese, expressed in milligrams per 100 grams of fresh matter from the various leaves of a tobacco plant grown on soil, plotted against the distance from the leaves to the soil, expressed in centimeters.

Figure 313. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the various leaves of a tobacco plant grown on soil, plotted against the distance from the leaves to the soil, expressed in centimeters.

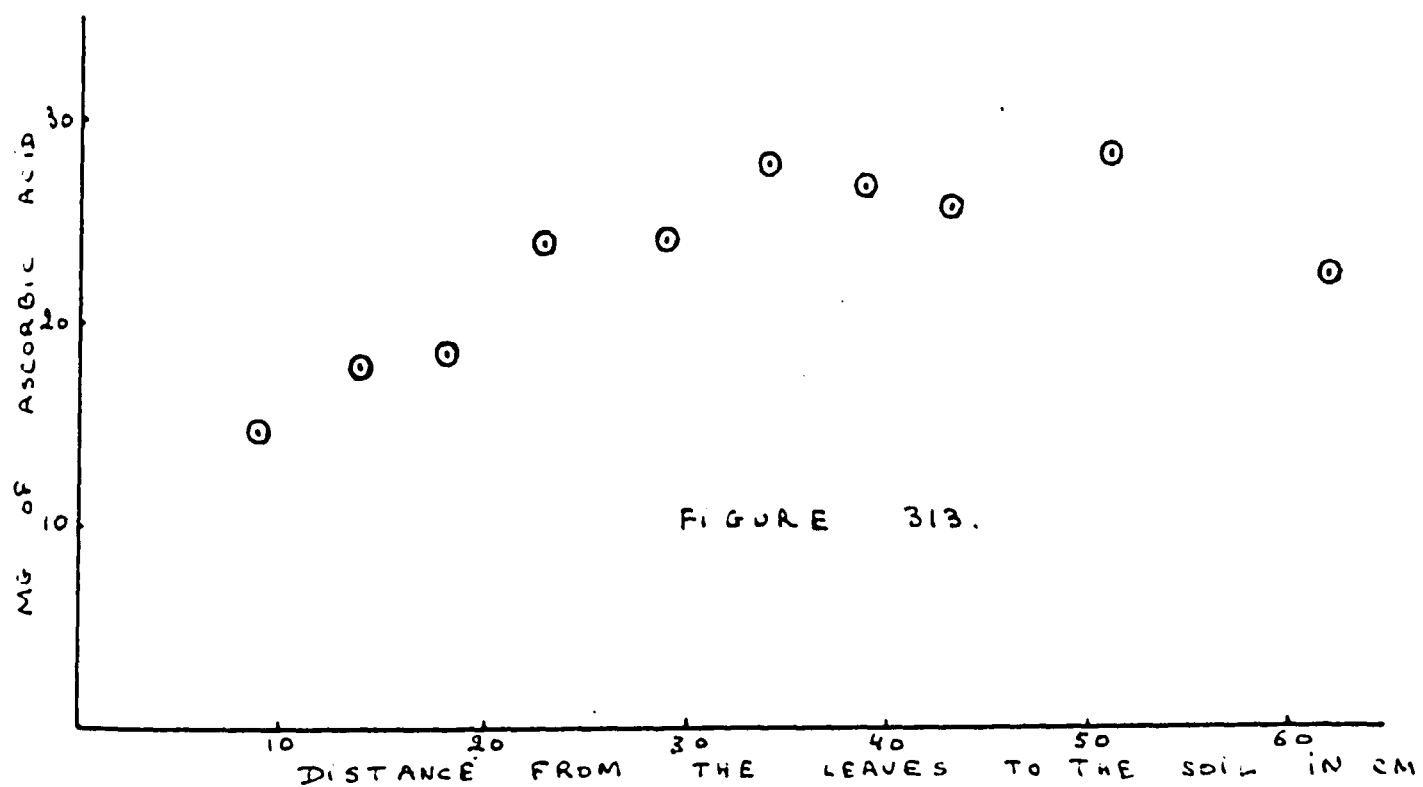
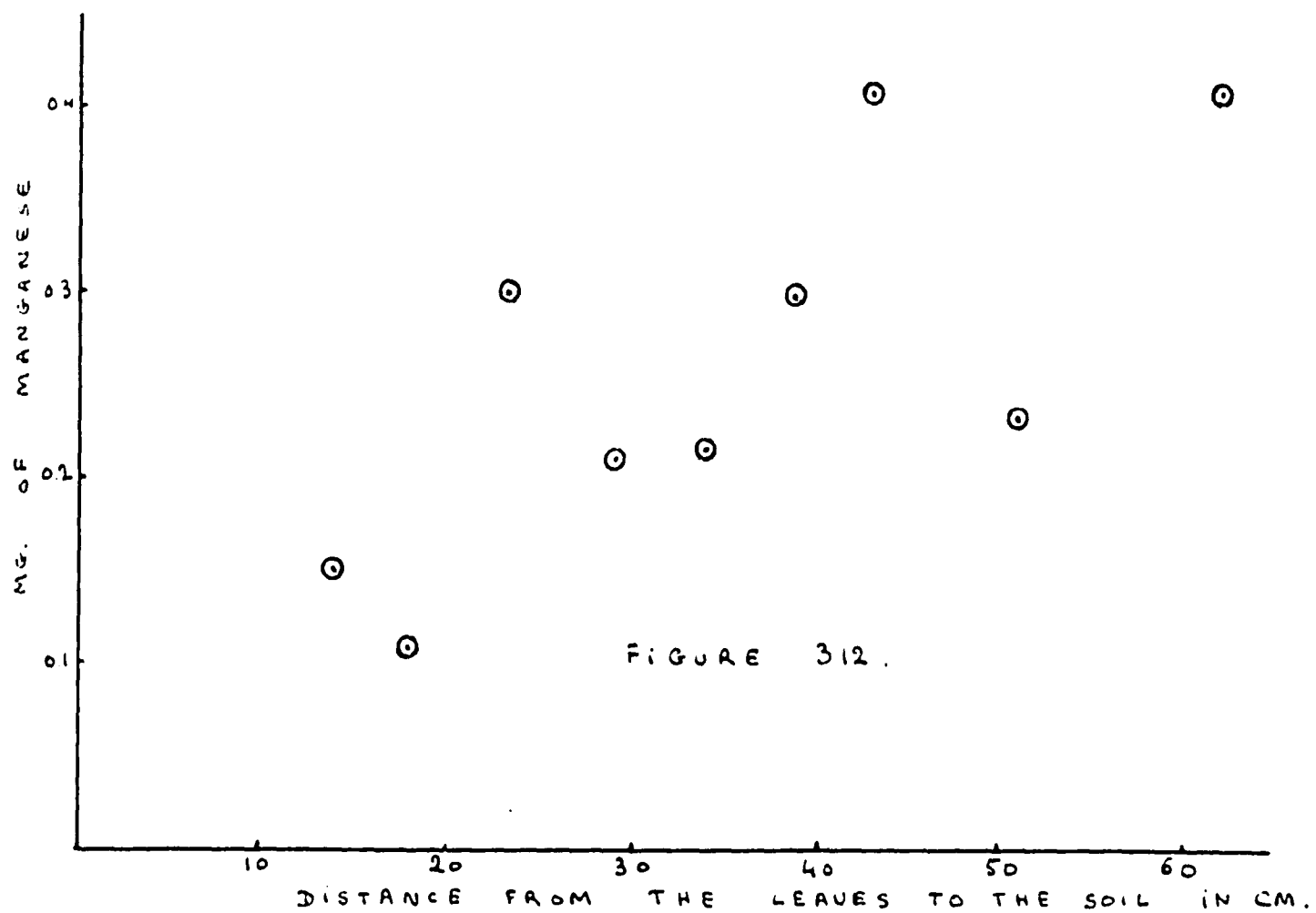
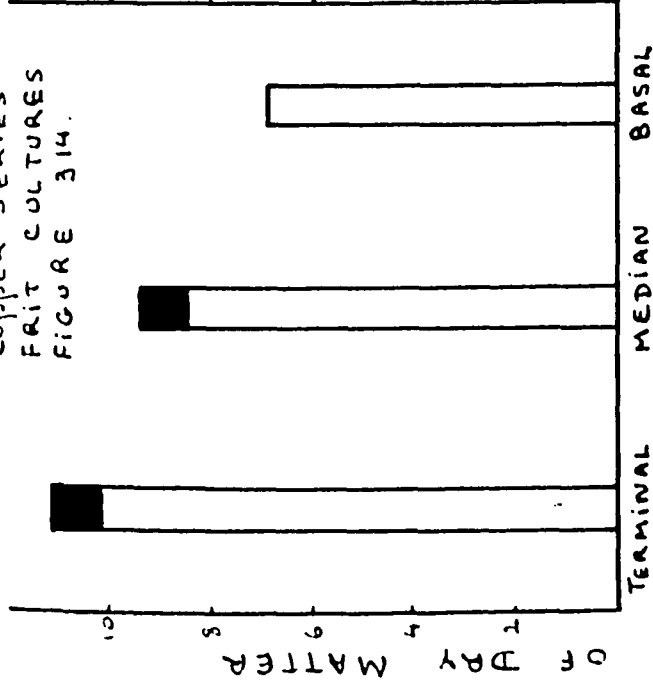
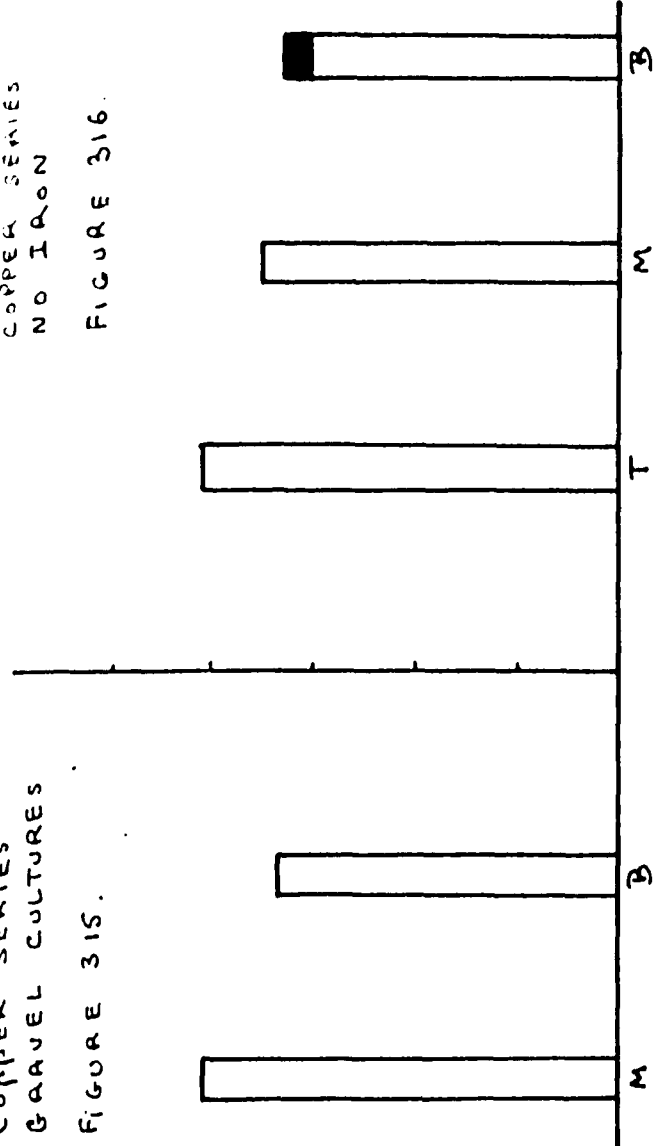


Figure 314 to figure 319 included. Average amounts of dry matter, expressed in grams per hundred grams of fresh matter from tobacco plants, grown on various cultures differing in iron availability without and with 0.08 parts per million of copper. The values for the basal, median and terminal leaves are represented by bar graphs. The shaded portions correspond to the increments of one copper series over the other.

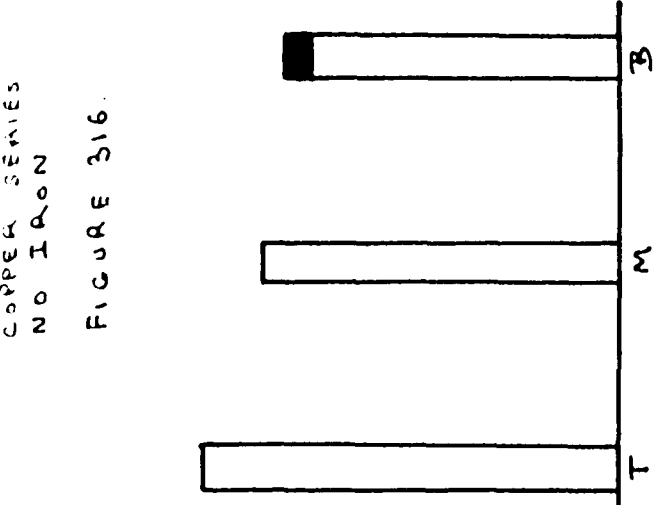
COPPER SERIES  
FRUIT CULTURES  
FIGURE 314.



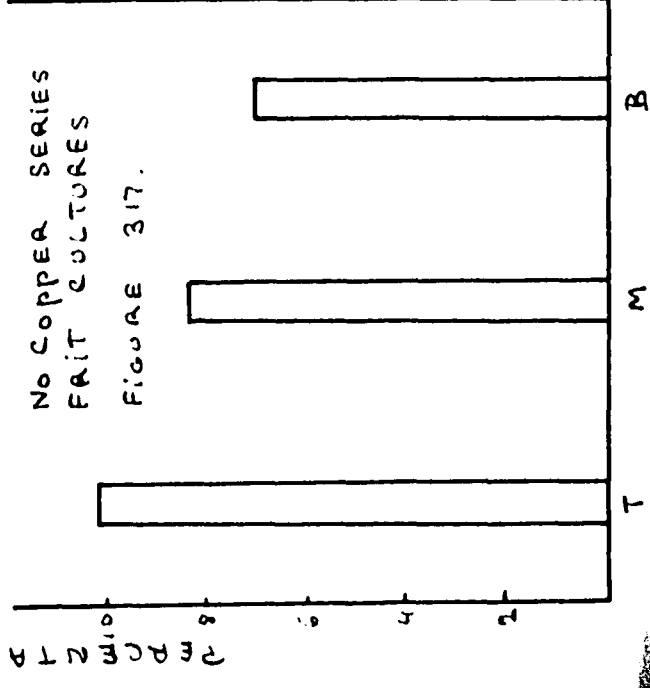
COPPER SERIES  
GRAVEL CULTURES  
FIGURE 315.



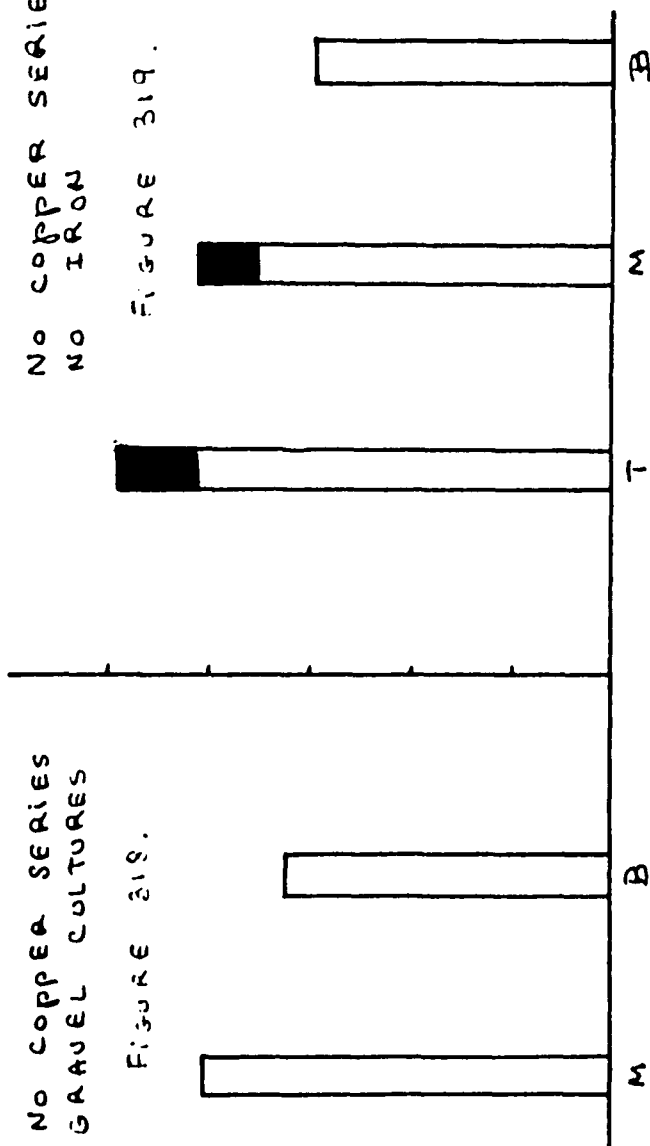
COPPER SERIES  
NO IRON  
FIGURE 316.



NO COPPER SERIES  
FRUIT CULTURES  
FIGURE 317.



NO COPPER SERIES  
GRAVEL CULTURES  
FIGURE 318.



NO COPPER SERIES  
NO IRON  
FIGURE 319.

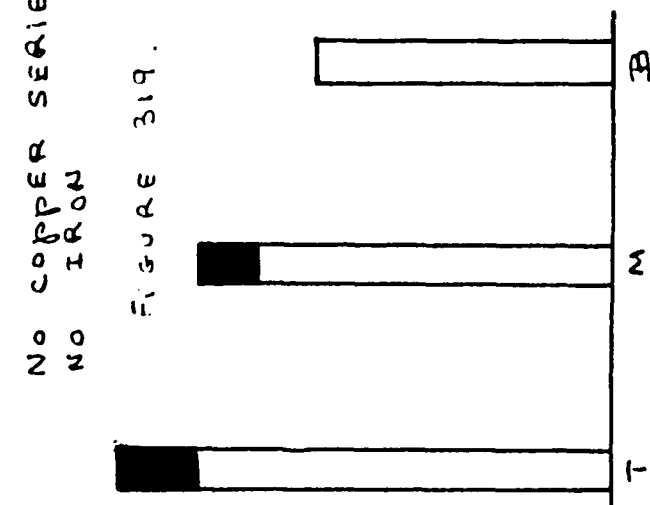
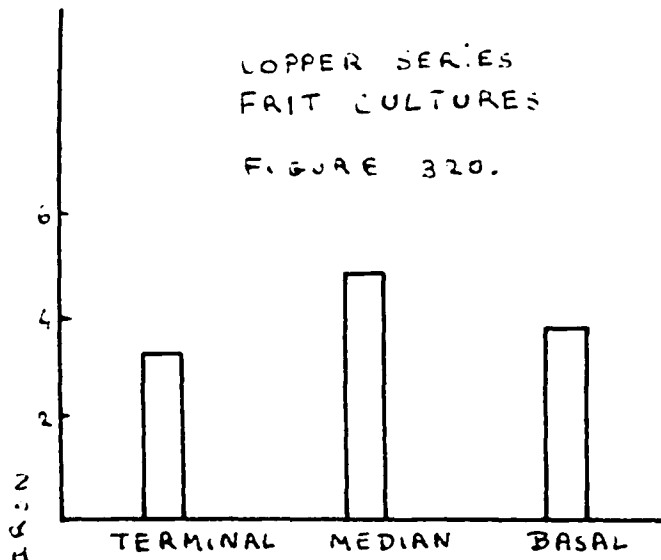
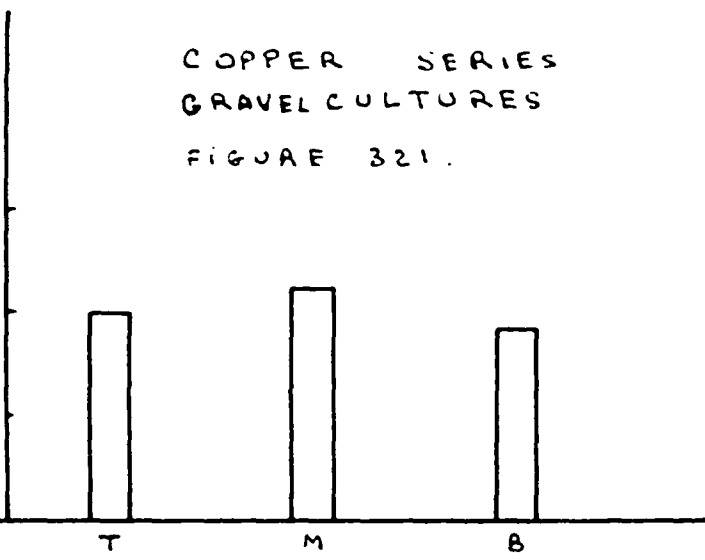


Figure 320 to figure 325 included. Average amounts of iron, expressed in milligrams per 100 grams of fresh matter from tobacco plants, grown on various cultures differing in iron availability without and with 0.08 parts per million of copper. The values for the basal, median, and terminal leaves are represented by bar graphs. The shaded portions correspond to the increments of one copper series over the other.

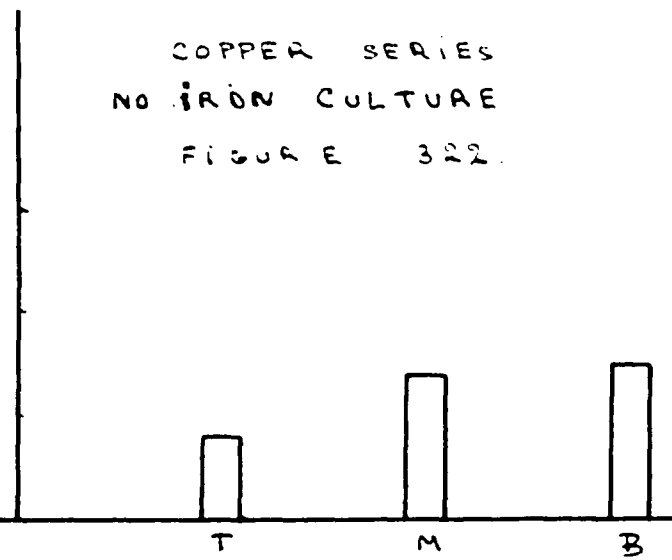
COPPER SERIES  
FAST CULTURES  
FIGURE 320.



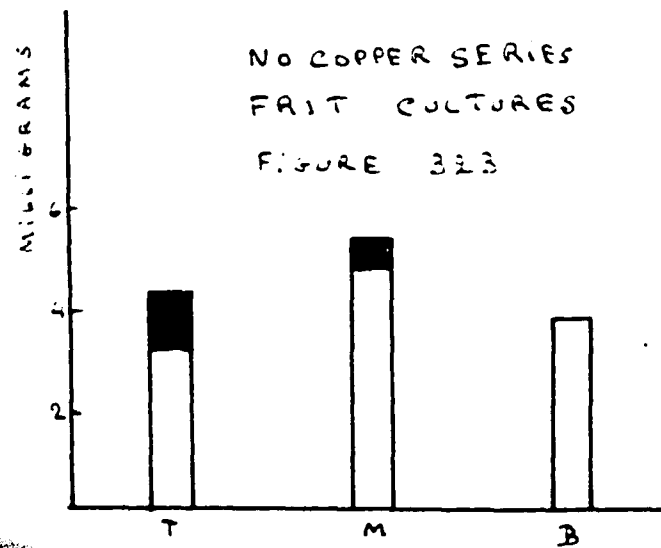
COPPER SERIES  
GRAVEL CULTURES  
FIGURE 321.



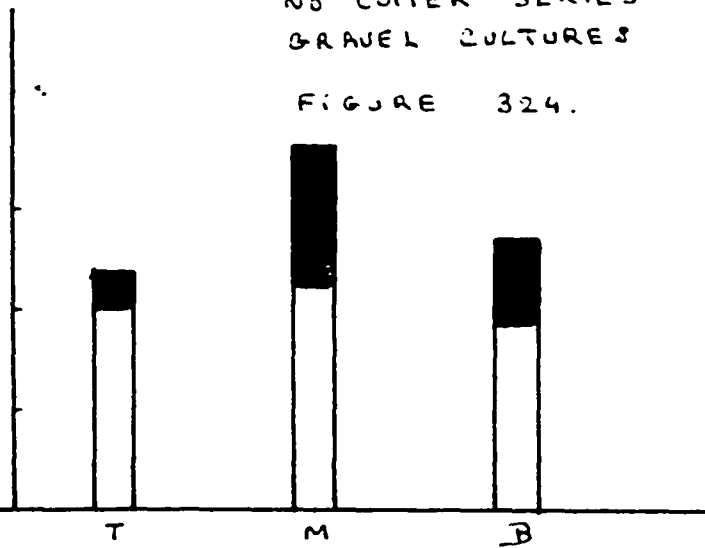
COPPER SERIES  
NO IRON CULTURE  
FIGURE 322.



NO COPPER SERIES  
FAST CULTURES  
FIGURE 323.



NO COPPER SERIES  
GRAVEL CULTURES  
FIGURE 324.



NO COPPER SERIES  
NO IRON  
FIGURE 325.

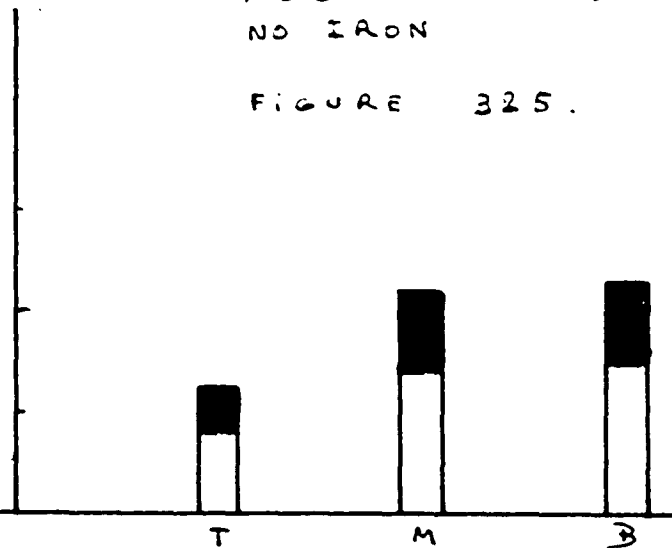
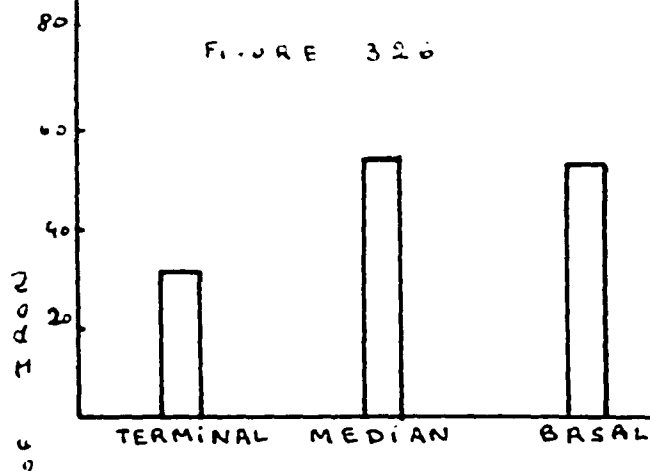


Figure 326 to figure 331 included. Average amounts of iron, expressed in milligrams per 100 grams of oven dry tissue from tobacco plants grown on various cultures differing in iron availability without and with 0.08 parts per million of copper. The values for the basal, median, and terminal leaves are represented by bar graphs. The shaded portions correspond to the increments of one copper series over the other.



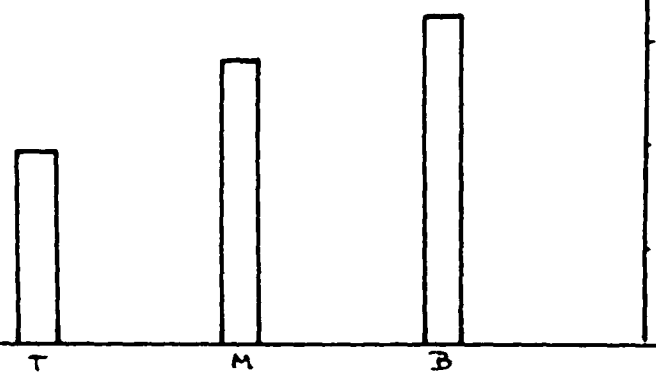
COPPER SERIES  
FRIT CULTURES

FIGURE 326



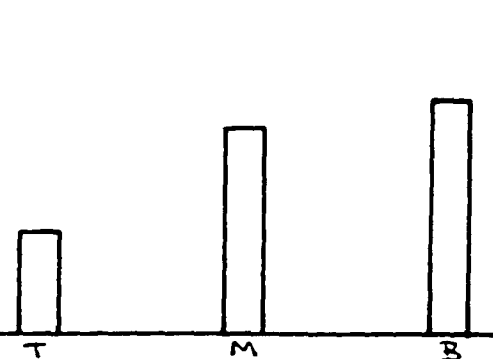
COPPER SERIES  
GRAVEL CULTURES

FIGURE 327.



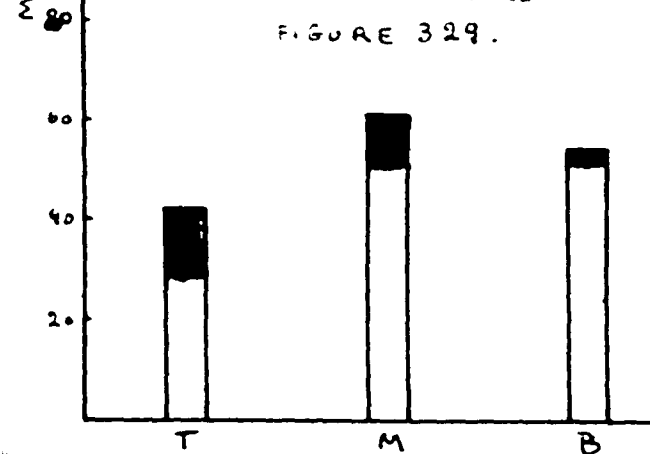
COPPER SERIES  
NO IRON CULTURES

FIGURE 328.



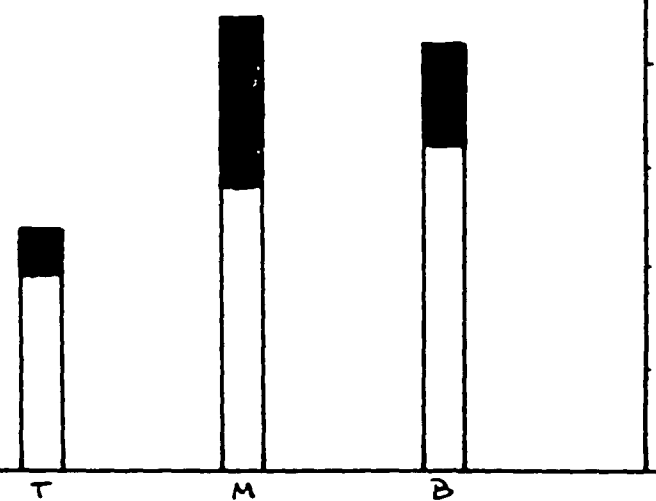
NO COPPER SERIES  
FRIT CULTURES

FIGURE 329.



NO COPPER SERIES  
GRAVEL CULTURES

FIGURE 330.



NO COPPER SERIES  
NO IRON

FIGURE 331

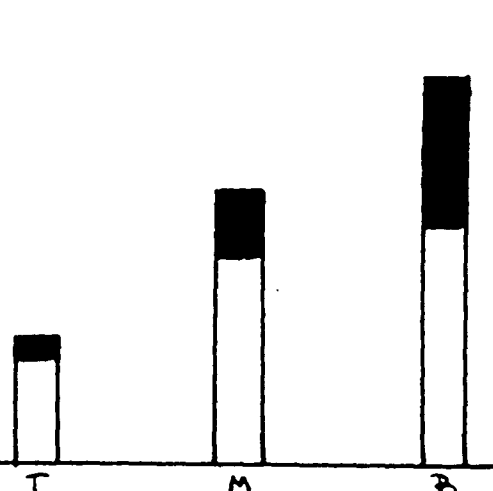
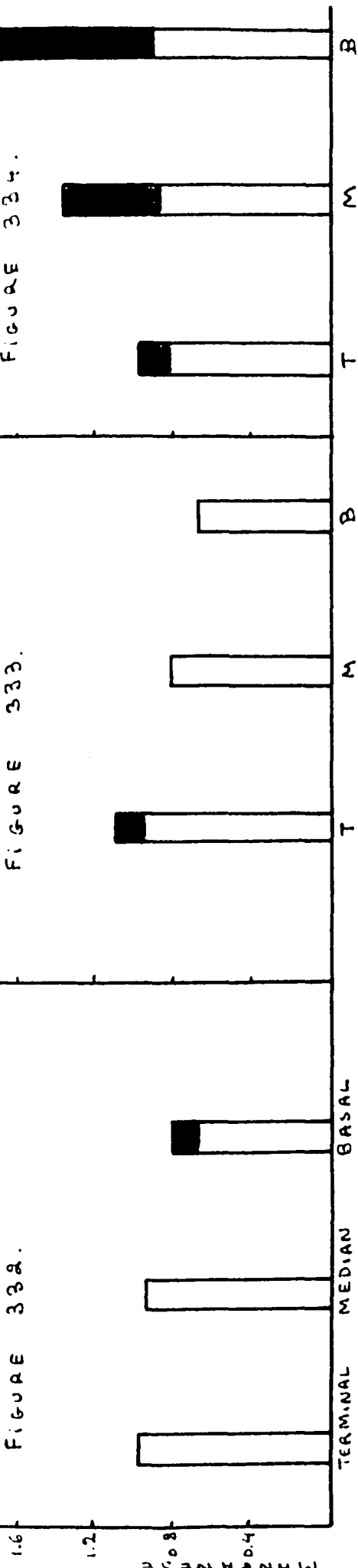
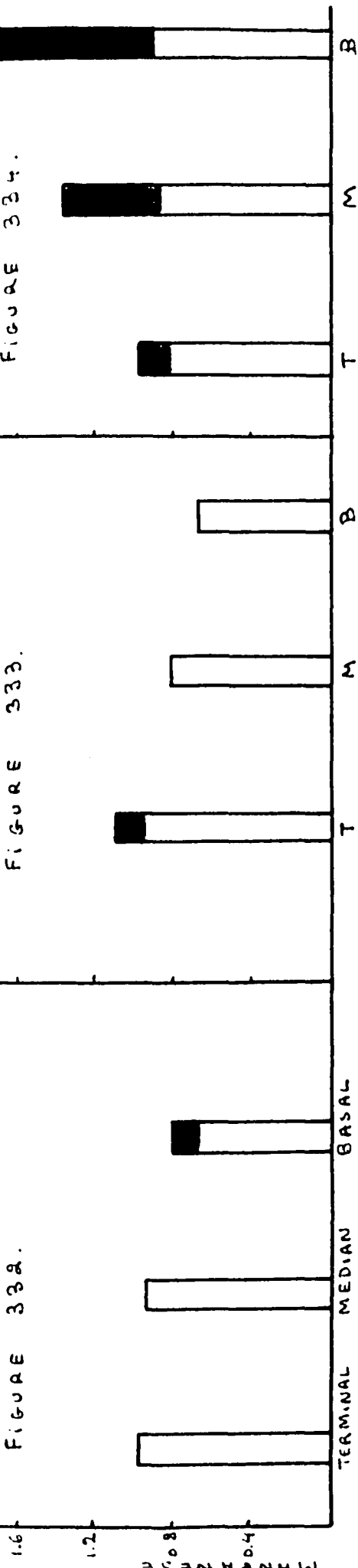


Figure 332 to figure 337 included. Average amounts of manganese, expressed in milligrams per 100 grams of fresh matter from tobacco plants grown on various cultures differing in iron availability without and with 0.08 parts per million of copper. The values for the basal, median, and terminal leaves are represented by bar graphs. The shaded portions correspond to the increments of one copper series over the other.

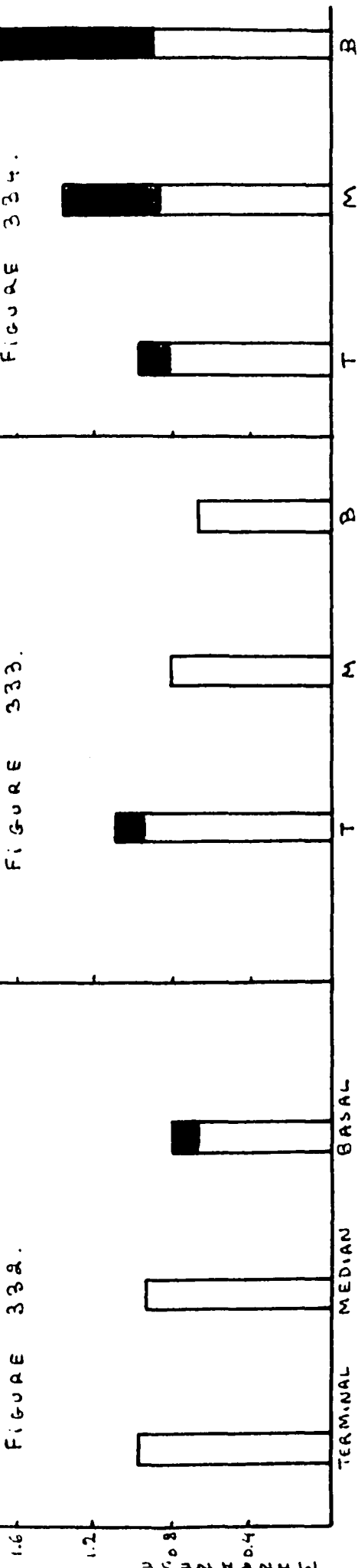
COPPER SERIES  
FRIT CULTURES  
FIGURE 332.



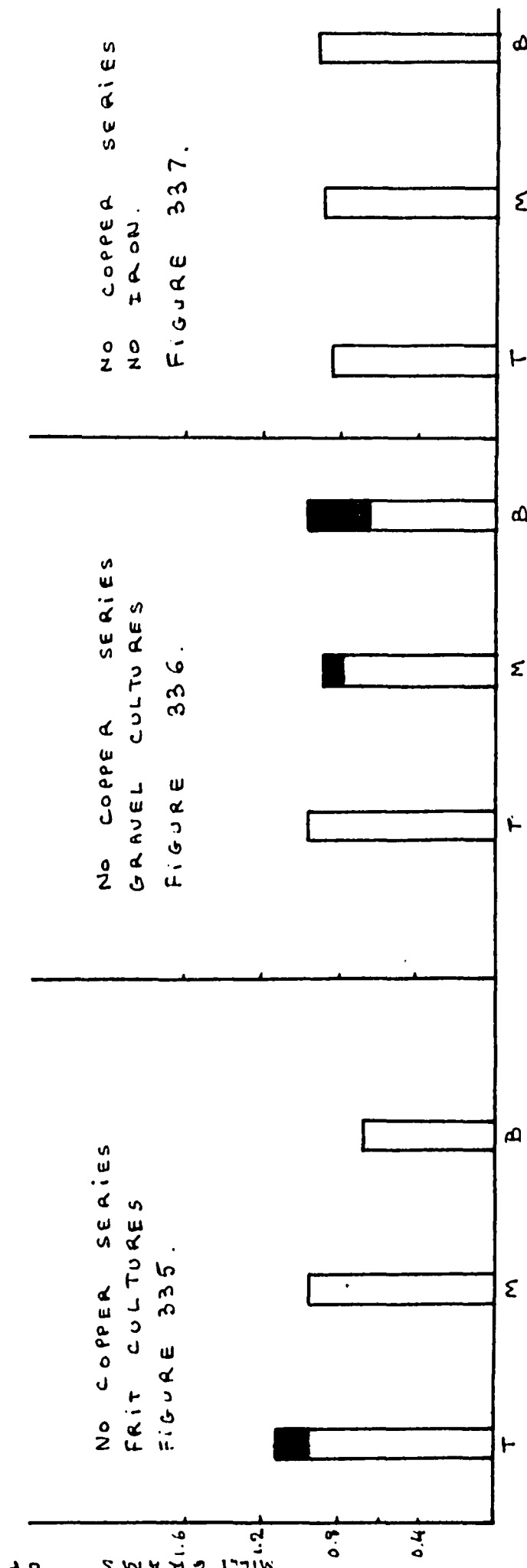
COPPER SERIES  
GRAVEL CULTURES  
FIGURE 333.



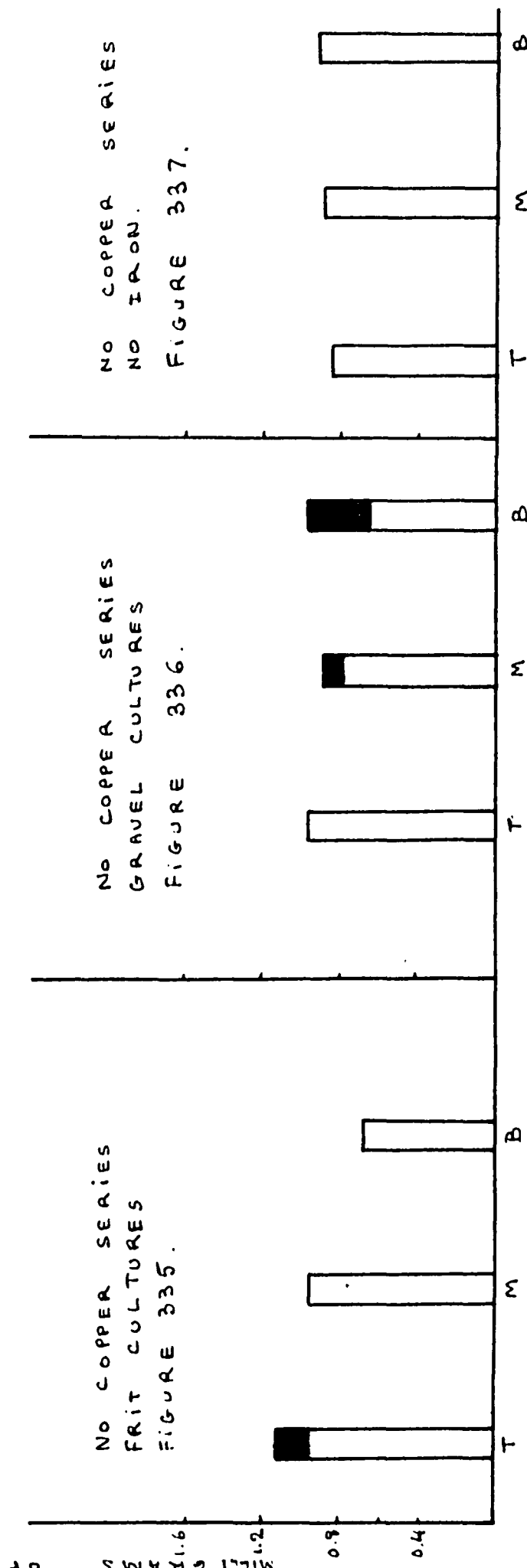
COPPER SERIES  
NO IRON  
FIGURE 334.



NO COPPER SERIES  
FRIT CULTURES  
FIGURE 335.



NO COPPER SERIES  
GRAVEL CULTURES  
FIGURE 336.



NO COPPER SERIES  
NO IRON  
FIGURE 337.

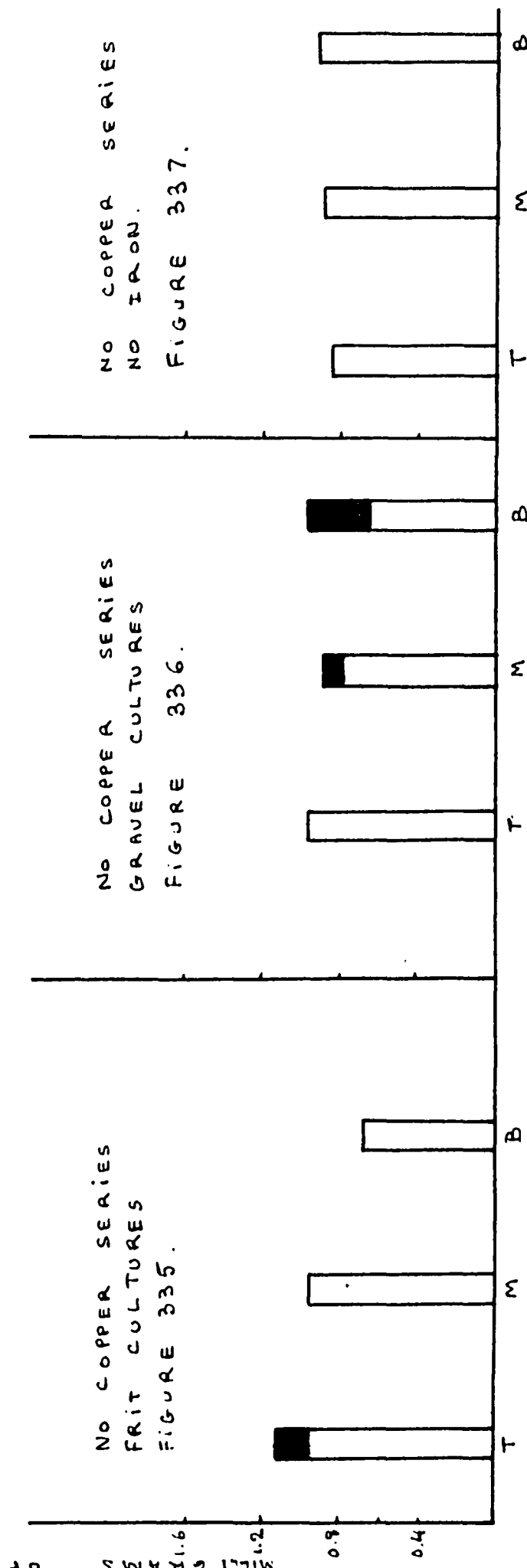


Figure 338 to 343 included. Average amounts of manganese, expressed in milligrams per 100 grams of oven dry tissue from tobacco plants grown on various cultures differing in iron availability without and with 0.08 parts per million of copper. The values for the basal, median, and terminal leaves are represented by bar graphs; the shaded portions correspond to the increments of one copper series over the other.

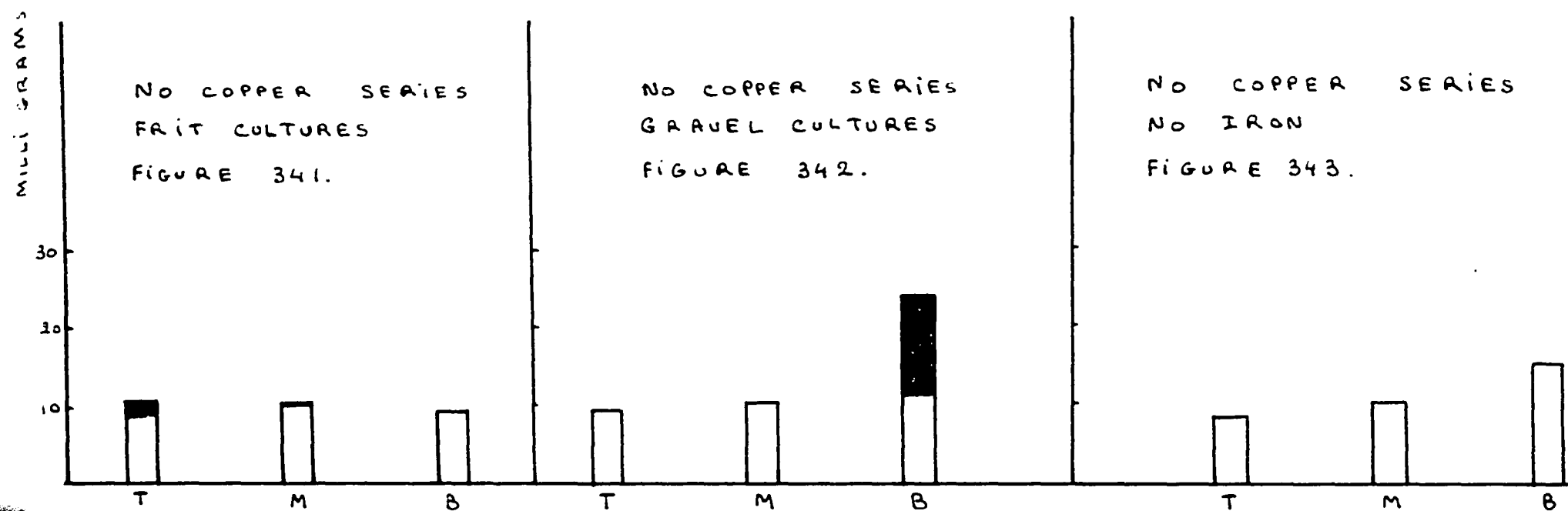
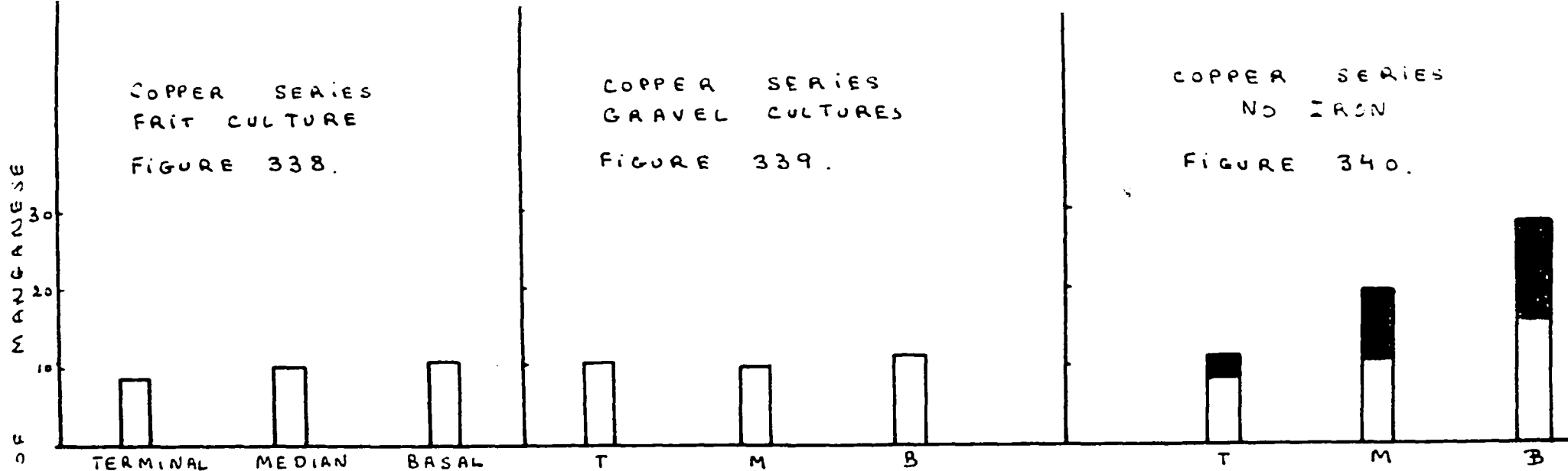


Figure 344. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the terminal, median, and basal leaves of tobacco plants grown on an iron-containing frit supplied with a nutrient solution with 0.08 parts per million of copper; plotted against the time of the various determinations during the drying of the plant material, expressed in hours.

Figure 345. Same as figure 344, for plants grown on gravel supplied with a nutrient solution containing 4 parts per million of iron and 0.08 parts per million of copper.

Figure 346. Same as figure 344, for plants grown on gravel supplied with a nutrient solution deprived of iron and with 0.08 parts per million of copper.

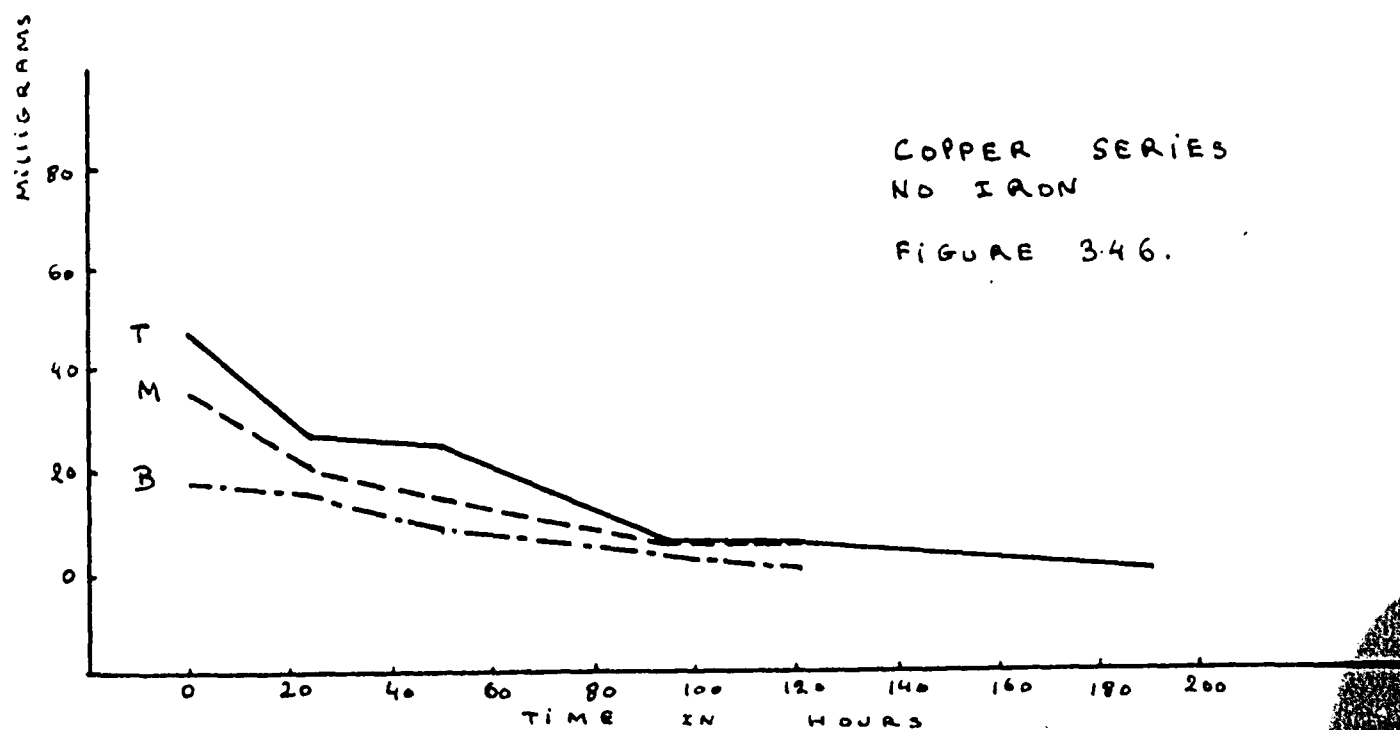
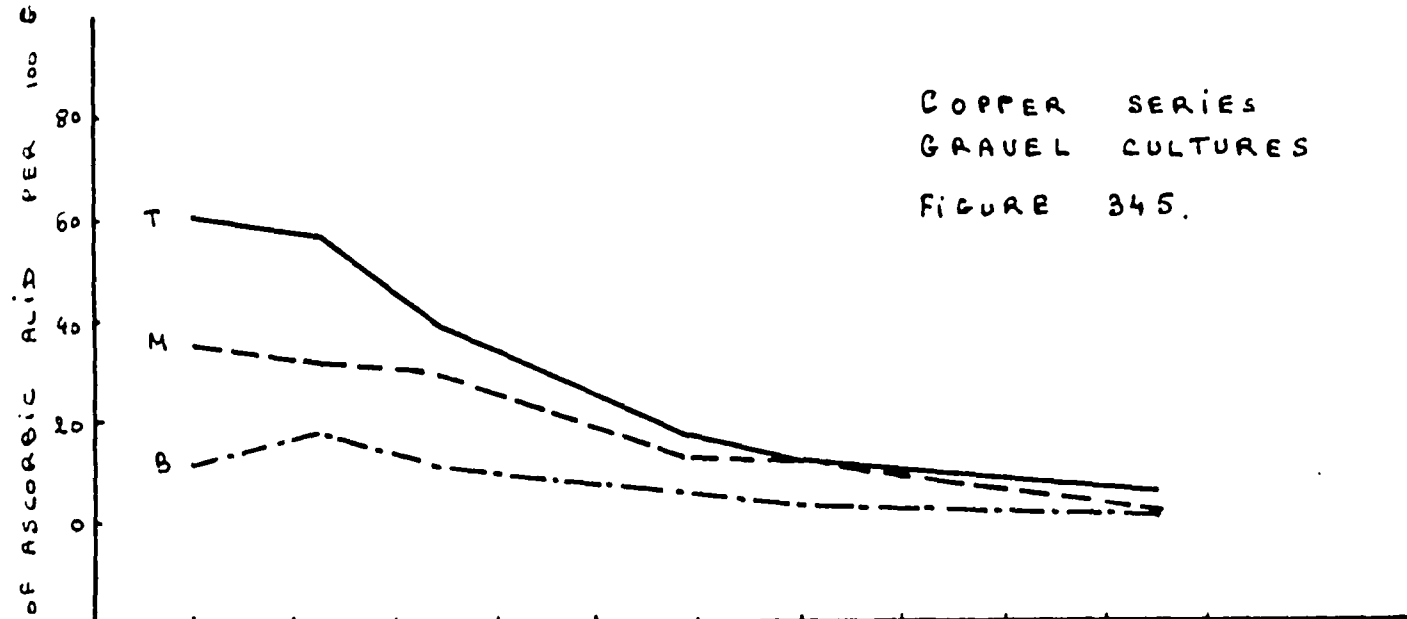
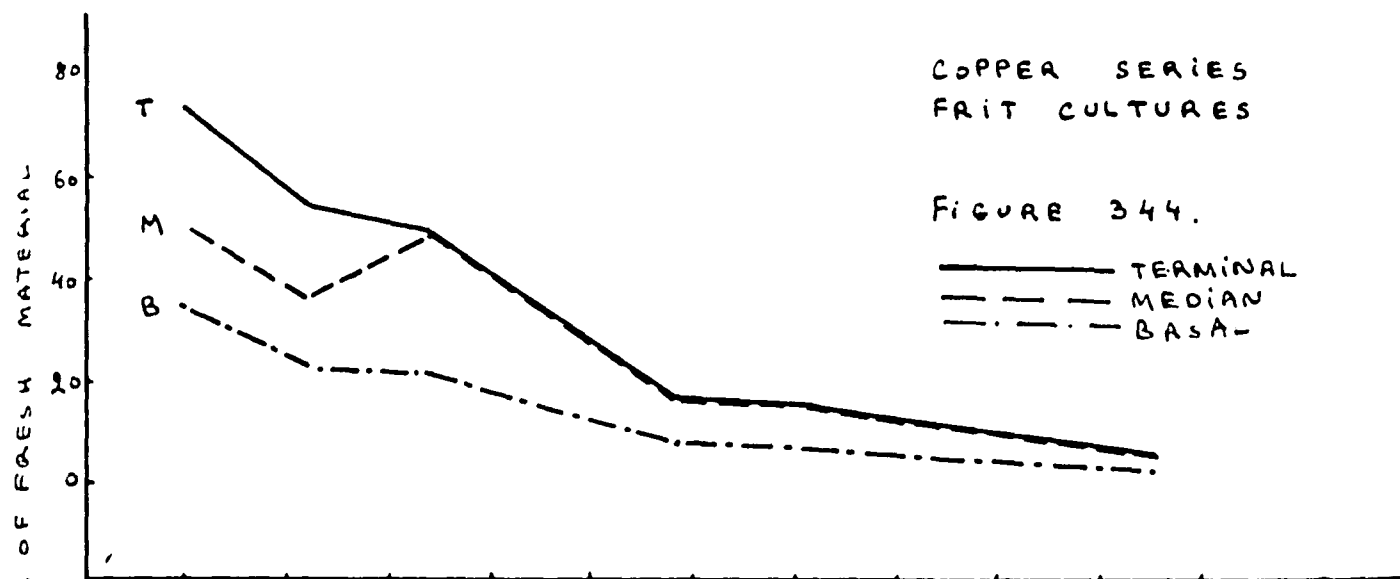


Figure 347. Average amounts of ascorbic acid, expressed in milligrams per 100 grams of fresh matter from the terminal, median and basal leaves of tobacco plants grown on an iron-containing frit supplied with a nutrient solution deprived of copper; plotted against the time of the various determinations during the drying of the plant material, expressed in hours.

Figure 348. Same as figure 341, for plants grown on gravel supplied with a nutrient solution containing 4 parts per million of iron and deprived of copper.

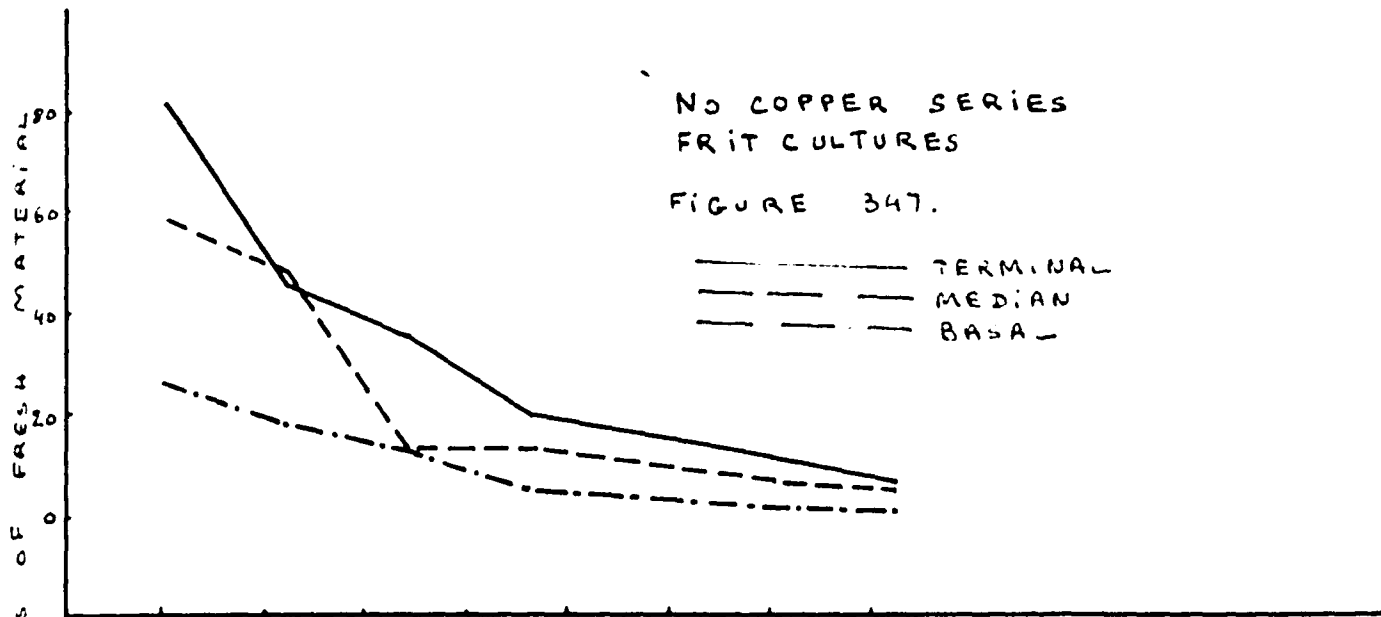
Figure 349. Same as figure 341, for tobacco plants grown on gravel supplied with a nutrient solution deprived of iron and copper.



NO COPPER SERIES  
FRIT CULTURES

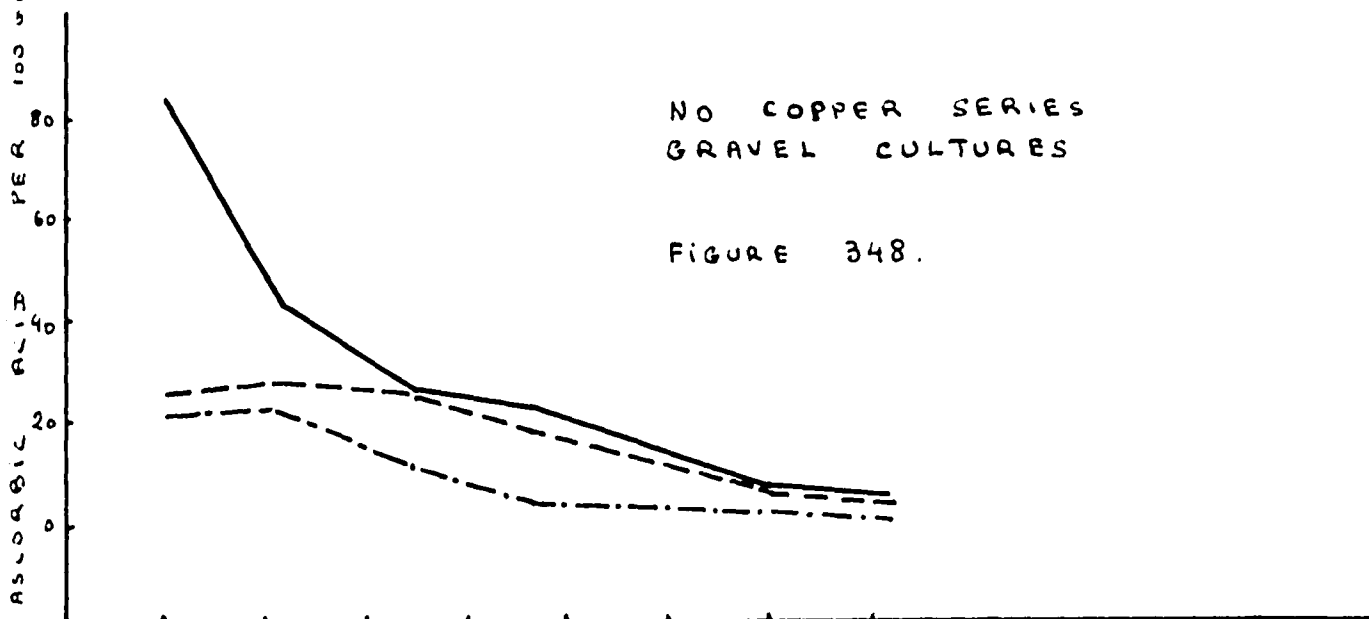
FIGURE 347.

— — — — — TERMINAL  
— — — — — MEDIAN  
— · — · — BASAL



NO COPPER SERIES  
GRAVEL CULTURES

FIGURE 348.



NO COPPER SERIES  
NO IRON

FIGURE 349.

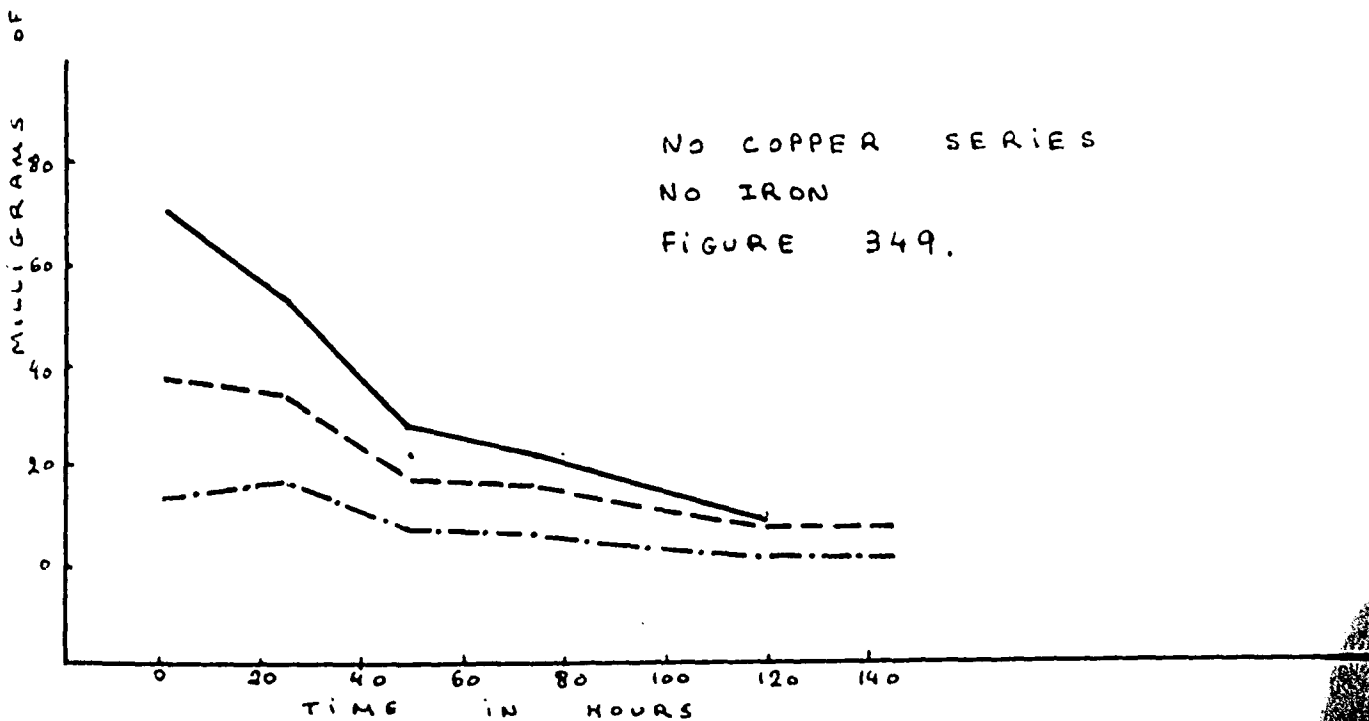


Figure 350. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from the terminal, median, and basal leaves of tobacco plants grown on various cultures differing in iron availability, supplied with a nutrient solution containing 0.08 parts per million of copper, plotted against the amounts of iron in the corresponding fresh tissue, expressed in milligrams.

Figure 351. Same as figure 350, for the plants grown on a nutrient solution deprived of copper.

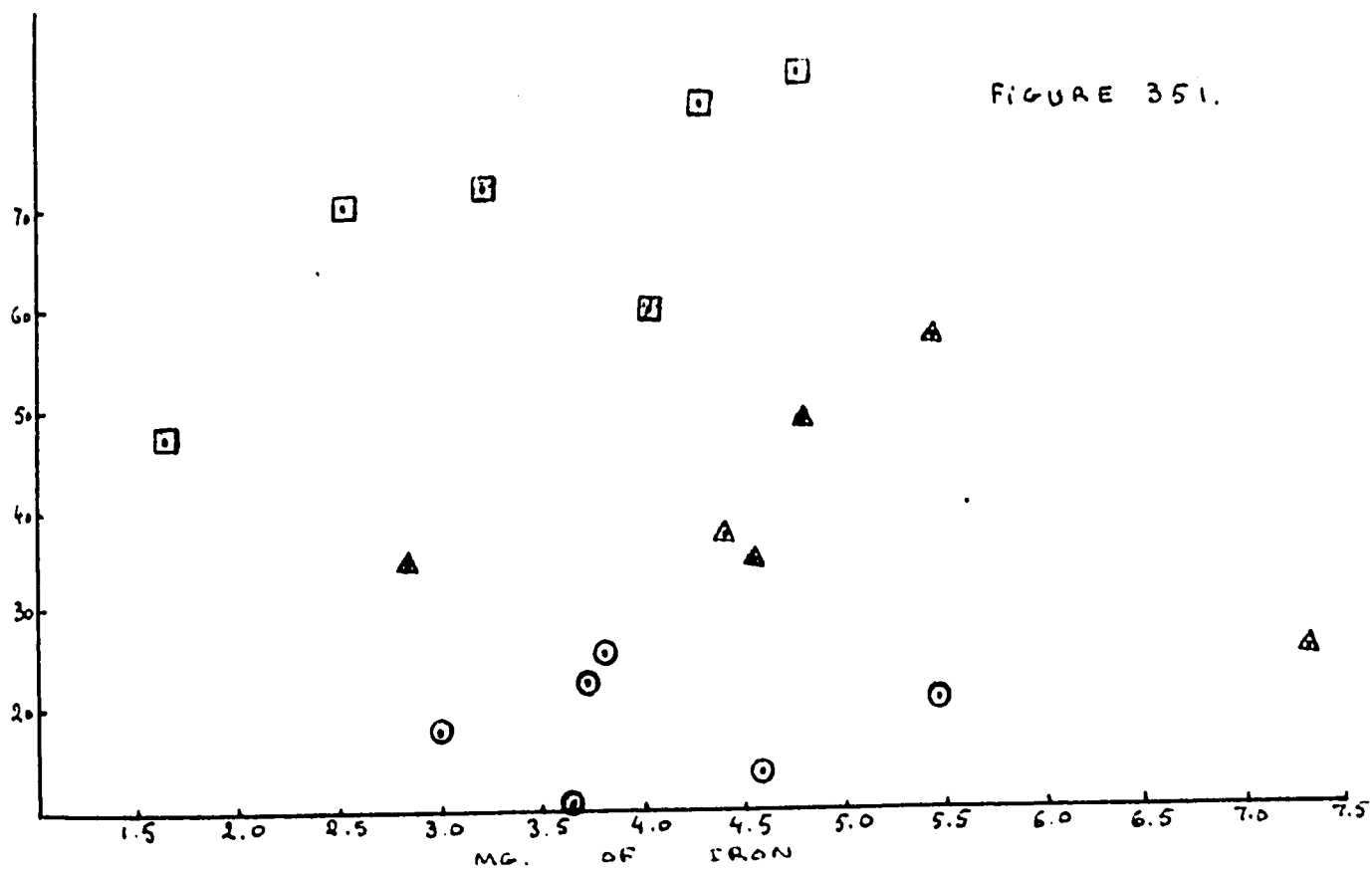
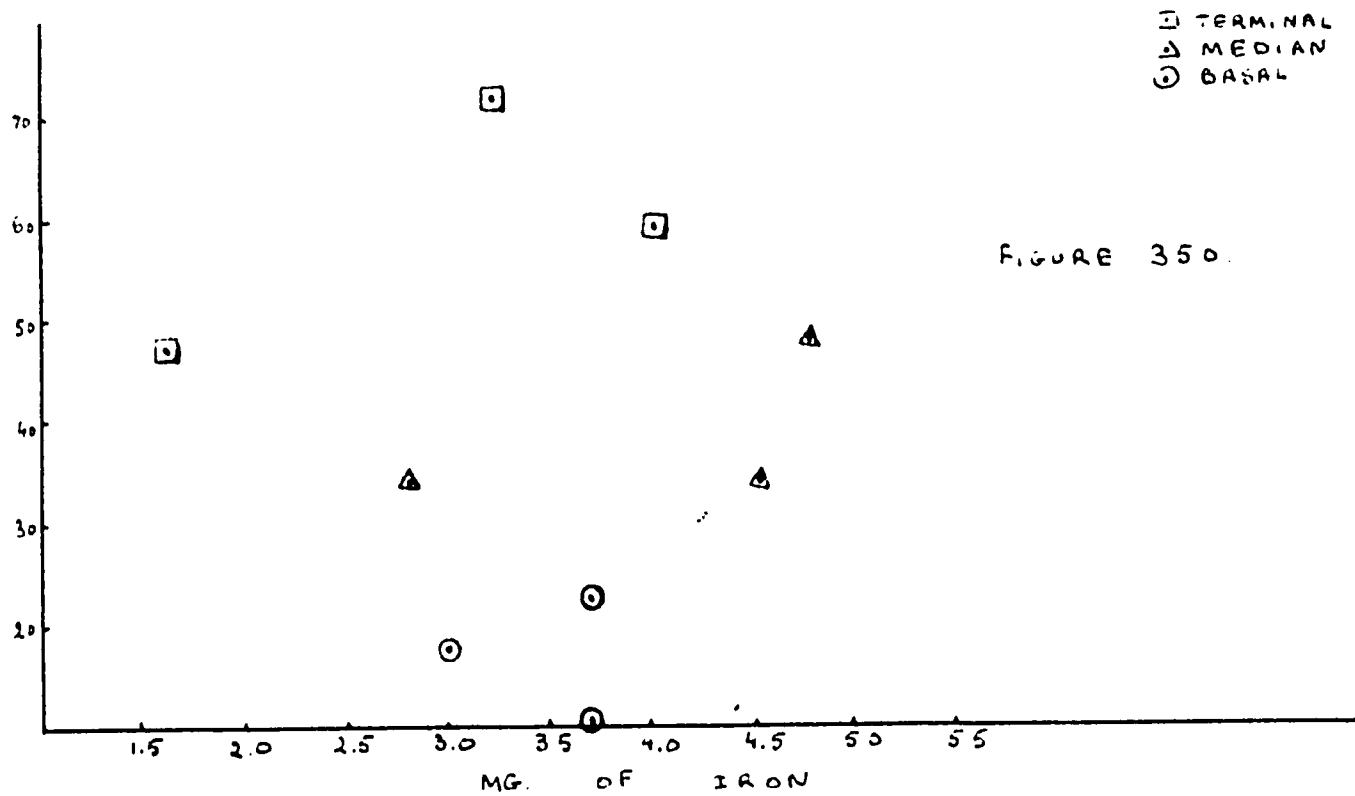


Figure 352. Average amounts of ascorbic acid at harvest, expressed in milligrams per 100 grams of fresh matter from the terminal, median, and basal leaves of tobacco plants grown on various cultures differing in iron availability, supplied with a nutrient solution containing 0.08 parts per million of copper, plotted against the amounts of manganese in the corresponding fresh tissue, expressed in milligrams.

Figure 353. Same as figure 352, for the tobacco plants grown on a solution deprived of copper.

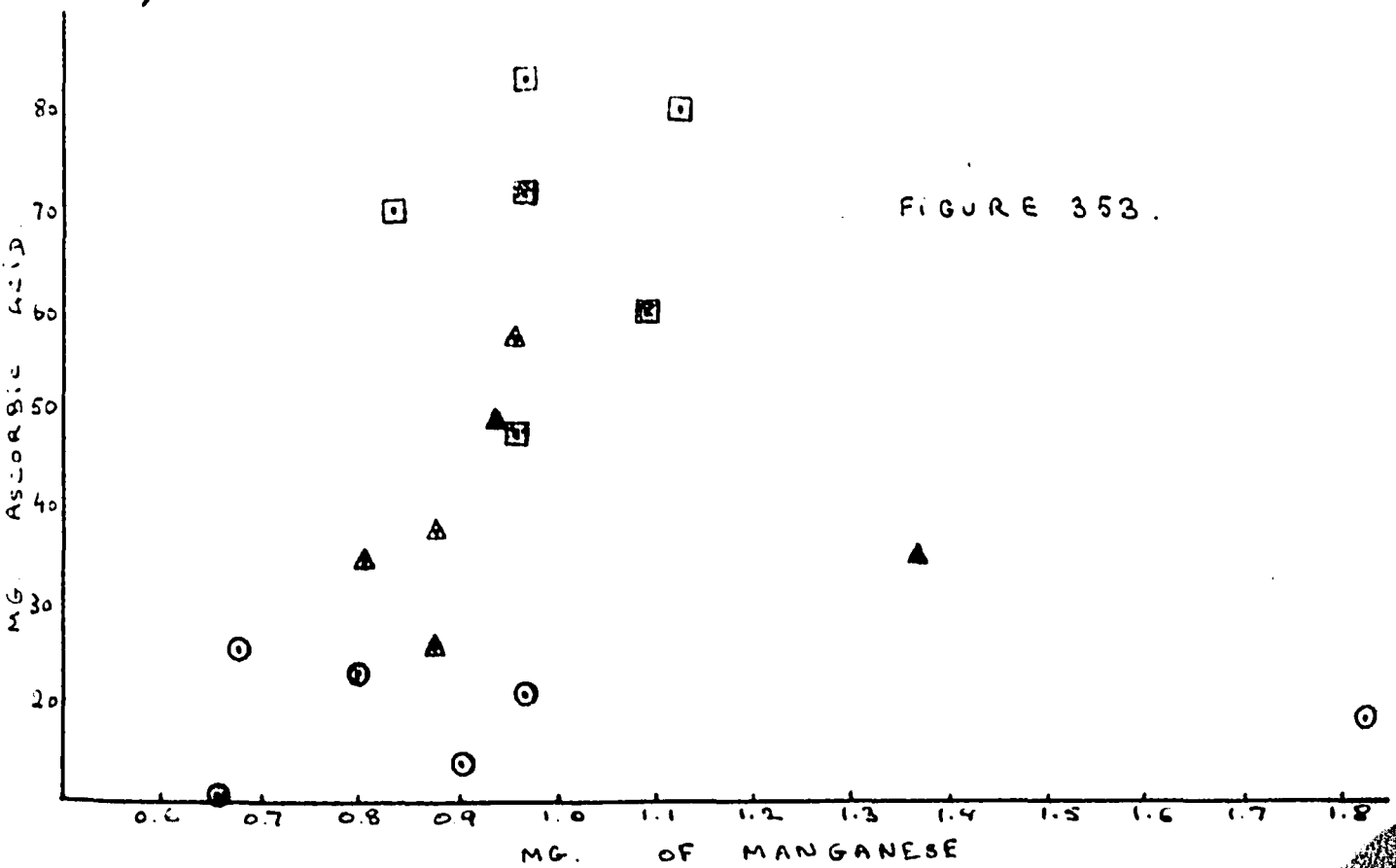
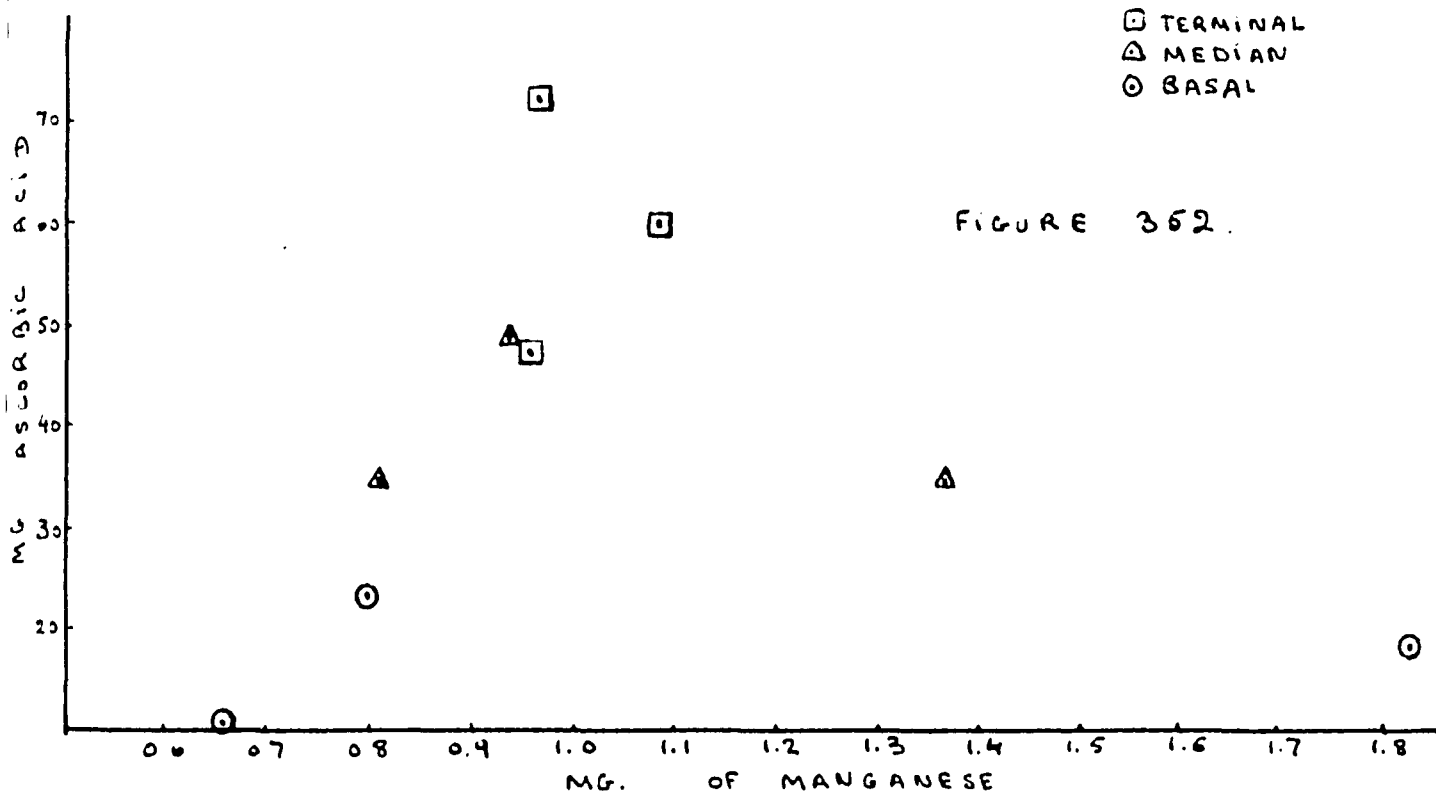


Figure 354. Average amounts of ascorbic acid at harvest, expressed in milligrams per hundred grams of fresh matter from the terminal, median, and basal leaves of tobacco plants grown on various cultures differing in iron availability, supplied without and with 0.08 parts per million of copper, plotted against the amount of dry matter, expressed in grams per hundred grams of fresh matter.

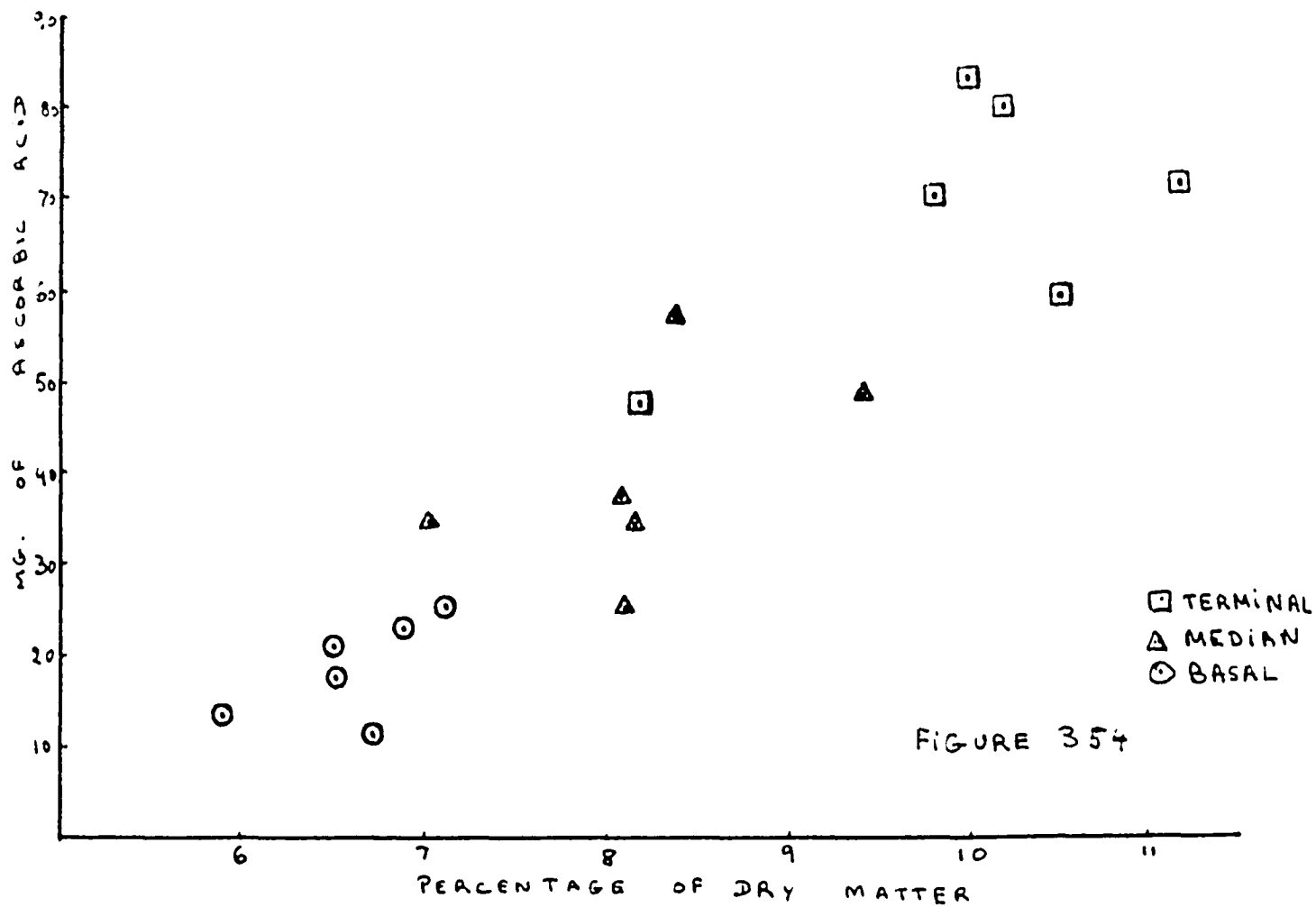


FIGURE 354

Figure 355 to figure 360 included. Amounts of ascorbic acid at harvest, 24, 48, and 120 hours after harvest, expressed in milligrams per 100 grams of fresh matter from the terminal, median and basal leaves of tobacco plants grown on various cultures differing in iron availability, supplied without and with 0.08 parts per million of copper. The shaded portions of the bar graphs correspond to the increments of one copper series over the other.



COPPER SERIES  
FAIT CULTURES

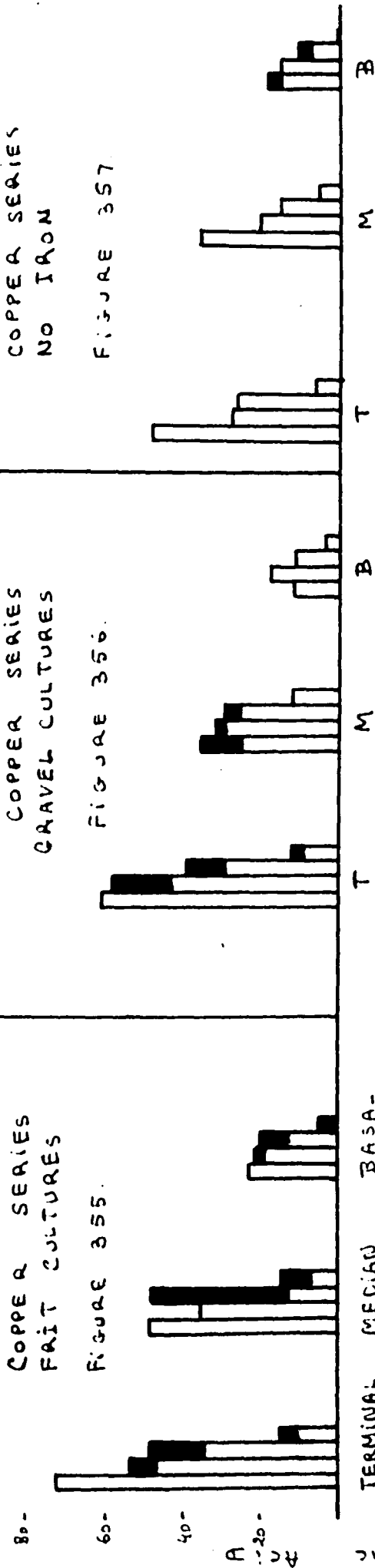
FIGURE 355.

COPPER SERIES  
GRAVEL CULTURES

FIGURE 356.

COPPER SERIES  
NO IRON

FIGURE 357.

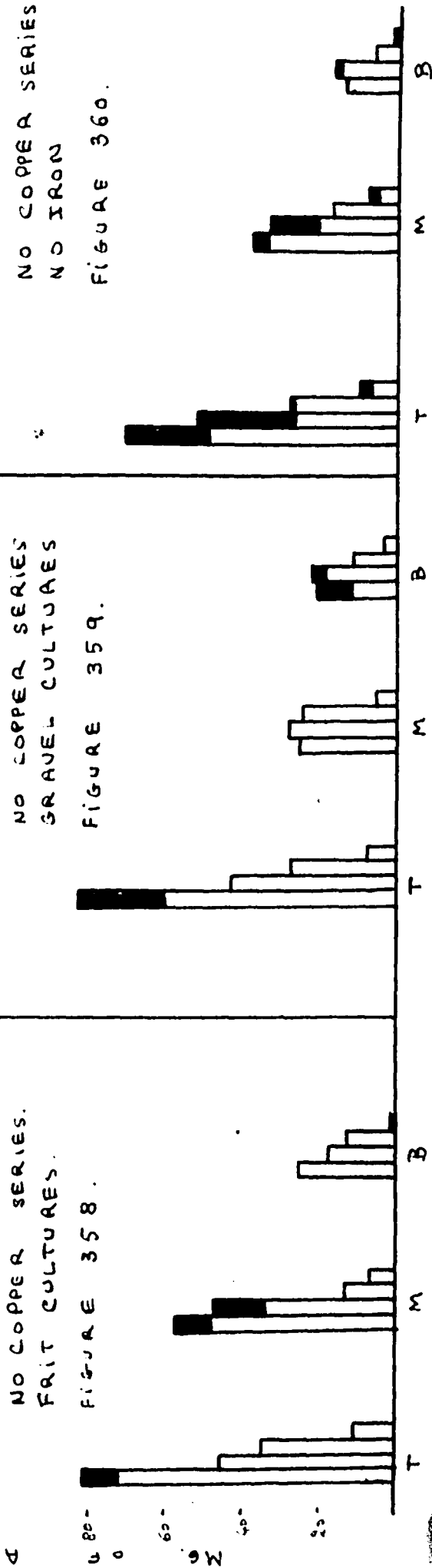


NO COPPER SERIES.  
FAIT CULTURES.

FIGURE 358.

NO COPPER SERIES  
GRAVEL CULTURES

FIGURE 359.



ASCORBIC ACID AT HARVEST, 24 HOURS, 48 and 120 HOUR AFTER HARVEST.

Figure 361. Percentages of the original amounts of ascorbic acid found in the fresh tissues from the terminal, median and basal leaves of tobacco plants grown on an iron-containing frit, supplied with a nutrient solution with 0.08 parts per million of copper; plotted against the time of the various determinations during the drying of the plant material, expressed in hours.

Figure 362. Same as figure 361, for the tobacco plants grown on gravel supplied with a nutrient solution containing 4 parts per million of iron and 0.08 parts per million of copper.

Figure 363. Same as figure 361, for the tobacco plants grown on gravel supplied with a nutrient solution deprived of iron and with 0.08 parts per million of copper

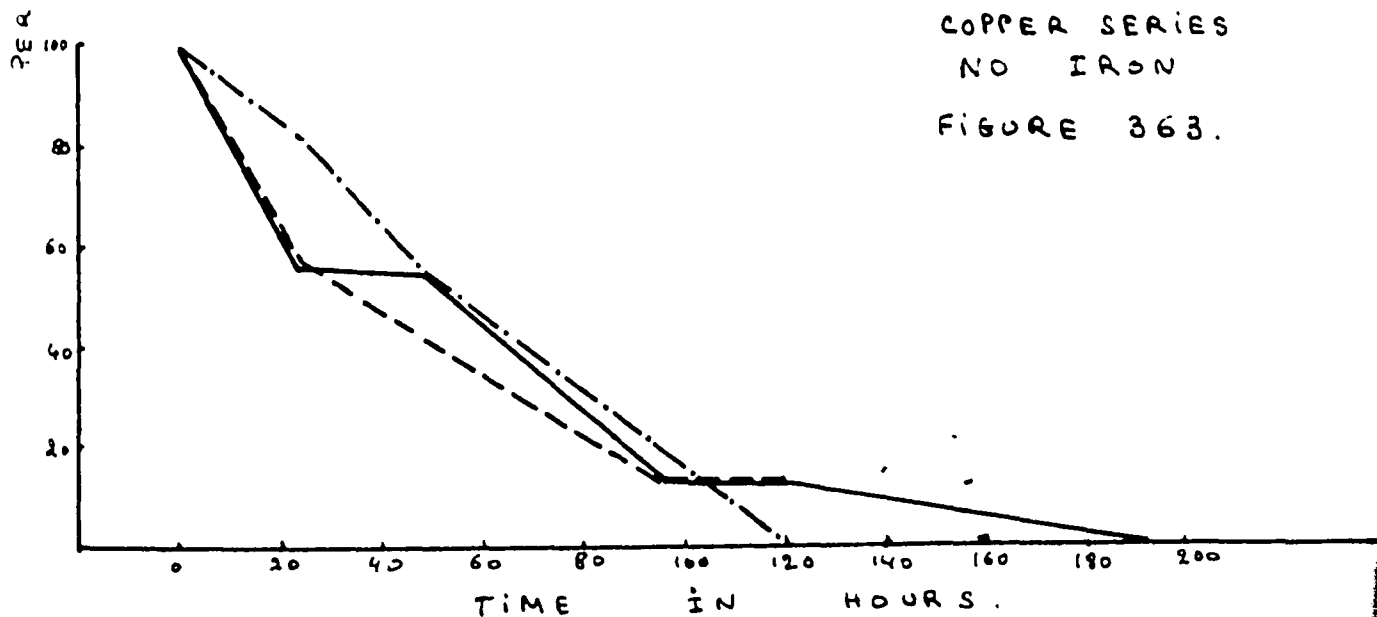
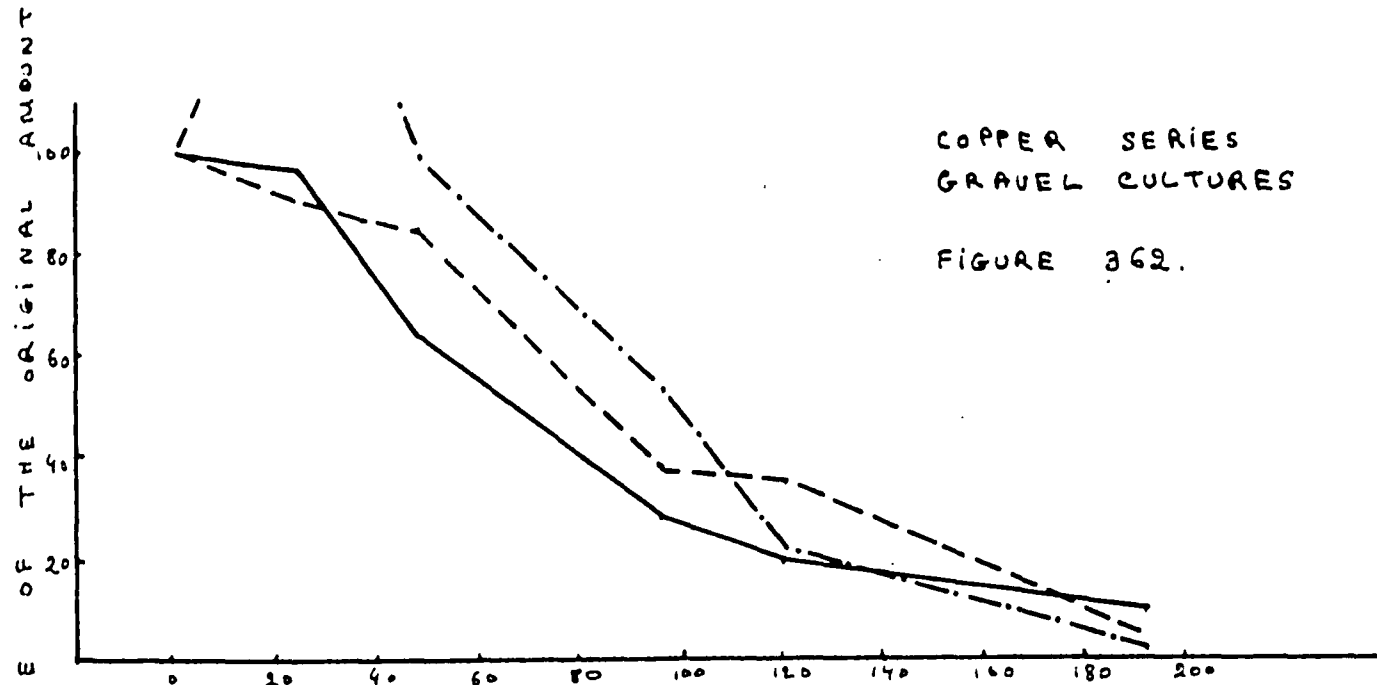
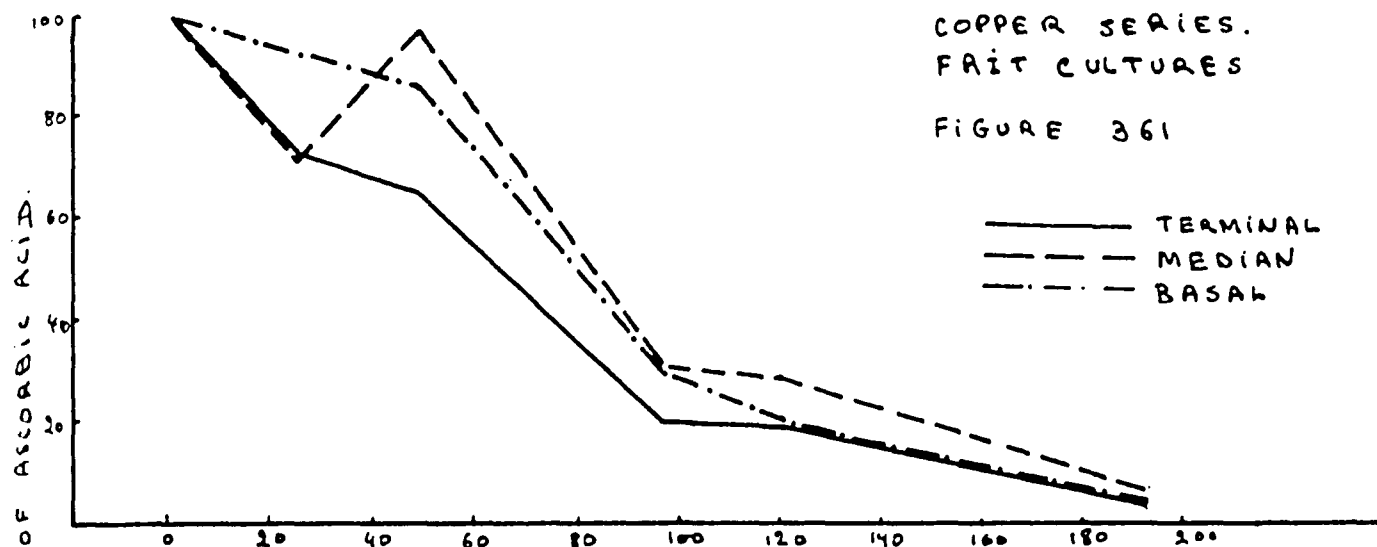


Figure 364. Percentages of the original amounts of ascorbic acid found in the fresh tissues from the terminal, median and basal leaves of tobacco plants grown on an iron-containing frit supplied with a nutrient solution deprived of copper; plotted against the time of the various determinations during the drying of the plant material.

Figure 365. Same as figure 364, for the tobacco plants grown on gravel supplied with a nutrient solution containing 4 parts per million of iron and deprived of copper.

Figure 366. Same as figure 364, for the tobacco plants grown on gravel supplied with a nutrient solution deprived of iron and copper.

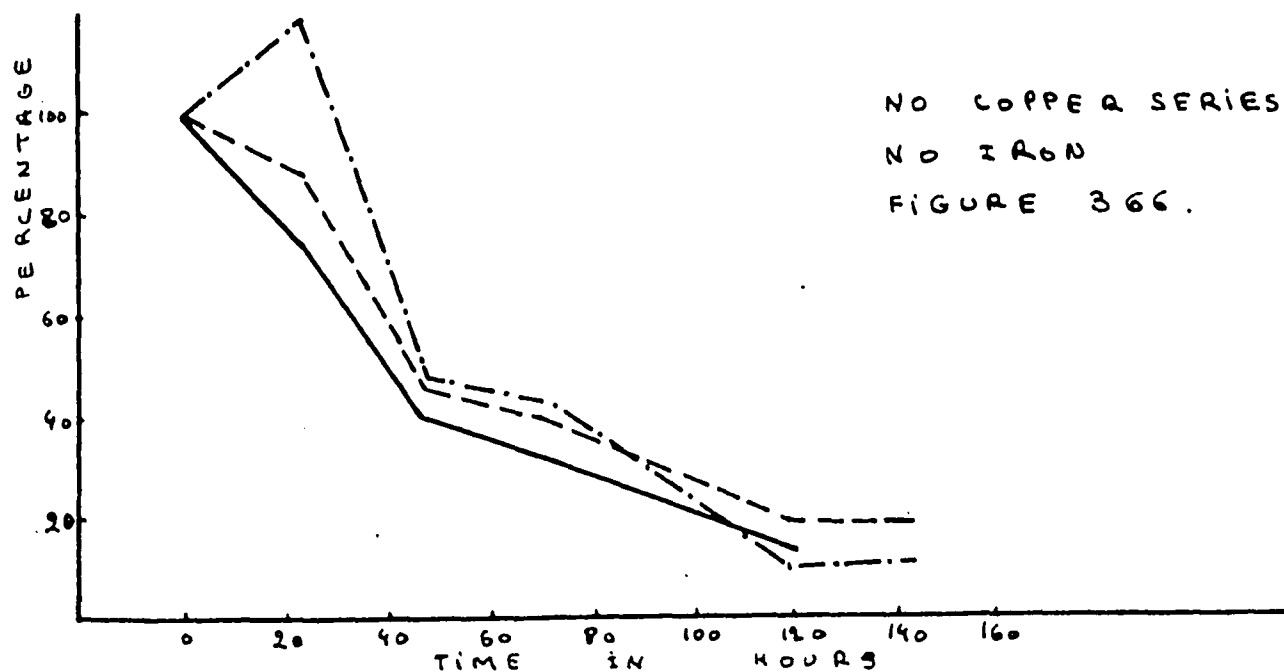
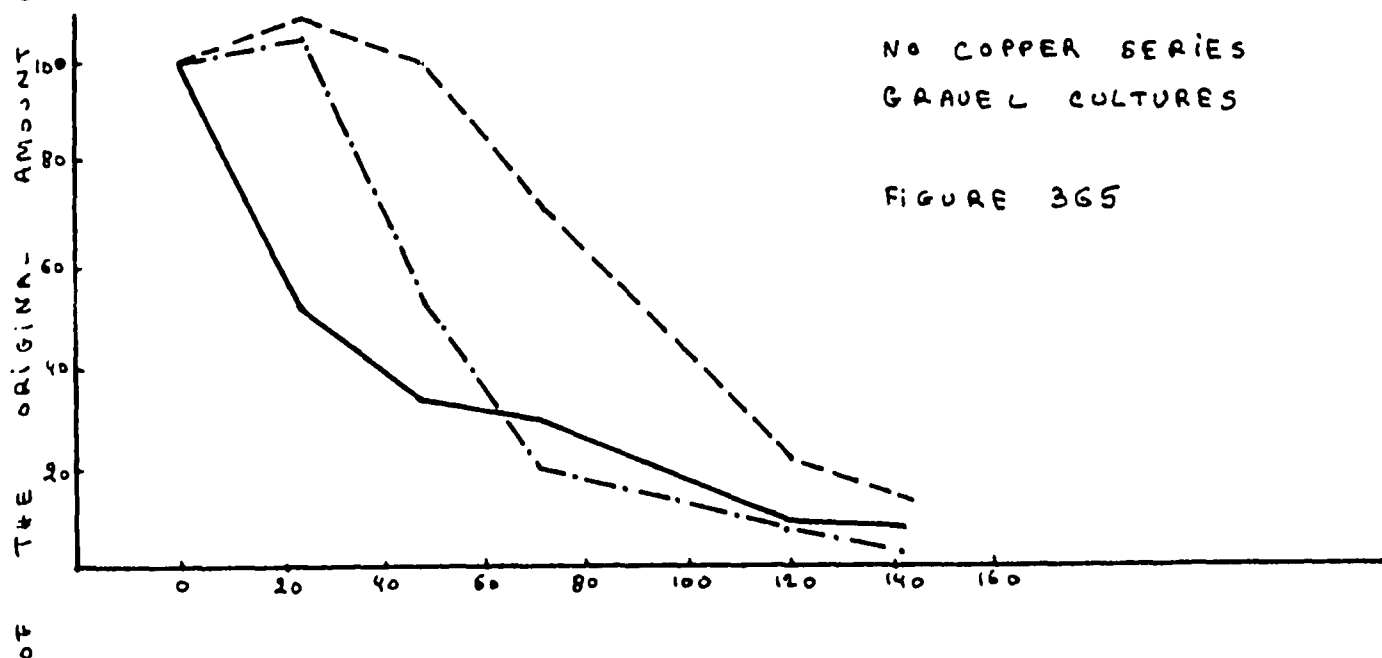
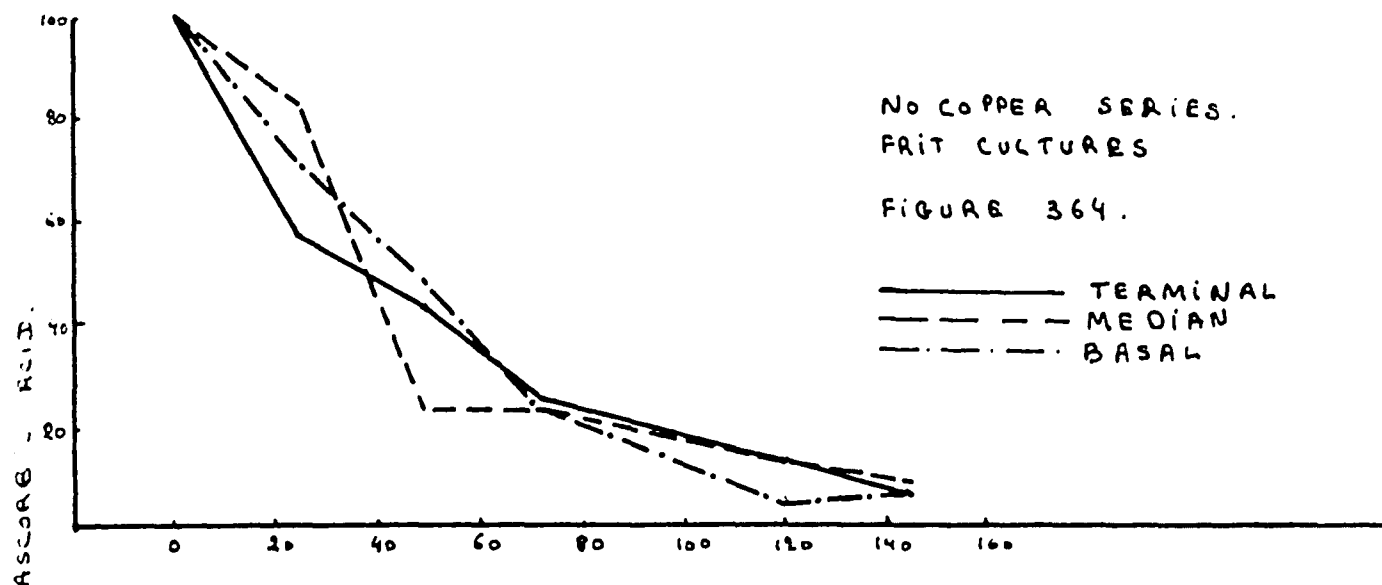


Figure 367. Differences between the percentages of the original amounts of ascorbic acid found at each determination in the terminal, median and basal leaves of tobacco plants grown on the iron-containing frit deprived of copper and the percentages obtained from the plants grown on the same media but supplied with 0.08 parts per million of copper, plotted against the time of each determination, expressed in hours after harvest.

Figure 368. Same as figure 367, for the tobacco plants grown on gravel supplied with 4 parts per million of iron.

Figure 369. Same as figure 367, for the plants grown on gravel supplied with a nutrient solution deprived of iron.

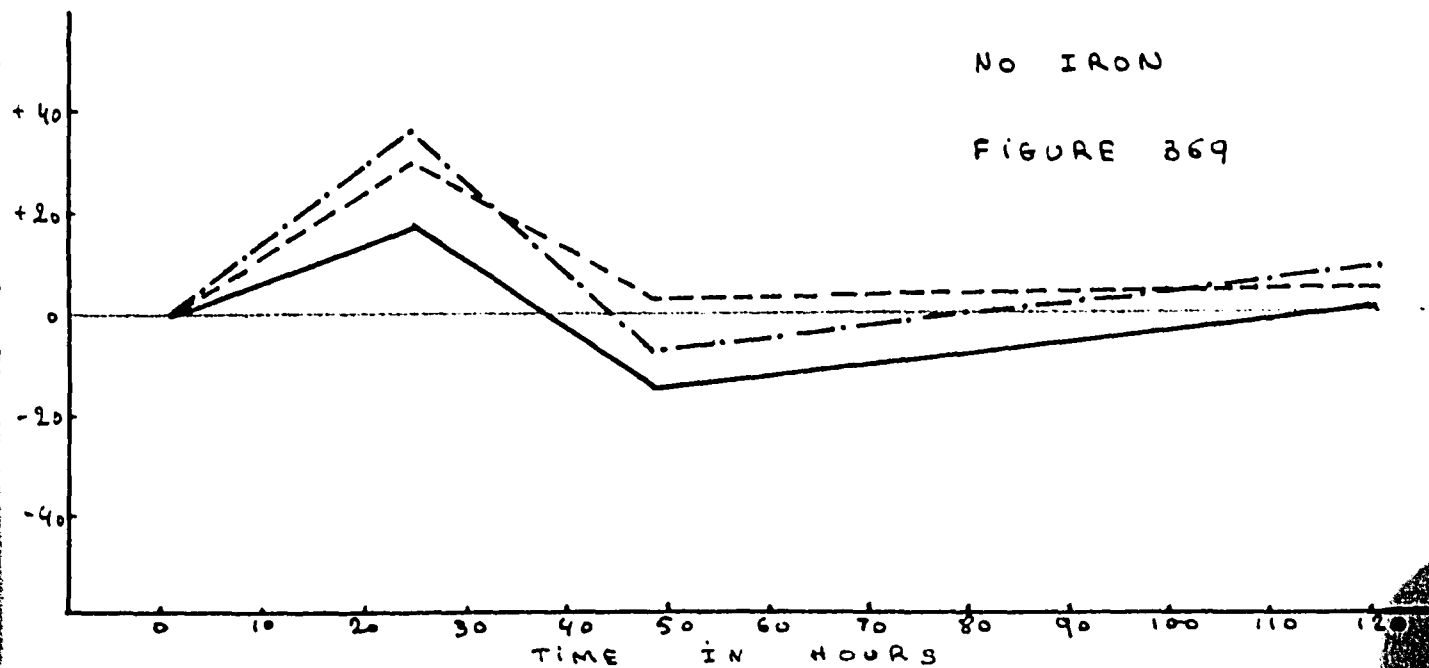
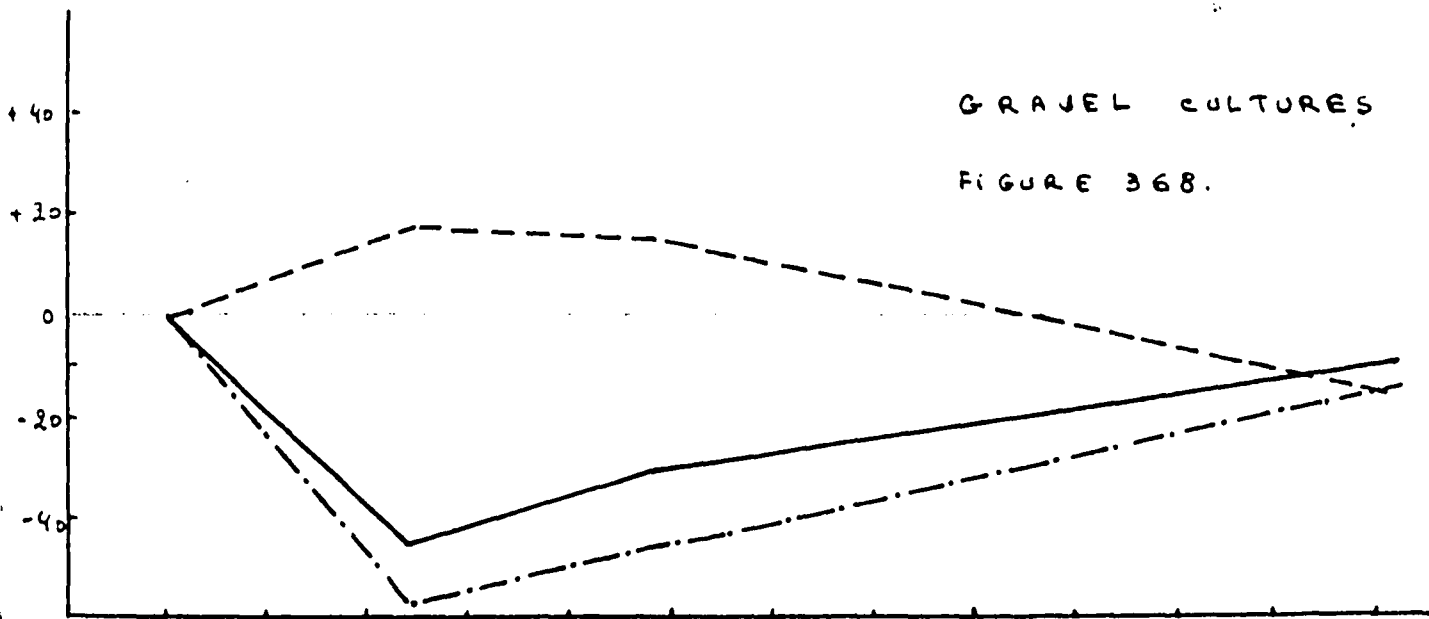
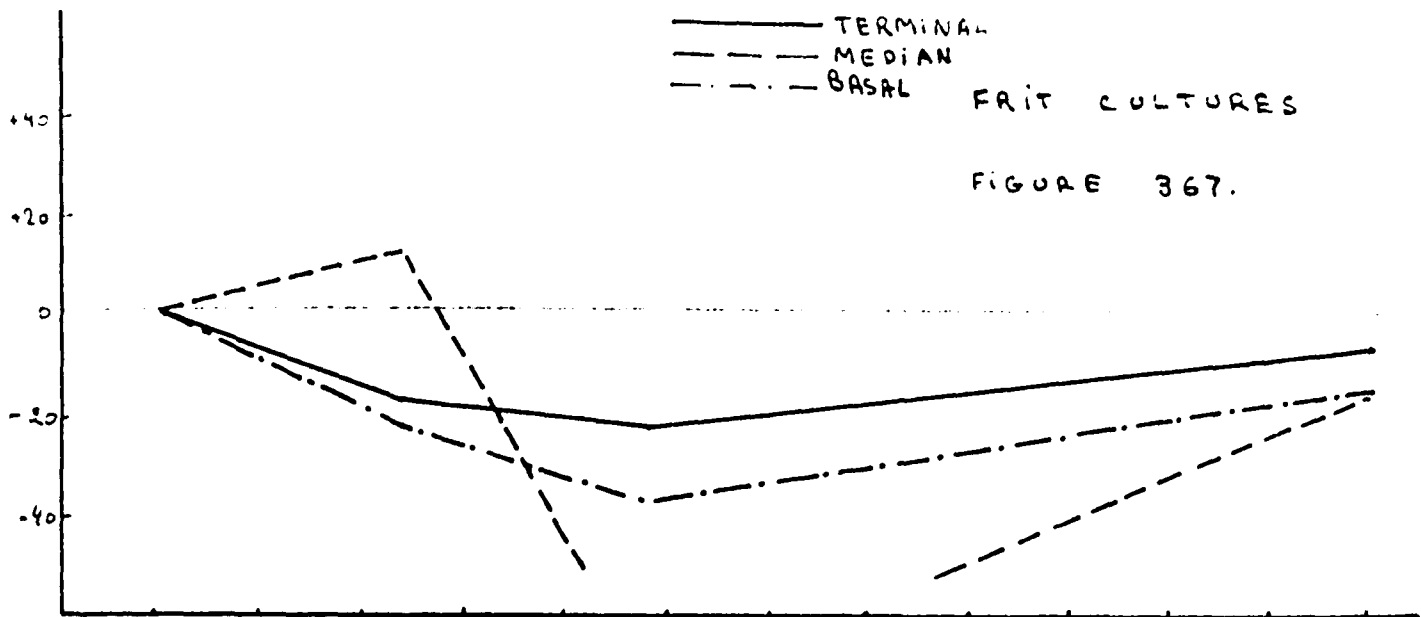


Figure 370. Percentages of the original amounts of ascorbic acid 24 hours after harvest, in the basal, median and terminal leaves of tobacco plants grown on various cultures differing in their iron availability and supplied with 0.08 parts per million of copper in the nutrient solution, plotted against the amounts of iron in the corresponding plant material, expressed in milligrams per 100 grams of fresh material, expressed in milligrams per 100 grams of fresh material.

Figure 371. Percentages of the original amounts of ascorbic acid 48 hours after harvest, in the basal, median and the terminal leaves of tobacco plants grown on various cultures differing in their iron availability and supplied with 0.08 parts per million of copper in the nutrient solution, plotted against the amounts of iron in the corresponding plant material, expressed in milligrams per 100 grams of fresh material.



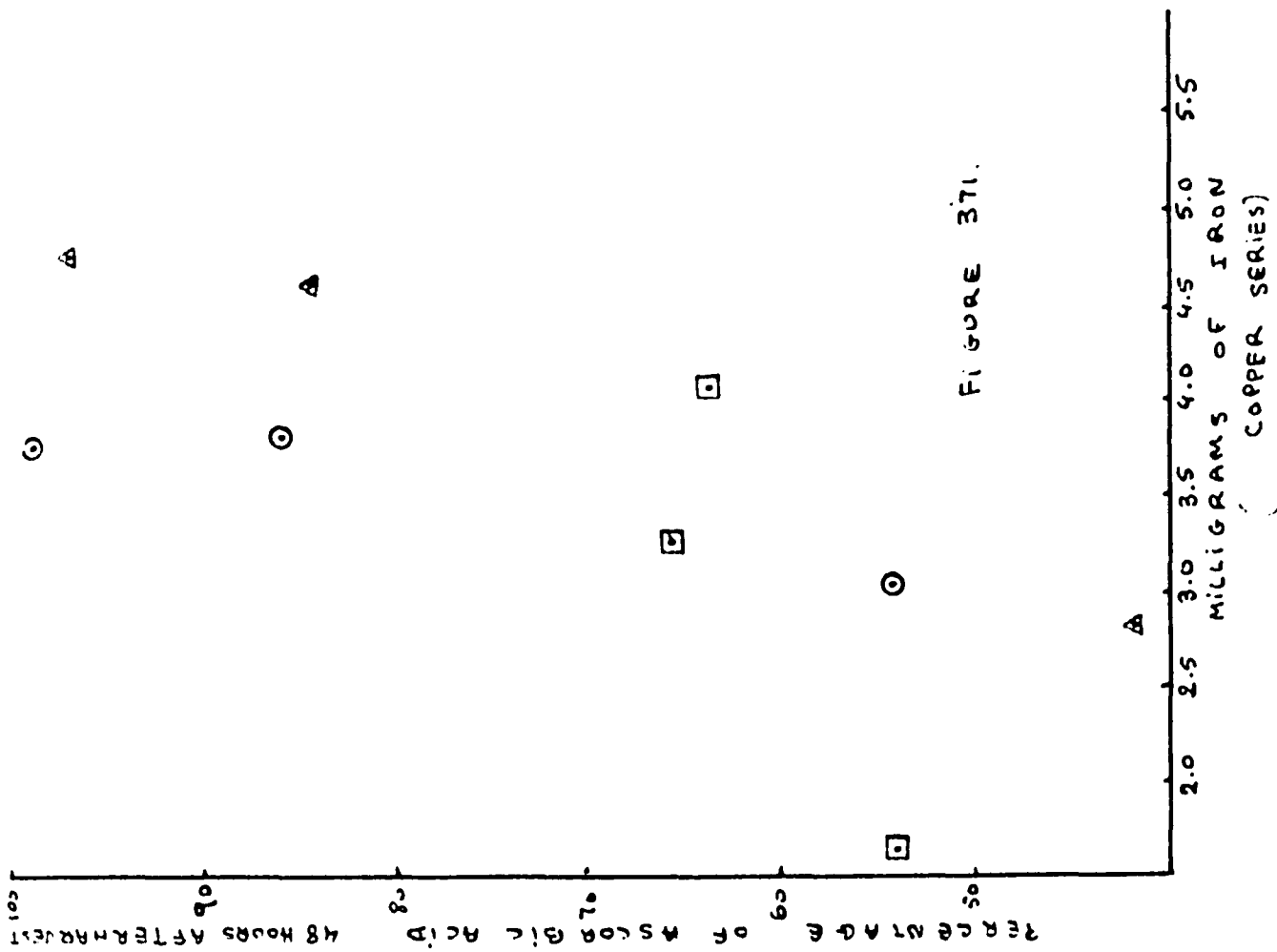
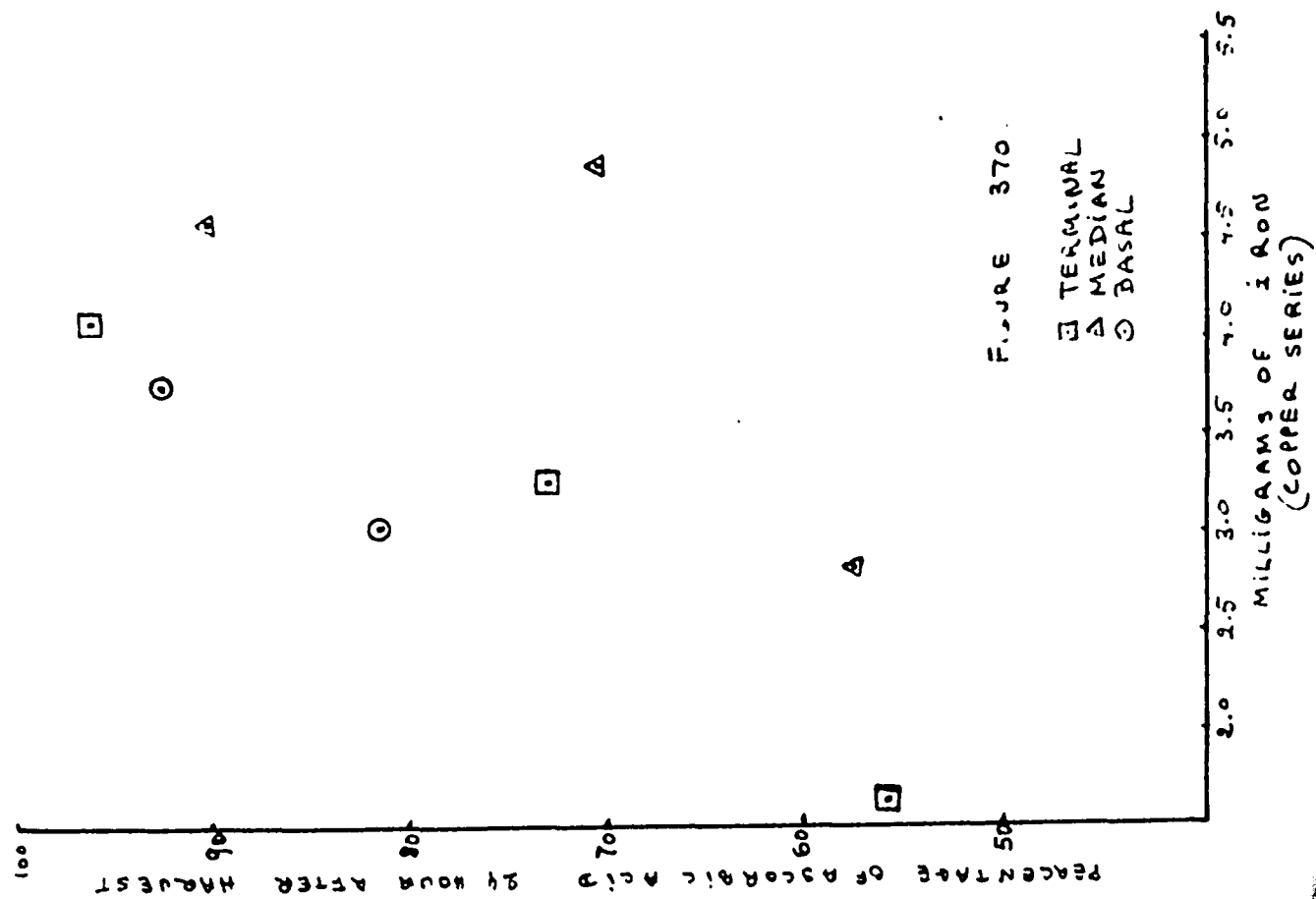


Figure 372. Percentages of the original amounts of ascorbic acid 96 hours after harvest, in the basal, median and terminal leaves of tobacco plants grown on various cultures differing in their iron availability and supplied with 0.08 parts per million of copper in the nutrient solution, plotted against the amounts of iron in the corresponding plant material, expressed in milligrams per 100 grams of fresh material.

Figure 373. Same as figure 372, 120 hours after harvest.

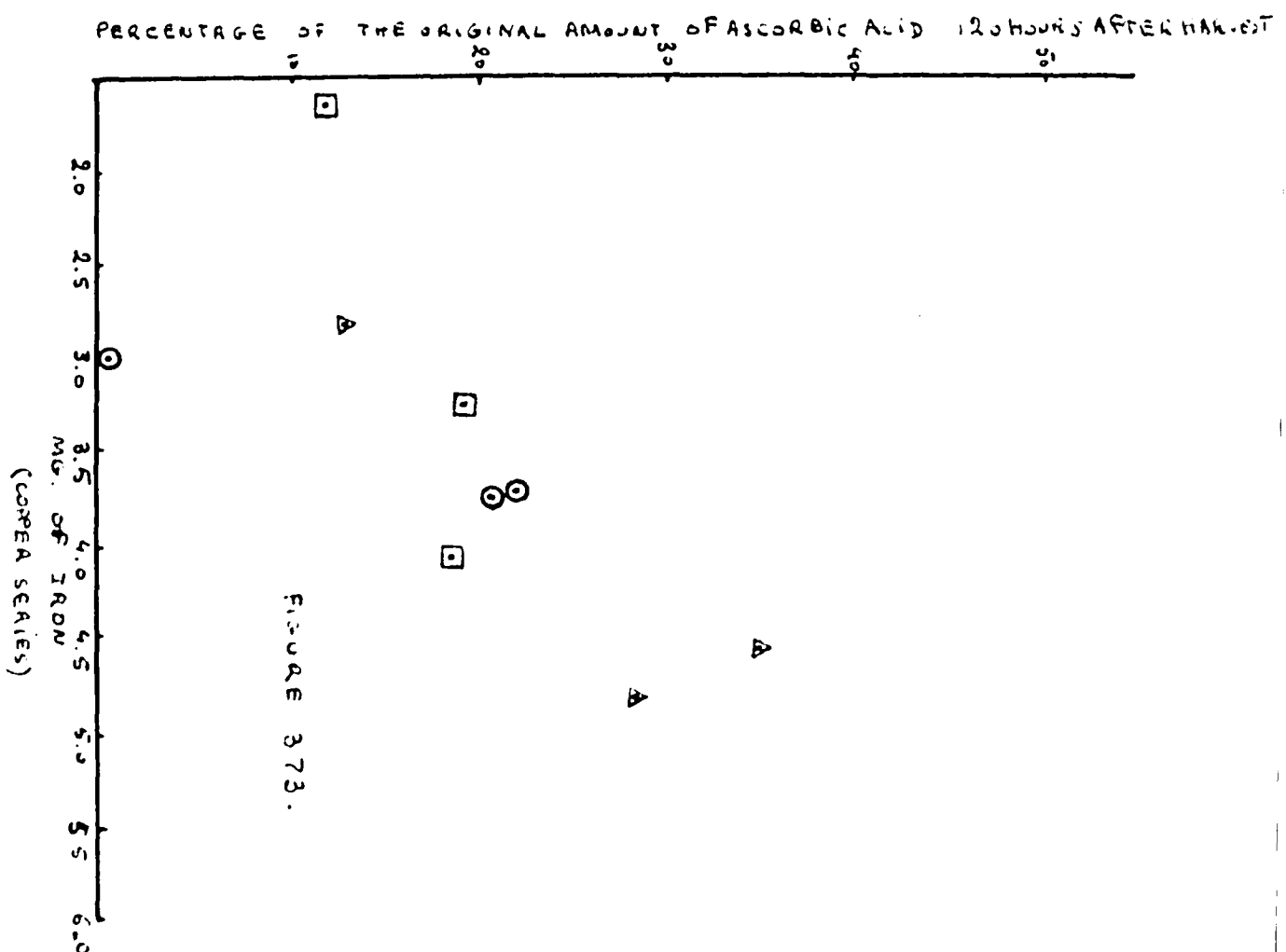
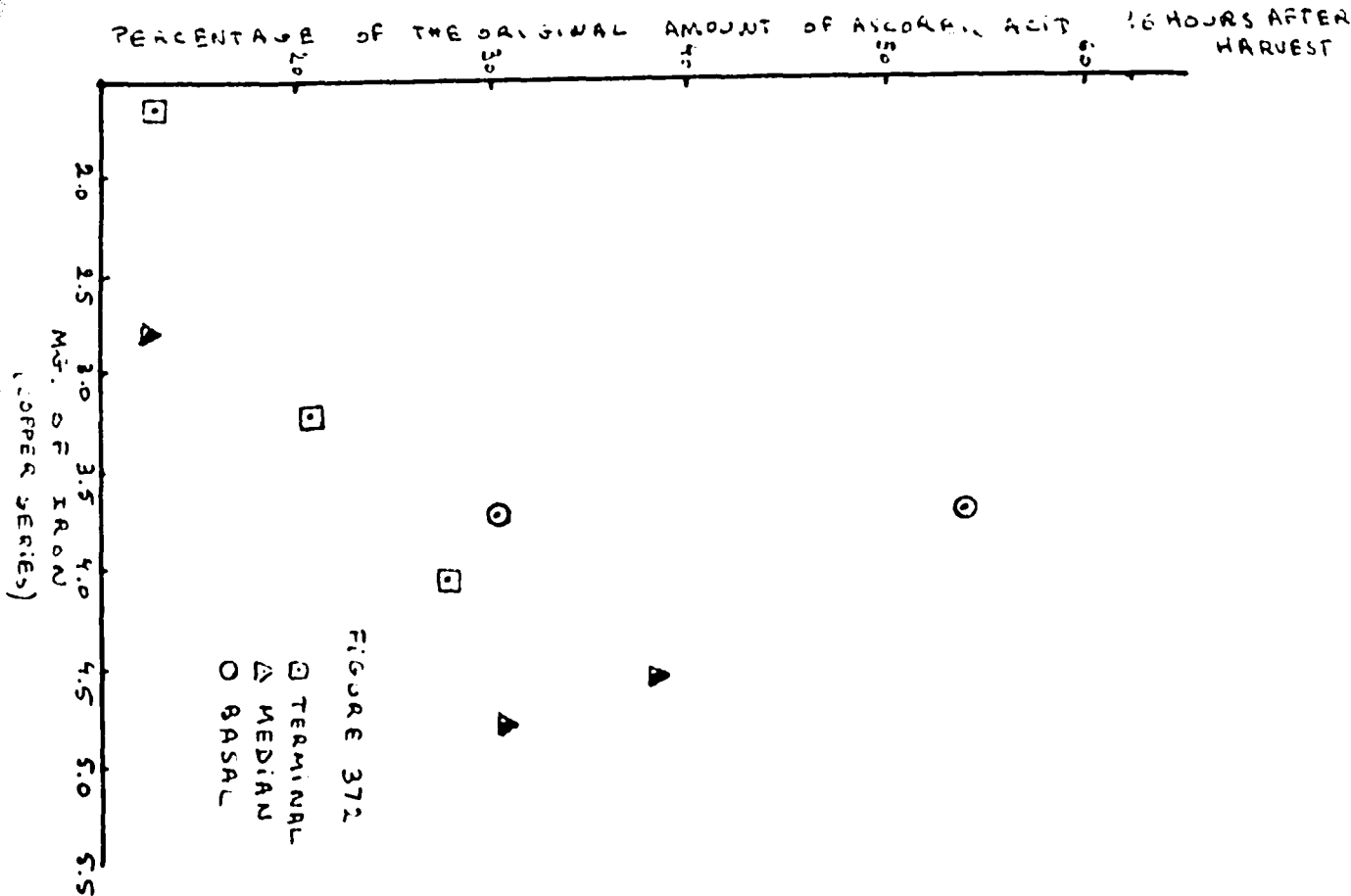


Figure 374. Percentages of the original amounts of ascorbic acid 24 hours after harvest in the basal, median and terminal leaves of tobacco plants grown on various cultures differing in their iron availability and deprived of copper in the nutrient solution, plotted against the amounts of iron in the corresponding plant material, expressed in milligrams per 100 grams of fresh material.

Figure 375. Same as figure 374, 48 hours after harvest.

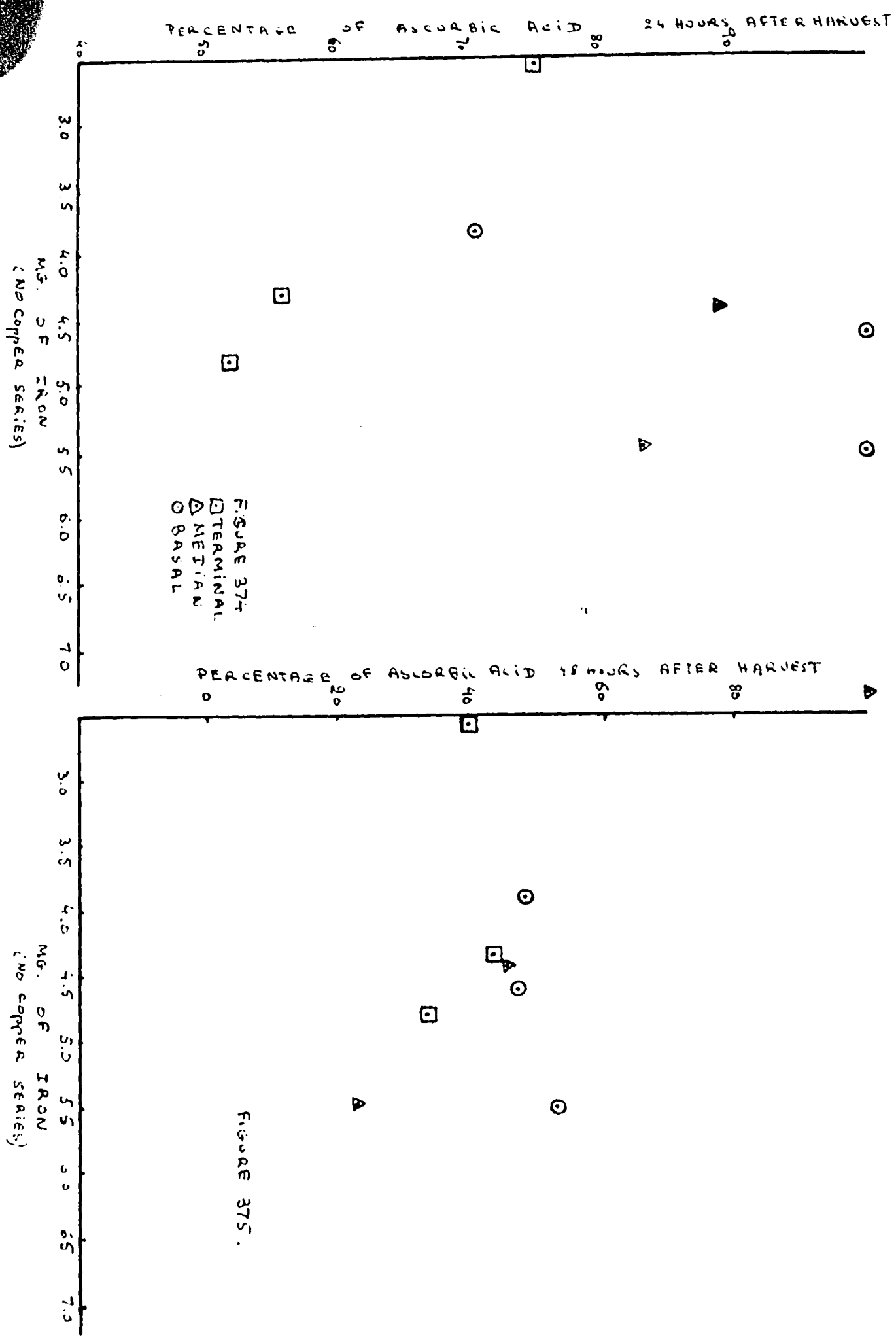


Figure 376. Percentages of the original amounts of ascorbic acid 72 hours after harvest in the basal, median, and terminal leaves of tobacco plants grown on various cultures differing in their iron availability and deprived of copper in the nutrient solution, plotted against the amounts of iron in the corresponding plant material, expressed in milligrams per 100 grams of fresh material.

Figure 377. Same as figure 376, 120 hours after harvest.

