ABSTRACT

Brown, John C. The Influence of Copper Compounds Applied to Muck Soil on the Yield, Growth Pattern and Composition of Certain Crops.

In view of the importance of a supply of copper for plant growth on certain acid muck soils of Michigan, a further study of copper's role in plant growth was undertaken.

Spring wheat, sudan grass, corn, tomatoes, sugar beets, oats and celery were grown on copper-deficient muck soil obtained from the Michigan State College Experimental Muck Farm. This muck contained 86% organic matter, had a pH of 6.0 and analysis for some of the elements showed a content of 3.3% nitrogen, .21% potassium, .12% phosphorus, 2.5% calcium, .27% magnesium, 1.3% iron and .0011% copper.

A 3-9-18 fertilizer was used at the rate of 3000 lbs. per acre, plus the principal micro-nutrient elements.

All the plants grown in this investigation gave a marked response to copper compounds as evidenced in greater yields. Copper deficiency symptoms were noted in each case where copper was not applied to the soil.

Beneficial effects upon growth were observed in growing spring wheat on sterilized muck soil where copper had not been applied. These plants produced good vegetative growth but showed copper deficiency at time of head formation. Very few heads were produced.

Brown, John C.

The absorption of copper by the plants studied, effected the uptake of iron, nitrogen, potassium, phosphorus, calcium, magnesium and silica.

The copper content of corn kernels was lower from plants grown in copper-deficient muck soil than in soils with sufficient copper. Seed from plants grown in the latter soil produced normal plants whereas seed from the copper-deficient soil produced dwarfed plants which showed permanent copper deficiency symptoms.

Copper aids in chlorophyll formation. Only where copper was supplied to the soil did the reducing sugar and nitrogen contents approach equal percentages in growing wheat. Without copper the nitrogen content of the plants remained high and the reducing sugar low.

The addition of copper to the soil resulted in a greater number and weight of fruit harvested from the tomato plants. Necrotic spots appeared on some of the tomato fruit where copper had not been included in the fertilizer. These necrotic areas were not found on the fruit harvested from plants where copper had been applied.

Sugar beet yields were higher where copper was applied to the soil and a red coloration appeared on the cut surfaces of the root. The red color did not appear when copper was not used.

A cross section of the beet showed the coloration to be concentrated in concentric rings. These rings corresponded to the secondary vascular tissue. The coloration is believed due to the presence of a copper enzyme and a suitable substrate.

Copper is believed necessary for the normal metabolic activity of the plant processes.

THE INFLUENCE OF COPPER COMPOUNDS APPLIED TO MUCK SOIL ON THE YIELD, GROWTH PATTERN AND COMPOSITION OF CERTAIN CROPS

By

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THE INFLUENCE OF COPPER COMPOUNDS APPLIED TO MUCK SOIL ON THE YIELD, GROWTH PATTERN AND COMPOSITION OF CERTAIN CROPS

INTRODUCTION

Plants grown on the acid muck soils of Michigan have benefited from an application of copper to the soil. The part which copper occupies in the metabolic processes of these plants has not been established. The purpose of this study was to investigate further the role which copper takes in plant growth.

REVIEW OF LITERATURE

The importance of copper in plant and animal metabolism has been expressed by Elvehjem (15) as follows: "A complete understanding of the action of copper will bring us distinctly nearer an understanding of the fundamental processes in living matter".

Elvehjem (15) reports Miessner in 1817 as probably the first worker to show that copper actually was a constituent of plants. Later Sarzeau (1830) found copper in numerous plants and determined the amount quantitatively. The work of Dischamps (1848) in the further study of vegetables revealed that a relationship existed between the copper in plants and that in the soil on which they were grown; while Chevreul (1868), in a more general statement, suggested that copper was quite widely distributed in organic matter.

Elvehjem (15) further reports the work of Maquenne and Demoussy, and Fleurent and Levi (1920) as establishing without question the universal distribution of copper in plant life. Maquenne and Demoussy found 3 to 40 p.p.m. of copper in all the plant materials they analyzed and suggested that copper must be an essential element in plant metabolism. The fact that copper was found in the more active tissues of the plant e.g. young shoots and leaves, led them to conclude that copper in some way aided in the vital functions of the plants.

Bain (5) in 1902 reports the beneficial action (over and above its fungicidal effects) of Bordeaux mixture used as a spray on the foliage of plants. The outstanding results secured in some cases from the use of Bordeaux resulted in its general acceptance. However, the immediate cause of this beneficial action and its mode of operation had to be established. He cites experiments with peach leaves in which copper was applied to the leaf in spots overnight and there was distinctly more starch under each of these spots. He contributes this greater starch content to the greater chlorophyll content of the plant where copper was applied.

Lutman (36) noted that the increase in yield of potatoes was directly proportional to the amount of copper sulfate used. A table is given showing yields of potatoes from sprayed (Bordeaux mixture) and unsprayed plots from 1891 to 1911. The sprayed pots had an average yield of 263 bushels as compared to 159 bushels per acre for the unsprayed. He also noted that the application of Bordeaux mixture brought about a daily increase in the amount of starch produced per plant.

Floyd (20) reports dieback or exanthema as one of the most important of the diseases of citrus trees. The severity of the disease was increased with increasing amounts of organic matter in the soil or large applications of nitrogenous fertilizers. Copper sulfate applied to the soil was found to correct this disease.

Upon establishment of the Florida Everglades Experiment Station in 1923, poor plant growth was rather general on the Experiment Station soils, as well as on other raw peat soils of the Everglades. The first corrective work was done in the summer of 1925. This work, reported by Bryan (8), shows the marked stimulating influence of copper and manganese on the peat soils of the Florida Everglades. The stimulating action of both copper and manganese seemed to be closely associated with chlorophyll development. The green coloration, presumably due to an increase in chlorophyll, was produced within ten days after treatment. The plants receiving copper and manganese produced mature seed, while the untreated ones failed entirely. These soils contained from 1.5 to 10 per cent iron.

Felix (18) found the addition of copper sulfate made possible the growth of onions and lettuce on unproductive peat soils of New York.

Harmer (23) has found copper sulfate markedly beneficial on the more acid mucks of Michigan. Crops most responsive included lettuce, spinach, dill, wheat, sudan grass, okra, carrots, onions, potatoes and tomatoes.

Copper has also been found to have a marked stimulating influence in animal life. Elvehjem (15) reports Harless in 1847 as detecting copper in the blood of marine animals and showing that it exists not as a free salt, but in combination with protein. This copper-containing protein which was named hemocyanin by Fredericq (1878) is apparently an oxygen carrier analogous to the hemoglobin of higher animals.

Rose and Bodansky (41) made a quantitative study of the copper in 35 marine animals. The amount of copper in the organisms was examined frequently and was found to diminish as the animals ascended from the comparatively simple to the more complex.

The factors controlling the affinities of hemoglobin and hemocyanin for oxygen are reported by Elvehjem (15) as being the same, and that these blood pigments are not fundamentally different because one contains iron and the other copper. Also, that the variation in the affinity of individual hemocyanins for oxygen is undoubtedly dependent upon the physicochemical changes taking place in the protein part of the molecule.

The chemical relationship existing between chlorophyll and hemoglobin is reported by Orth, Wickwire and Burge (39). One point of difference however, is that magnesium is the element in chlorophyll corresponding to iron in the hemoglobin.

Elvehjem and Sherman (17) showed that copper does not influence iron assimilation but it is indispensable for the transformation of the absorbed iron into blood hemoglobin. Their results showed that in the absence of copper, inorganic iron is readily assimilated and stored in the liver and spleen of rats. The iron so stored cannot be used for hemoglobin formation until copper is supplied. After copper is supplied the greater part of the iron in the liver is removed and built into hemoglobin of the blood. Copper as reported here is not necessary for iron assimulation but it is necessary for the synthesis of hemoglobin.

A similar experiment carried out by Elvehjem and Hart (16) on young chicks gave comparable results.

Cook and Spilles (11) showed that the addition of copper to an iron-low diet of rats resulted in a depletion of the reserve iron in the spleen.

Copper has also been found present in certain enzyme systems. Oxidative enzymes that contain copper are divided into two groups by Ball (6) according to their substrates. One group would contain those enzymes which oxidize mono or polyphenols, the other, those enzymes whose substrate is ascorbic acid. Enzymes in one group do not appear to be able to catalyze the oxidation of the substrate for the other group. However, enzymes of both groups presumably catalyze the oxidation of their substrates by reason of the change of copper from the cupric to the cuprous form, the latter being reoxidized by oxygen.

In a proposed classification of the chemical elements by Thatcher (45), he believes there is sufficient experimental evidence to justify the hypothesis that manganese and iron, copper and zinc are two pairs of mutally coordinating catalysts for oxidation-reduction reactions; the former, for biological reactions in which the addition or removal of oxygen is the basis of the energy exchange and the latter, for those in which loss or gain of hydrogen is involved.

An interesting paper by Shear, Crane and Myers (42) on the nutrient element balance, as a fundamental concept in plant nutrition, adds additional importance to the role any one element may play in plant nutrition.

Shear, et al. point out that the concentration of salts in leaves is regulated by the absorption power of the roots. The growth of the whole plant, including the formation of seeds, is again controlled by the transformation of the nutrient salts as they are assimulated by the green leaves. Leaf analysis is an integration of all circumstances which influence the availability of the salts in the soil. Quoting the above authors, "It has been the experience of the authors, both under field and greenhouse conditions, that there are no symptoms the cause of which can be accurately diagnosed visually". They hold further that magnesium deficiency may result from an excess accumulation of potassium in the leaves. Calcium or magnesium accumulation or a combination of both may produce potassium deficiency. Excess of phosphate, copper and zinc or of manganese and cobalt; or too low a level of potassium, magnesium or calcium in relation to either or both of the others, may cause iron deficiency.

All other factors being constant, plant growth is a function of two variables of nutrition (42), intensity and balance, as they are reflected in the composition of leaves when the plants are in the same stages of growth or development. Maximum growth and yield occur only upon the coincidence of optimum intensity and balance.

Interpretation of the nitrogen content of leaves in terms of the nitrogen status of plants is complicated in that, when nitrogen is supplied, it accumulates only to the extent to which its available supply will permit growth. In other words, if the intensity and balance of the other elements are favorable for a high growth potential, growth will be in proportion to the available nitrogen supply and accumulation of nitrogen will be prevented by its utilization in additional growth. A specific example of the effect of an essential element, namely copper, on the accumulation and utilization of nitrogen, can be cited from the work of Gilbert, Sell and Drosdoff (21). They state that nitrogen applications to tung trees in early stages of copper deficiency failed to increase linear growth, but greatly increased the incidence of copper deficiency.

It has been definitely established that copper is essential for plant growth in a general review of the literature on this subject by Sommer (43).

EXPERIMENTAL PROCEDURE

A copper-deficient muck soil was obtained from the Michigan State College Experimental Muck Farm. This was a virgin muck which had never been fertilized. The muck consisted of 86 per cent organic matter, had a pH value of approximately 6.0 and contained 3.3 per cent total nitrogen, .21 per cent potassium, .12 per cent phosphorus, 2.5 per cent calcium, .27 per cent magnesium, 1.3 per cent iron and .0011 per cent copper. Unless otherwise indicated this muck was used throughout the following experiments and all treatments were made in triplicate or quadruplicate.

The fertilizer used consisted of a 3-9-18 applied at the rate of 3000 lbs. per acre in all cases except where indicated in special experiments. The major elements were applied in the form of chemically pure potassium-di-phosphate, potassium nitrate and potassium chloride.

The micro-nutrient elements were applied in all experiments unless otherwise indicated as follows: Ferric sulfate 50 lbs./A., cobaltous acetate 25 lbs./A., manganous acetate 50 lbs./A., zinc acetate 25 lbs./A., boric acid 25 lbs./A., calcium oxide 200 lbs./A. and copper sulfate 200 lbs./A. The term copper will be used throughout this experiment to mean copper sulfate unless otherwise stated.

These constituents were all weighed out separately and mixed thoroughly with six thousand grams of muck soil of uniform moisture content, which was then placed in two-gallon glazed jars. In one experiment with corn four-gallon jars were used employing twelve thousand grams of soil.

This procedure was used throughout these experiments in an attempt to obtain as much uniformity as possible. For each experiment new virgin muck with the above stated fertilizer applications was used. Every precaution was taken to avoid contamination of jars with copper.

The crop in each jar was harvested, weighed and immediately dried in a hot air oven. After drying the crop was weighed again and the loss in moisture determined. In the first two experiments analyses were made of the plants from each jar. Each jar contained an equal number of plants and, in most experiments using wheat, the number of plants was maintained at fifteen. In the experiments following the two mentioned above, the plant material from each jar was weighed separately, and those from the replicated treatments were composited and ground in a Wiley mill to pass through a 20 mesh sieve. This material was used in the determination of some of the elements of the plant.

All plant material was thoroughly ground and mixed before samples were taken. A three-gram sample of the plant material representing each treatment was ashed, the ash heated gently with HCl for one-half hour and then filtered. The filtrate was diluted to 200 cc. Aliquots for analysis were taken from this dilution. Separate samples were used in determining total nitrogen. The above procedure holds true for all experiments in this investigation.

Methods of analysis used in these experiments were as follows:

(1) Silica - according to Piper, C. S. 1944 Soil and plant analysis. Interscience Publishers, Inc. N. Y. 263-265.

(2) Copper - Carbamate method 1940 Methods of Analysis Assoc. of Off. Agr. Chemists, Fifth Edition 122.

(3) Iron - Thiocynate method 1940 Methods of Analysis Assoc. of Off. Agr. Chemists, Fifth Edition 126.

(4) Nitrogen - Kjeldahl Method 1940 Methods of Analysis Assoc. of Off. Agr. Chemists, Fifth Edition 26-27.

(5) Potassium - according to the Determination of Exchangeable Potassium in Soils using Hexanitrodyphenylamine by Lawton, K. 1945 Soil Sci. of America 10: 126-128.

(6) Phosphorus - using ammonium molybdate and Fiske-Subbarow reducing agent. The colorimetric determination of phosphorus by Fiske, C. H. and Subbarow, Y. 1925 Jour. Biol. Chem. 66: 375-400.

(7) Calcium - according to Peech, M. and English, L. 1944 "Microchemical Soil Tests" Soil Science 57: 167-195.

(8) Magnesium - according to Peech, M. and English, L. 1944 Titan Yellow Procedure. Microchemical Soil Tests. Soil Science 57: 167-195.

(9) Reducing sugar - according to "A study of methods for the determination of sugar in crop plants". By Waldron, D. R., Ball, C. D., Miller, E. J. and Benne, E. J. 1948 Jour. of the Assoc. of Agr. Chemists 31: 708-714.

The results of the analysis made are reported in table form appearing with each experiment.

EXPERIMENT 1

This experiment was set up as described above with the objective of noting the growth habits of plants with and without copper added to the soil.

Other variables included:

(1) Different ways of applying copper to the soil as used by Willis and Piland (47) which led to the question in their work as to whether copper was effective in the soil or in the plants. In their study they applied copper half-way down in the soil of the jars.

In this experiment the following variations were used:

(a) Copper was applied half-way down in the jar.

(b) Copper was mixed thoroughly in the soil.

(c) Copper not applied.

(2) Silicic acid was applied in increasing amounts from 25 pounds up to and including 3000 pounds per acre for the purpose of observing any effect it might have on the availability of the copper.

Each treatment was triplicated in two-gallon jars containing 6000 grams of muck soil and 3000 pounds per acre of 3-9-18 fertilizer plus the micro-nutrient elements. All treatments received 200 pounds of calcium hydroxide except treatment twenty-one. A description of treatments and yields are given in Figure 1. Tables 1, 2 and 3 show the results of the analysis of the wheat straw and heads. Where copper was not used in the soil, no heads were produced. Thus, no comparisons can be made with the weight and composition of the heads produced where

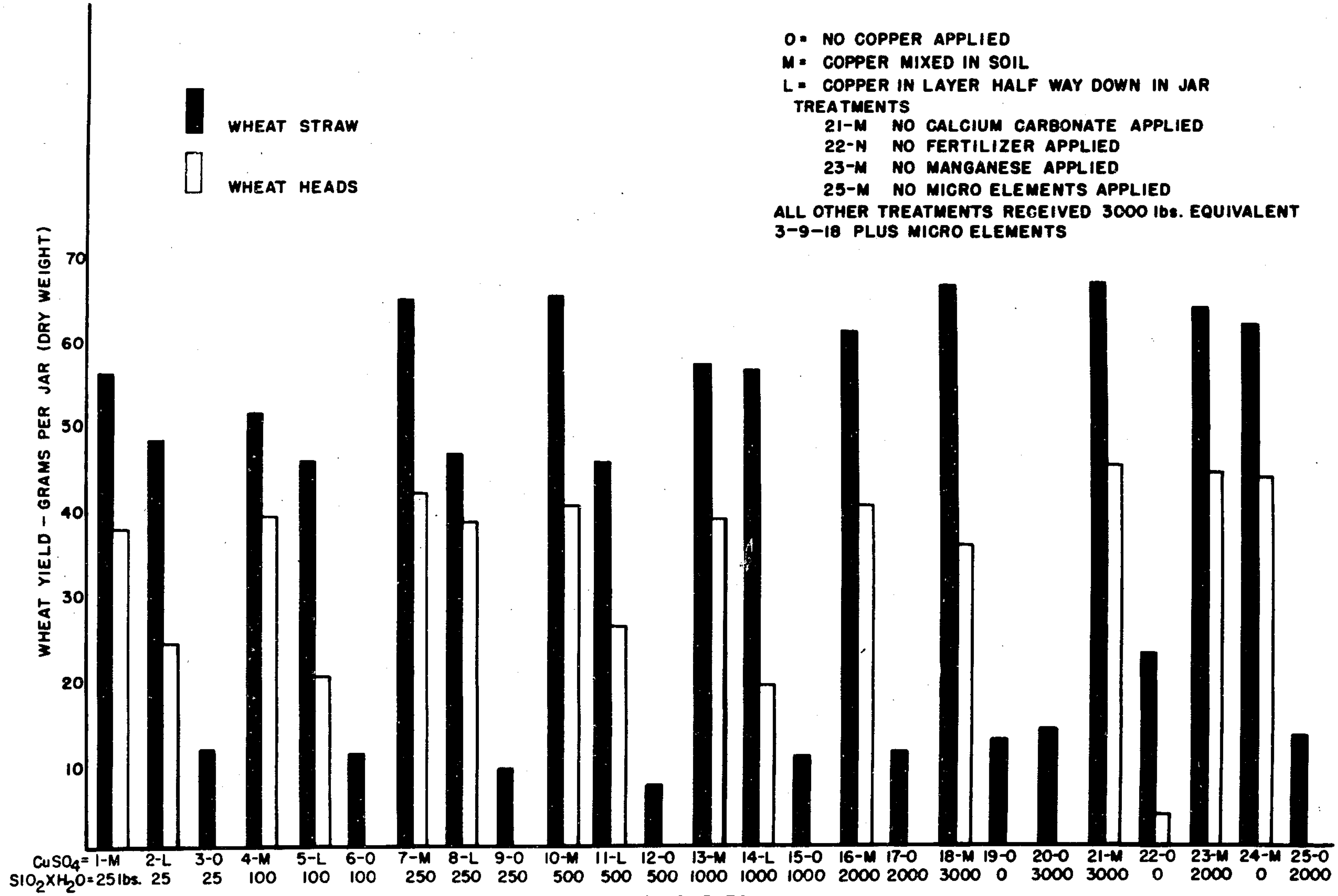

Fig. 1 RESPONSE OF SPRING WHEAT TO COPPER AS APPLIED WITH VARIED RATES OF SILICIC ACID TO MUCK SOIL

TABLE 1

Effects of two rates of copper and several rates of application of silica on the percentage content of certain elements in mature wheat

Treatments Lbs./A.		Yield gms. dry wt.			Per cent composition in straw									% in heads		
Cu	Si	Straw	Hds.	Total	Ash	Cu	Fe	Si	N	K	P	Ca	Mg	Cu	N	K
200	25	56	38	94	2.68	.0018	.011	.45	.31	.56	.09	.28	.07	.0050	1.55	.43
200	100	52	36	88	2.82	.0012	.009	.19	.29	.24	.10	.20	.11	.0052	1.47	.60
200	250	64	42	106	2.56	.0016	.011	.26	.30	.25	.08	.21	.10	.0053	1.45	.53
200	500	65	41	106	2.96	.0016	.012	.31	.32	.30	.09	.22	.10	.0052	1.56	.52
200	1000	57	39	96	2.88	.0012	.010	.55	.31	.25	.08	.23	.11	.0061	1.53	.55
200	2000	61	41	102	4.33	.0004	.002	3.58	.36	.20	.04	.08	.05	.0062	1.55	.49
200	3000	66	35	101	5.01	.0007	.002	3.97	.33	.20	.07	.08	.03	.0053	1.59	.46
200	None	62	43	105	2.57	.0013	.012	.66	.31	.39	.08	.12	.11	.0048	1.54	.51
No Cu	25	12	0	12	14.30	.0022	.026	1.72	4.07	6.27	.91	.65	.34	-	-	-
0	100	12	0	12	15.96	.0027	.020	1.71	3.95	4.42	.78	.58	.28	-	-	-
0	250	10	0	10	15.66	.0022	.023	2.16	4.56	5.34	.99	.71	.30	-	-	-
0	500	8	0	8	17.99	.0017	.029	2.30	4.51	4.96	1.00	.72	.30	-	-	-
0	1000	12	0	12	16.52	.0031	.020	3.25	4.17	5.52	.92	.61	.30	No heads produced		
0	2000	12	0	12	20.33	.0026	.021	8.65	3.81	5.63	1.02	.66	.30	-	-	-
0	3000	14	0	14	20.59	.0024	.021	8.55	3.92	6.37	.81	.54	.25	-	-	-
0	None	14	0	14	13.86	.0017	.026	.54	4.07	5.46	.94	.59	.34	-	-	-
0	None*	23	5	28	4.57	.0018	.015	.68	1.84	.14	.15	.89	.49	.0021	3.01	-

* No fertilizer used

TABLE 2

Effects of two rates of copper and several rates of application of silica on the total content of certain elements in mature wheat

Treatments Lbs./A.		Yield gms. dry wt.			Total content in straw (gms.)									Total content in heads		
Cu	Si	Straw	Hds.	Total	Ash	Cu	Fe	Si	N	K	P	Ca	Mg	Cu	N	K
200	25	56	38	94	1.49	.0010	.006	.25	.17	.31	.05	.16	.04	.0019	.59	.16
200	100	52	36	88	1.46	.0006	.005	.10	.15	.12	.05	.10	.06	.0019	.53	.22
200	250	64	42	106	1.65	.0010	.007	.17	.19	.16	.05	.14	.06	.0022	.61	.22
200	500	65	41	106	1.92	.0010	.008	.20	.21	.20	.06	.14	.07	.0021	.64	.21
200	1000	57	39	96	1.65	.0007	.006	.32	.18	.14	.05	.13	.06	.0024	.60	.21
200	2000	61	41	102	2.64	.0002	.001	2.18	.22	.12	.02	.05	.03	.0025	.64	.20
200	3000	66	35	101	3.29	.0005	.001	2.60	.22	.13	.05	.05	.02	.0019	.56	.16
200	None	62	43	105	1.58	.0008	.007	.41	.19	.24	.05	.07	.07	.0021	.66	.22
No Cu	25	12	0	12	1.73	.0003	.003	.21	.49	.76	.11	.08	.04	-	-	-
0	100	12	0	12	1.84	.0003	.002	.20	.45	.51	.09	.07	.03	-	-	-
0	250	10	0	10	1.51	.0002	.002	.21	.44	.52	.10	.07	.03	-	-	-
0	500	8	0	8	1.37	.0001	.002	.18	.34	.38	.08	.06	.02	-	-	-
0	1000	12	0	12	1.92	.0004	.002	.38	.48	.64	.11	.07	.04	No heads produced		
0	2000	12	0	12	2.52	.0003	.003	1.07	.47	.70	.13	.08	.04	-	-	-
0	3000	14	0	14	2.92	.0003	.003	1.21	.56	.91	.12	.08	.04	-	-	-
0	None	14	0	14	1.87	.0002	.004	.07	.55	.74	.13	.08	.05	-	-	-
0	None*	23	5	28	1.06	.0004	.004	.16	.43	.03	.04	.21	.11	.0001	.15	-

* No fertilizer used

TABLE 3

The response of spring wheat to copper layered in middle of jar as compared to copper mixed throughout soil

Treatments Lbs./A.		Yield gms. dry wt.			Per cent composition in straw									% in heads		
Cu	Si	Straw	Hds.	Total	Ash	Cu	Fe	Si	N	K	P	Ca	Mg	Cu	N	K
200	25	48	24	72	4.02	.0012	-	.50	.66	.46	-	-	-	.0058	2.52	.32
200	100	47	20	67	3.76	.0020	-	.35	.57	.99	-	-	-	.0047	2.17	.62
200	250	47	39	86	3.73	.0016	.014	.53	.44	.65	.11	.30	.12	.0056	1.88	.50
200	500	46	25	71	4.03	.0013	.012	.27	.69	.46	.19	.30	.10	.0056	2.16	.54
200	1000	57	19	76	4.24	.0021	-	1.22	.66	.68	-	-	-	-	2.55	-
200	3000	67	46	113	4.21	.0004	-	3.26	.29	-	-	-	-	.0059	1.61	.21
200	2000	64	44	108	3.52	.0020	-	1.81	.30	.24	-	-	-	.0054	1.58	.48
No Cu	1000	13	0	13	19.51	.0016	-	8.33	3.75	5.53	-	-	-	-	no heads	-
					Total composition in grams											
200	25	48	24	72	1.93	.0006	-	.24	.32	.22	-	-	-	.0014	.61	.08
200	100	47	20	67	1.76	.0009	-	.16	.27	.46	-	-	-	.0009	.43	.12
200	250	47	39	86	1.76	.0008	.007	.25	.21	.31	.05	.14	.06	.0022	.73	.20
200	500	46	25	71	1.85	.0006	.006	.12	.32	.21	.09	.14	.05	.0014	.54	.14
200	1000	57	19	76	2.43	.0012	-	.70	.38	.39	-	-	-	-	.49	-
200	3000	67	46	113	2.82	.0003	-	2.19	.20	-	-	-	-	.0027	.74	.10
200	2000	64	44	108	2.25	.0013	-	1.16	.19	.15	-	-	-	.0024	.70	.21
No Cu	1000	13	0	13	2.58	.0002	-	1.10	.50	.73	-	-	-	-	no heads	-

copper was applied. These are the only tables of such data that will be presented.

This wheat was planted March 1, 1947. Three weeks later a retardation in growth was noted where copper had not been applied. On April 5, 1947 a pronounced difference in color of plants receiving different treatments was noted as follows:

(a) No fertilizer - grayish green.

(b) Complete fertilizer minus copper - light yellowish green.

(c) Complete fertilizer plus copper - dark natural green.

Where silica had been applied from 2000 to 3000 pounds per acre the plants grew much more erect and the straw was more brittle.

Of particular interest (Figure 1, treatment 2), is the plant produced by placing the copper in a layer "half-way" down in the jar instead of mixing it thoroughly in the soil. This relationship holds in all cases of layering instead of mixing copper in this experiment.

The plants that did not receive copper (Fig. 2) produced no heads and soon dried up. Figures 3, 4 and 5 show in more detail the response of spring wheat to copper.

No marked differences in yield were noted with increasing amounts of silica applied. But, differences in the composition of the plants are noted in Tables 1, 2 and 3 with increasing silica content. It can be seen that where silica was applied at the rate of 2000 lbs./A. the plants contained much more silica. At the 2000 and 3000 lbs./A. applications of silica

Fig. 2 Response of spring wheat to copper. The wheat producing heads received copper, those jars of wheat not producing heads did not receive copper.

Fig. 3 Response of spring wheat to copper. Treatments are left to right: (66) No fertilizer - No copper, (67) No fertilizer - Copper, (58) Fertilizer - No copper.

Fig. 4 Response of spring wheat to copper. Treatments are: (Left) No fertilizer - No copper, (Center) No fertilizer - Copper added, (Right) Fertilizer - Copper added.

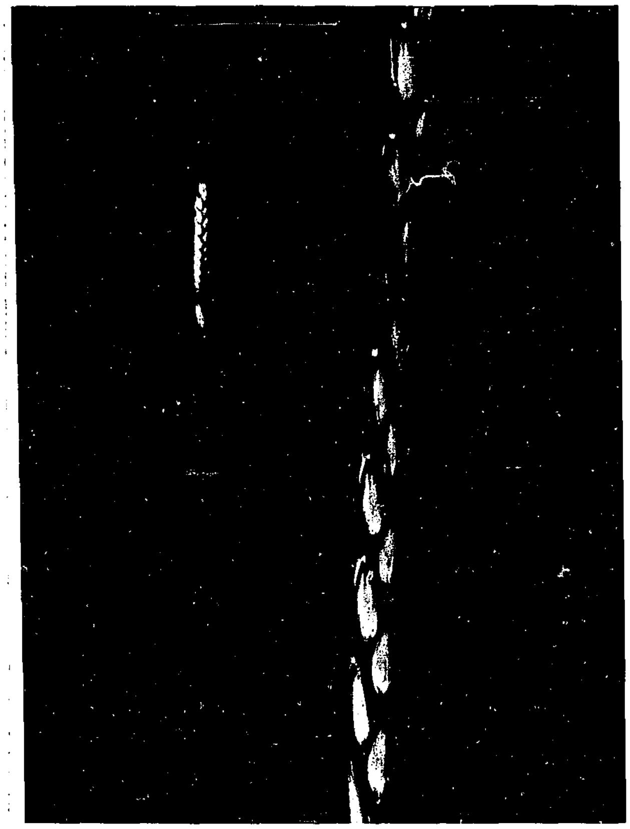

Fig. 5 Development of wheat heads as effected by a copper application. (Left) No fertilizer - No copper, (Center) Fertilizer - No copper, (Right) Fertilizer - Copper.

(where copper had been applied), the percentage copper and iron was reduced as compared to those treatments receiving the lower applications of silica. The percentage (Table 1) copper and iron was higher in similar treatments which did not receive copper. The content of the major elements found in these treatments are shown in Figure 6.

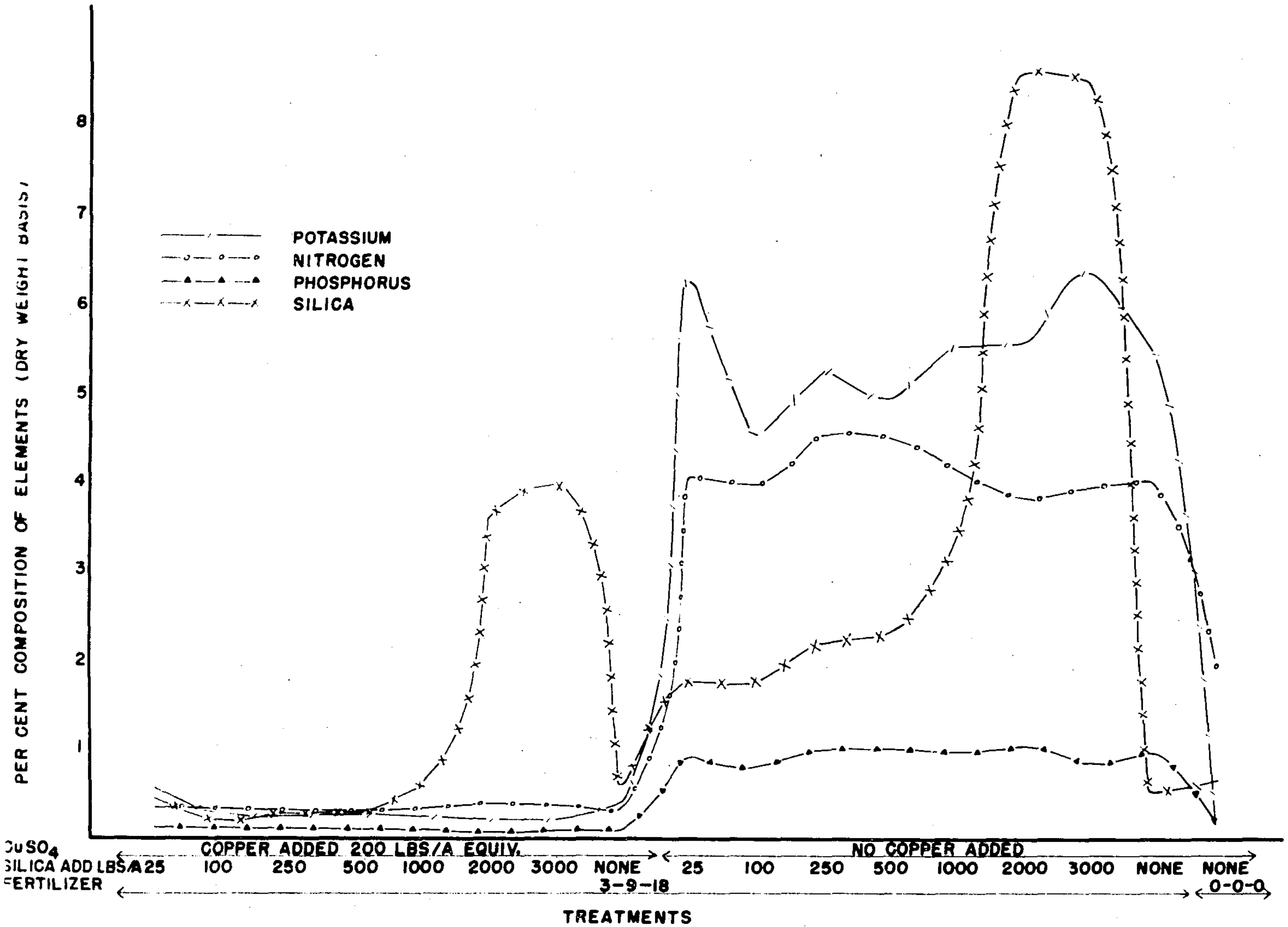

Fig. 6 PERCENT COMPOSITION OF ELEMENTS FOUND IN SPRING WHEAT STRAW AS AFFECTED BY VARIED COPPER AND SILICA CONTENT ON MUCK SOIL

SUMMARY

Copper applied "half-way" down in the soil of the jars did not stimulate plant growth as it did in those jars where the copper was thoroughly mixed with the soil.

Silica applied in the form of silicic acid to the soil influenced the uptake by the plant of Cu, Fe, Ca and Mg where copper had been applied. The uptake of silica was markedly increased at the 2000 and 3000 lb./A. applications.

The copper-deficient wheat plants were characterized by a lighter green color, retarded growth, curling and dying-back of the tips and no head production.

EXPERIMENT 2

This experiment was designed to further investigate the findings in Experiment 1. Copper was applied (200 lbs./A.) to three jars as follows: (1) At the bottom of the jar, (2) in middle of jar, (3) broadcast at the top of jar, (4) mixed thoroughly in the soil and (5) mixed in soil plus 2500 lbs./A. silicic acid. These treatments with their effect on yields and element composition are given in Tables 4 and 5.

Figure 7 shows the average yields of straw and heads for these treatments. Yields from jars in which copper was mixed in the soil were slightly better than yields of jars where copper was applied at the bottom or top of soil, and much better than where copper was applied as a layer "half-way" down in the soil of the jar. Plants from this latter treatment

TABLE 4

The effect of copper, applied at different depths in the soil, on yield and composition of spring wheat

Treatment	Yield gms. dry wt. Straw	Heads	Total	Per cent composition in straw Ash	Cu	Fe	SiO_2	N	K	P	Ca	Mg
1 200 Cu bottom jar	70	24	94	8.96	.0018	.026	1.17	1.95	2.78	.45	.36	.27
2 200 Cu middle jar	56	15	71	10.57	.0022	.029	.97	2.47	3.74	.51	.47	.28
3 200 Cu top jar	65	25	90	8.22	.0014	.021	1.50	1.92	2.58	.52	.32	.20
4 200 Cu mixed in soil	69	26	95	8.77	.0021	.029	.88	2.09	3.02	.47	.35	.20
5 200 Cu 2500 Si	72	32	104	12.87	.0009	.003	5.38	1.74	1.13	.39	.15	.02
6 200 Cu 8-9-0 $NH_4C_2O_4$*	69	24	93	7.35	.0012	.017	1.54	3.46	.70	.58	.82	.42
7 No Cu	40	0	40	12.64	.0019	.031	.71	3.21	4.26	.65	.65	.31

* 1,000 lbs./A. ammonium oxalate added

TABLE 5

The effect of copper, applied at different depths in the soil, on yield and composition of spring wheat

Treatment	Yield gms. dry wt. Straw	Heads	Total	Total content in straw (gms.) Ash	Cu	Fe	SiO_2	N	K	P	Ca	Mg
1 200 Cu bottom jar	70	24	94	6.27	.0017	.024	1.10	1.84	2.62	.42	.34	.25
2 200 Cu middle jar	56	15	71	5.91	.0016	.021	.69	1.76	2.66	.36	.34	.20
3 200 Cu top jar	65	25	90	5.34	.0013	.019	1.35	1.73	2.33	.47	.29	.18
4 200 Cu Mixed in soil	69	26	95	5.07	.0020	.028	.84	1.99	2.88	.45	.34	.19
5 200 Cu 2500 Si	72	32	104	5.06	.0009	.003	5.61	1.82	1.18	.41	.16	.02
6 200 Cu 8-9-0 $NH_4C_2O_4$*	69	24	93	9.27	.0011	.016	1.43	3.21	.65	.54	.76	.39
7 No Cu	40	0	40	6.05	.0008	.012	.28	1.28	1.70	.26	.26	.12

* 1,000 lbs./A. ammonium oxalate added

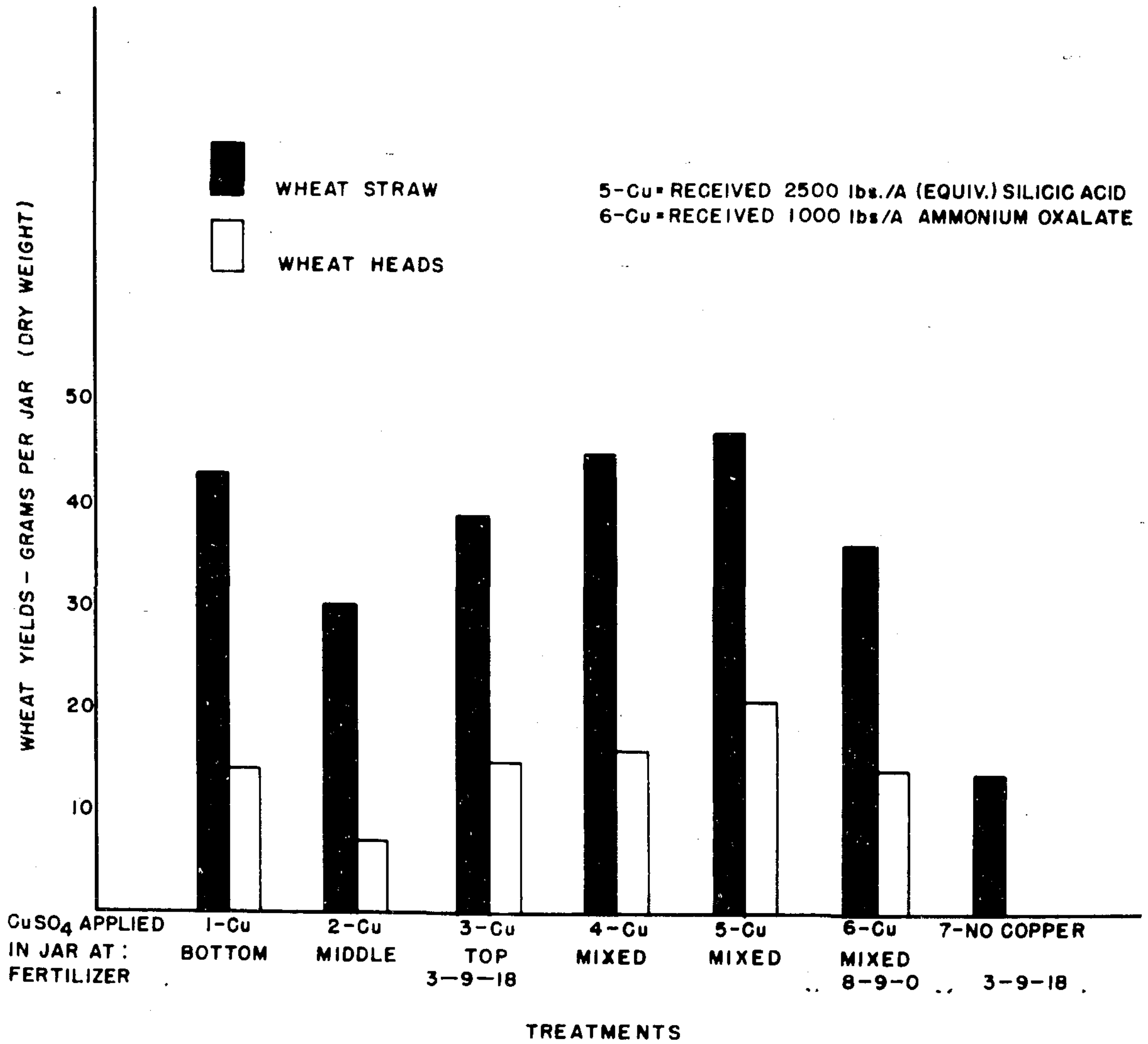

Fig. 7 RESPONSE OF SPRING WHEAT TO COPPER AS APPLIED IN VARIOUS POSITIONS IN JAR

showed copper deficiency symptoms (curling and dieing-back of terminal growth) similar to wheat leaves of Figure 8 in an early stage of its growth. These symptoms later disappeared at what is believed to be about the time the roots of the wheat reached the copper. Growth had been retarded and the plants never entirely recovered as indicated by the yields shown in Figure 7.

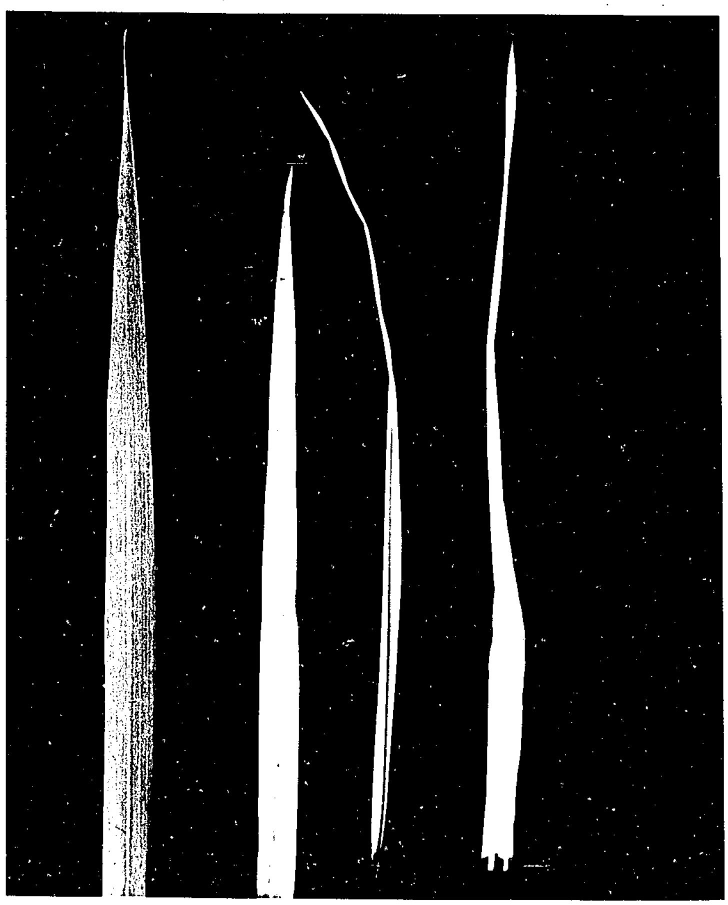

Fig. 8 Comparison between two wheat leaves on right showing copper deficiency symptoms; and two normal leaves on left.

The first roots developed by the young wheat seedlings were observed to grow rapidly and tended to spread along the bottom of jar. Thus, in treatment 1, Figure 7 the roots would be in contact with the copper at an early stage of their growth. Applications broadcast on the top would also be in close contact with newly developing fibrous roots. It would appear that the roots of the wheat seedlings were not able to obtain the copper layered in the jar. These differences in yield and plant growth observed are believed to be a result of the differences in accessibility of the copper.

INTRODUCTION TO EXPERIMENTS 3 AND 4

Hoffer (27), working with corn, found that when corn plants grow in a soil without adequate available potassium, iron compounds accumulate in the joint tissues. He considered this effect as a characteristic symptom of potassium starvation.

Willis and Piland (46) working on certain unproductive acid peats of North Carolina, in growing corn, found an excessive absorption of iron and lodgement of iron in the nodes. Either heavy potash applications or the addition of copper sulfate corrected this condition. They indicated that the symptom of iron accumulation in the nodes of corn is therefore not specific for potash deficiency.

Heald (25) describes the general effects of a shortage of potassium as follows:

(a) A reduced photosynthetic activity and consequently a retarded or dwarfed growth of storage organs, such as fleshy roots or tubers, or in cereal crops the development of vegetative structures at the expense of the grains.

(b) In woody plants a suppressed or weak development of the terminal shoots which may end in a "die-back" as has been shown for both wild species and cultivated fruits.

(c) The appearance of yellowish, brownish or white spots in leaves at first near the margin and later blighting of the foliage and premature death if the shortage is not relieved. The amount of stored carbohydrates, such as sugars and starches, is in direct proportion to the amount of available potash.

Excessive nitrogen applications are reported by Camp and Fudge (10) as accentuating copper deficiency. They cite other authors who have made similar observations.

In view of the varied opinions concerning the response of crops to copper as affected by the presence of excessive nitrogen, the possibility of a relationship existing between potassium and copper and a possible tie-up by calcium of the copper applied to the soil, prompted these two experiments.

EXPERIMENT 3

In the absence or presence of copper how do varying amounts and different forms of nitrogen (organic and inorganic), different amounts of potassium, sugar and acetates affect plant growth?

The treatments for this experiment with the yields of straw and heads of wheat are given in Figure 9.

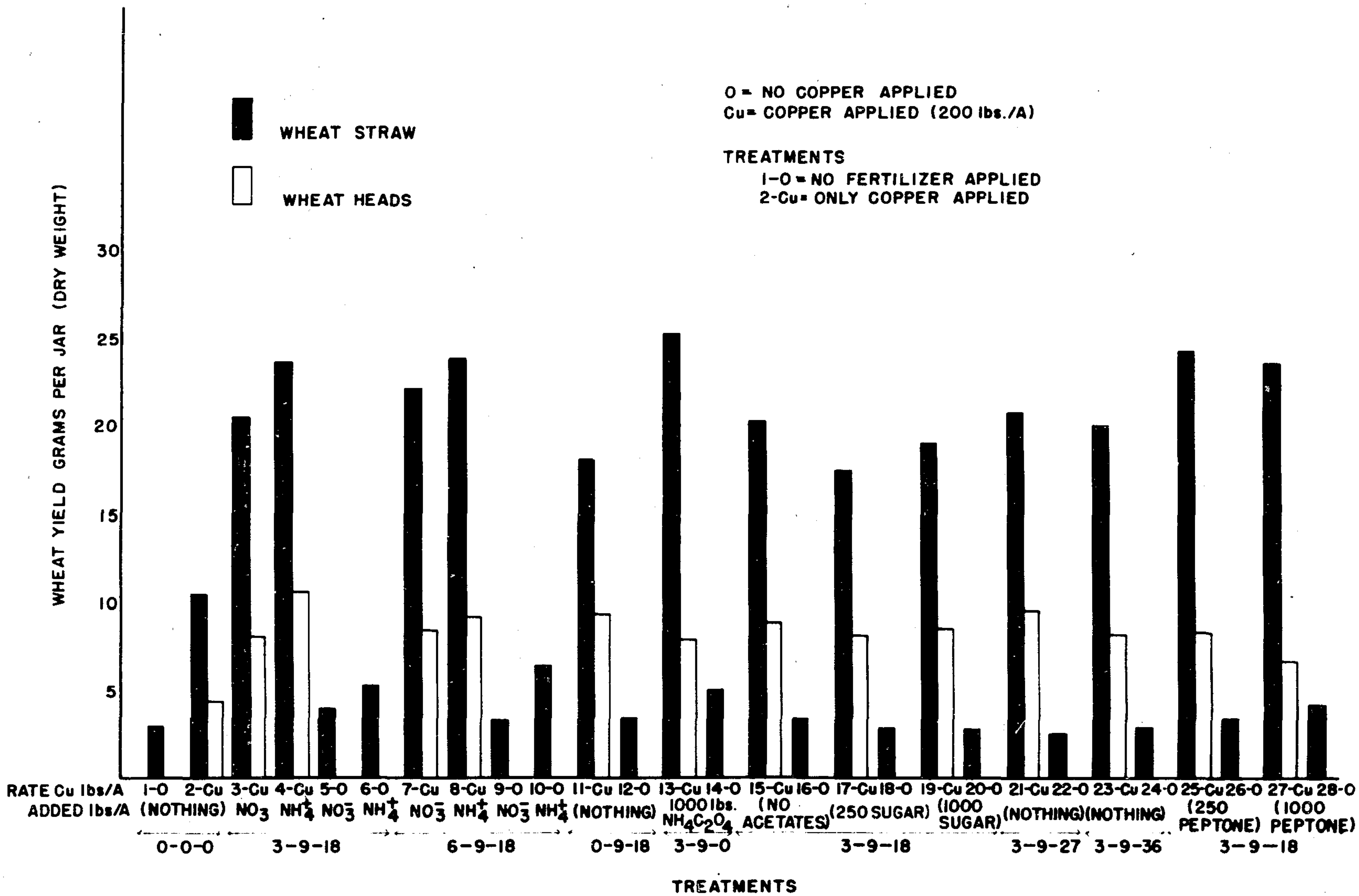

Fig. 9 RESPONSE OF SPRING WHEAT TO COPPER AS APPLIED WITH VARIOUS AMOUNTS AND KIND OF FERTILIZER TO MUCK SOIL

The plants of this experiment responded as anticipated to the treatments applied. Large applications of nitrogen produced more vegetative growth in the plant and vice versa, whether applied in the organic or inorganic form. Increasing amounts of potassium seemed to have no pronounced effect on the growth of the plants in this experiment. The plants did not produce heads and made very poor growth with all treatments where copper was not added. The characteristic curling of leaves and dying-back from the tips was typical.

EXPERIMENT 4

Sudan grass, a copper-responsive crop, was used in this experiment. The objective was to observe any relationship which might exist between potassium and treatments with and without copper; increasing the copper sulfate application up to 2500 lbs./A. Could extremely large applications of copper stimulate plant growth in the absence of potassium? What effect would the application of calcium, at varying rates, have on the above relationship?

The data from this experiment is presented in Figure 10 and Table 6. In Figure 10 it is noted that where potassium had not been applied there was very little response to increasing copper applications. All plants showed stunted growth and produced very few heads. It is interesting at this point to mention that the deficiency symptoms produced where potassium had not been applied (but with copper added) were outwardly similar to those produced where potassium was applied (but

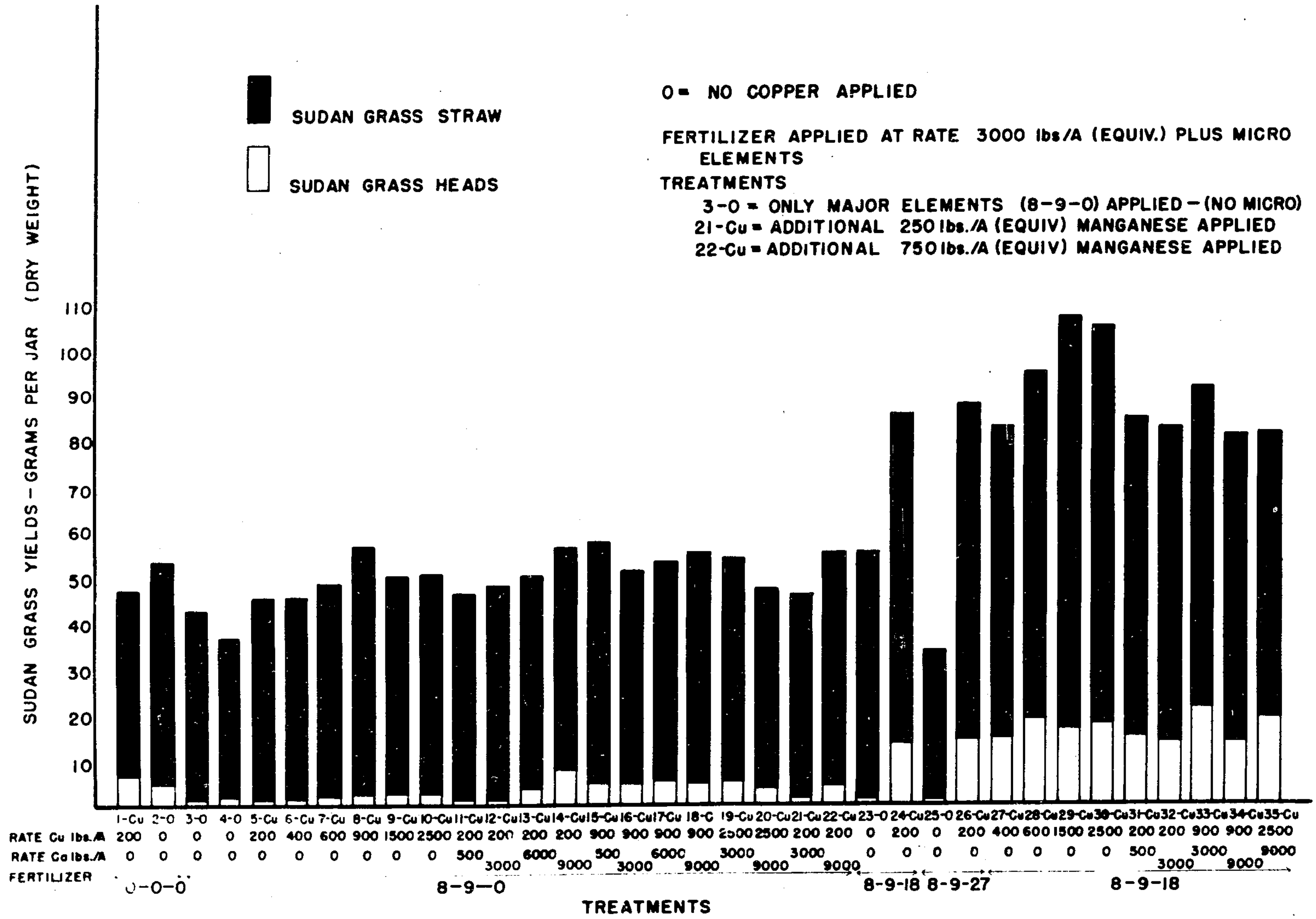

FIG 10 RESPONSE OF SUDAN GRASS TO COPPER, POTASSIUM AND CALCIUM AS APPLIED AT VARIED AMOUNTS ON MUCK SOIL WITH AND WITHOUT A HIGH LEVEL OF NITROGEN

TABLE 6

Response of sudan grass to copper, potassium and calcium applied in varied amounts on muck soil with and without a high level of nitrogen

	Treatment Lbs./A.		Yield gms. dry wt. Straw	Hds.	Total	Per cent composition in straw Cu	Fe	N	K	P	Ca	Mg
1	0-0-0	Cu	48	8	56	.0031	.020	.95	.17	.11	1.06	.65
2	0-0-0	No Cu	53	6	59	.0026	.019	1.21	.17	.12	1.05	.69
3	0-6-8	No Cu*	42	2	44	.0026	-	2.15	.73	-	-	-
4	0-6-8	No Cu	38	2	40	.0022	.021	2.32	1.42	.70	1.54	1.39
5	0-6-8	200 Cu	47	2	49	.0044	.026	2.17	.68	.73	1.65	1.39
6	0-6-8	400 Cu	47	2	49	.0041	-	2.39	.09	-	-	-
7	0-6-8	600 Cu	49	3	52	.0035	-	1.95	.17	-	-	-
8	0-6-8	900 Cu	57	3	60	.0036	.023	2.26	.41	.69	1.76	1.51
9	0-6-8	1500 Cu	51	2	53	.0051	-	2.15	.15	-	-	-
10	0-6-8	2500 Cu	51	2	53	.0043	.016	2.03	.25	.63	1.36	1.29
11	0-6-8	200 Cu** 500 Ca**	47	1	48	.0029	.025	2.20	.28	.67	1.50	1.41
12	0-6-8	200 Cu 3000 Ca	49	2	51	.0025	-	2.02	.14	-	-	-
13	0-6-8	200 Cu 6000 Ca	50	5	55	.0045	-	2.00	.93	-	-	-
14	0-6-8	200 Cu 9000 Ca	56	8	64	.0035	.023	1.94	.89	.18	1.96	.88
15	0-6-8	900 Cu 500 Ca	57	5	62	.0034	.020	1.78	.48	.51	1.21	1.08
16	0-6-8	900 Cu 3000 Ca	52	5	57	.0027	-	1.76	.12	-	-	-
17	0-6-8	900 Cu 6000 Ca	54	6	60	.0035	-	1.61	.15	-	-	-
18	0-6-8	900 Cu 9000 Ca	55	5	60	.0040	-	1.72	.12	-	-	-
19	0-6-8	2500 Cu 3000 Ca	54	5	59	.0042	-	1.50	.24	-	-	-
20	0-6-8	2500 Cu 9000 Ca	48	3	51	.0056	.020	1.90	.93	.22	1.58	1.01
21	0-6-8	250 Mn 3000 Ca 200 Cu	47	2	49	.0026	-	1.87	.63	-	-	-
22	0-6-8	750 Mn 9000 Ca 200 Cu	55	6	61	.0038	-	1.62	.17	-	-	-

TABLE 6 (Cont'd.)

		Treatment Lbs./A.	Yield gms. dry wt. Straw	Hds.	Total	Per cent composition in straw Cu	Fe	N	K	P	Ca	Mg
23	8-9-18	No Cu	55	2	57	.0020	-	1.65	1.16	-	-	-
24		200 Cu	86	14	100	.0026	.022	.98	.89	.26	.31	.30
25	8-9-27	No Cu	34	1	35	.0016	.027	2.49	2.01	.66	.89	.69
26		200 Cu	87	14	101	.0035	.019	1.19	.59	.28	.51	.47
27	8-9-18	400 Cu	83	15	98	.0037	.017	.96	.73	.30	.47	.45
28		600 Cu	95	19	114	.0022	.016	.78	1.00	.25	.44	.37
29		1500 Cu	107	16	123	.0036	-	.78	.98	-	-	-
30		2500 Cu	105	18	123	.0029	.018	.69	.64	.24	.39	.31
31		200 Cu 500 Ca	85	15	100	.0025	.019	.83	.60	.27	.56	.47
32		200 Cu 3000 Ca	83	13	96	.0029	.027	1.16	1.53	.25	.36	.32
33		900 Cu 3000 Ca	92	21	113	.0028	.028	.96	1.42	.23	.39	.28
34		900 Cu 9000 Ca	81	13	94	.0028	-	1.16	1.13	-	-	-
35		2500 Cu 9000 Ca	81	19	100	.0028	.022	1.13	1.08	.11	.50	.30

* No micro-nutrient elements applied

** Calcium applied as calcium hydroxide; copper applied as copper sulfate copper not added). This condition (shown in Figure 11) was characterized by a firing (red) of the tips and edges of the leaves with some curling and dying-back. The relative response of the plants to the fertilizer treatments is shown in Figure 12.

Highest yields were obtained with a complete fertilizer and 1500 to 2500 lbs./A. applications of copper without adding calcium. Upon adding calcium to similar treatments there was a marked decrease in yields as shown in Figure 10.

Fig. 11 Copper-deficient sudan grass leaves. The dark areas on leaves were red in color.

Fig. 12 Response of sudan grass to copper and potassium. Treatments were: (1) "Cu" plus "K", (2) "K" without "Cu", (3) "Cu" but no "K", (4) No "Cu" and no "K", (0) No fertilizer applied.

SUMMARY

(1) Sudan grass did not respond to increasing amounts of copper in the absence of potassium.

(2) Sudan grass did not respond to an application of potassium in the absence of copper.

(3) Maximum yields were obtained with the addition of 1500 and 2500 lbs./A. equivalent of copper sulfate in the absence of calcium. With the addition of calcium to similar treatments the yields were decreased.

EXPERIMENT 5

At this stage of the investigation no correlation had been observed between the fertilizer treatments used and any stimulating effect from a treatment which had not received copper. Fertilizer applications in the absence of copper increased copper deficiency symptoms.

Even though the wheat plants had developed die-back and appeared straw-colored in the absence of copper; there was a rapid response when copper was applied at that stage of maturity. This is brought out vividly in Figure 13 in which the plant on the left did not receive copper, the center jar received copper one week and the jar on the right two weeks before picture was taken. All plants had previously appeared as those in the jar on the left having received a complete fertilizer but no copper.

This die-back of new growth in a copper-deficient plant is further shown in celery Figure 14.

Fig. 13 Response of spring wheat to copper after developing copper deficiency symptoms. Treatments were: Jar (58) No copper, (19) received copper one week and (29) received copper two weeks before this picture was taken.

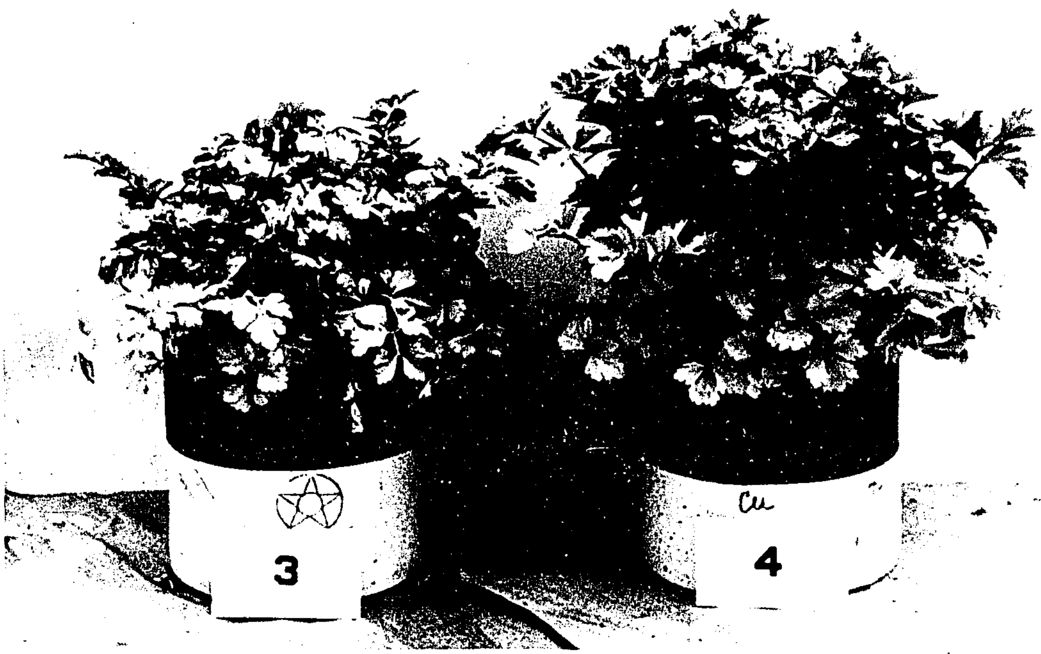

Fig. 14 Response of celery to copper. Plants on left show dying-back of new growth, copper was <u>not</u> applied. The plants on right received copper and maintained normal growth.

Experiment 5 was designed to investigate the availability and use of some different copper compounds as affecting plant growth.

Table 7 and Figure 15 give an outline of the treatments and data secured. The copper-containing compounds were mixed into the soil and each jar contained equivalent amounts of copper. Figure 16 shows the uniform growth of the spring wheat. Yields from jars receiving twenty-five pounds of copper sulfate per acre were as high as those yields from 100 lbs. per acre applications.

The cupferron was added as a possible means of tying up some of the iron in the soil.

Finely divided electrolytic copper (C.P.) gave beneficial effects. Copper applied as the metal gave results almost equal to the applications of copper salts.

The results from the copper pyrophosphate catalysts are in agreement with those found by Armiger and co-workers (2).

The results with treatment 11, Fig. 15, where no fertilizer had been applied except 100 lbs./A. of copper sulfate, brings out the importance of copper as an aid to head formation on wheat.

SUMMARY

Spring wheat gave favorable response in growth to the different copper-containing compounds used in this experiment. The per cent nitrogen in the plants where copper was not applied continued to be high. Only where copper alone was applied to

TABLE 7

Response of spring wheat to different kinds and rate of application of copper compounds; the copper added in equivalent amounts.

	Treatment		rate #/A.	Yield gms. dry wt. Straw	Heads	Total	Per cent composition in straw Cu	Fe	N	K	P	Ca	Mg.
1	3-9-18	Cu-S	25	28	26	54	.0019	.011	.43	.51	.17	.28	.11
2	3-9-18	Cu-S	100	28	26	54	.0010	.012	.45	.58	.14	.22	.11
3	3-9-18		None	12	1-	13	.0011	.021	3.03	1.45	.65	.46	.24
4	3-9-18	Cu-P	25	25	23	48	.0013	—	.41	.41	—	—	—
5	3-9-18	Cu-P	100	25	23	48	.0017	—	.40	.24	—	—	—
6	3-9-18	Cu-A	25	25	23	48	.0013	—	.39	.37	—	—	—
7	3-9-18	Cu-A	100	26	24	50	.0031	—	.41	.57	—	—	—
8	3-9-18	Cu-H	25	25	23	48	.0008	—	.38	.60	—	—	—
9	3-9-18	Cu-H	100	26	24	50	.0010	—	.40	.70	—	—	—
10	0-0-0		None	11	10	21	.0016	.016	1.14	.25	.10	.62	.35
11	0-0-0	Cu-S	100	12	14	26	.0013	.011	.76	.25	.07	.49	.30
12	3-9-18	Cu-E	100	21	20	41	.0017	—	.60	.84	—	—	—
13	3-9-18		None*	14	1	15	.0021	.028	2.77	2.44	.61	.51	.24

* Cupferron was added (200 lbs./A.)

Cu-S = Copper sulfate
Cu-A = Tetra copper calcium oxychloride
Cu-H = Copper hydroxide
Cu-E = Electrolytic copper (CP)
Cu-P = Copper pyrophosphate catalysts

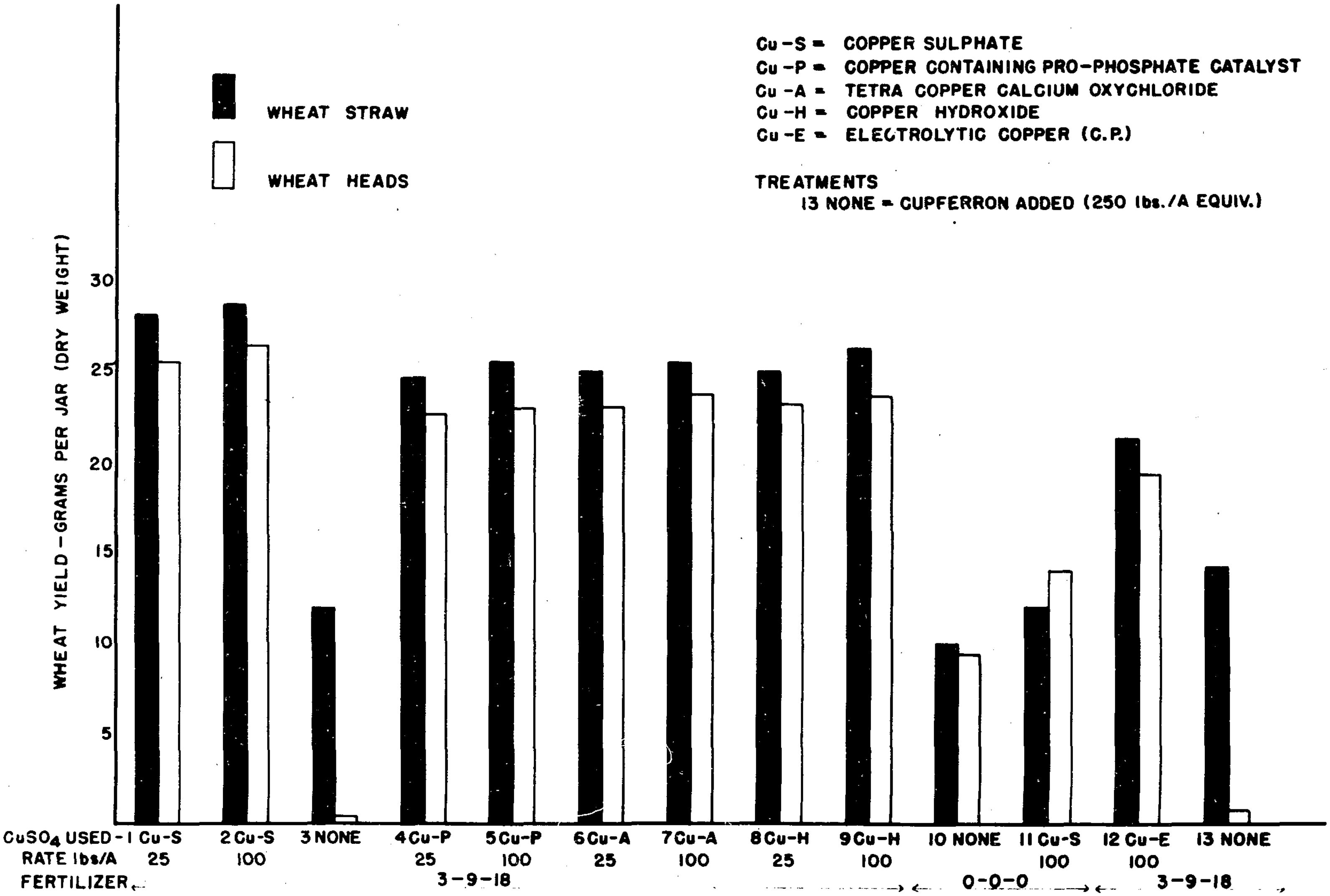

Fig. 15 RESPONSE OF SPRING WHEAT TO EQUIVALENTLY ADDED DIFFERENT KINDS OF COPPER CONTAINING SUBSTANCES

Fig. 16 Response of spring wheat to different copper compounds. Treatments were: (Left to right) (1) Copper not used, (2) copper sulfate 100 lbs./A., (3) copper pyrophosphate catalysts 100 lbs./A., (4) tetra-copper calcium oxychloride 100 lbs./A., (5) copper hydroxide 100 lbs./A., (6) electrolytic copper (C.P.) 100 lbs./A., (7) copper not used but 200 lbs./A. cupferron added.

the soil (no other fertilizer) did the head yields exceed that of the straw.

EXPERIMENT 6

Experiment 6 was set up, as a result of the work by Piper (40) on partial sterilization of the soil which increased growth and copper uptake of the plant, to determine whether muck would react in the same manner as the mineral soil investigated. He concluded that the increase in availability of copper, as a result of partial sterilization, indicates that the deficiency in the Dobe soil (highly calcareous shelly sand) was one of unavailability rather than lack of total copper. Also, that copper becomes necessary for growth in the early seedling stage and apparently remains essential as long as active growth is proceeding.

Each treatment was set up in triplicate. Six jars were sterilized by steam at 10 pounds pressure for eight hours; three jars with copper applied, and three jars containing no copper. This sterilization process was repeated three days later to kill any microbes that might have been in the spore state previously. Six other jars were prepared comparable to those above but were not sterilized. Spring wheat was planted in all jars. The treatments are shown in Table 8.

TABLE 8

Response of spring wheat to copper as affected by sterilized and unsterilized muck soil

Treatment			Yield gms. dry wt. Straw	Heads	Cu	Fe	N	K	P	Ca	Mg
1	Un-S**	No Cu	19	None	.0016	.021	2.93	4.36	.79	.62	.32
2		Cu	51	29	.0010	.011	1.55	1.12	.39	.22	.14
3	S*	No Cu	63	8	.0018	.012	3.00	2.14	.52	.59	.38
4		Cu	85	32	.0027	.010	2.13	1.21	.37	.53	.34

*S - Sterilized muck soil
**Un-S - Unsterilized muck soil

Where copper had not been applied, but the soil sterilized, plant growth was rapid and vigorous in the early stages. It was not until these plants had started to head that copper deficiency symptoms appeared. These symptoms appeared suddenly and very few heads were produced. This is reflected in the yields shown in Table 8 and in Figure 17. Sterilized muck produced greater yields of straw both with and without copper than did the unsterilized soil. Copper notably favored head formation. Figure 18 shows the appearance of the plants. The differences in size and fullness of wheat kernels produced are shown in Figure 19.

SUMMARY

Sterilization of muck soil stimulated the growth of wheat plants. When copper was not applied, the sterilized muck produced plants with abundant vegetative growth but very few heads and showed copper deficiency symptoms at time of heading. In comparison, a muck not sterilized (no copper applied) produced very little vegetative growth, no heads, and the plants showed copper deficiency symptoms early in their growth cycle.

The results of this experiment indicate that sterilization possibly makes some nutrient elements more available (including copper) and that the deficiency of copper in this soil is one of lack of availability rather than lack of total supply.

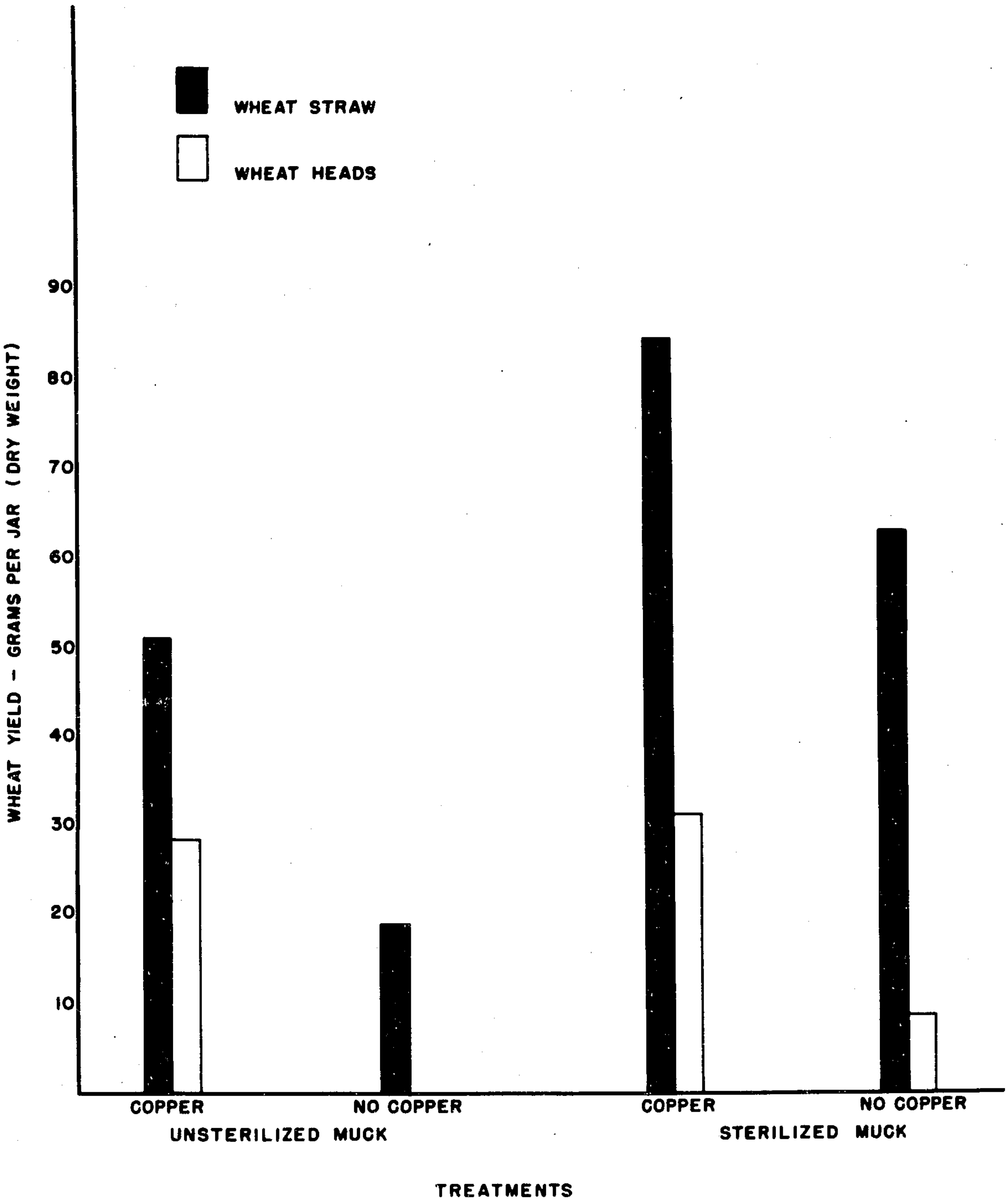

Fig. 17 RESPONSE OF SPRING WHEAT TO COPPER GROWN IN STERILIZED AND UNSTERILIZED MUCK SOIL

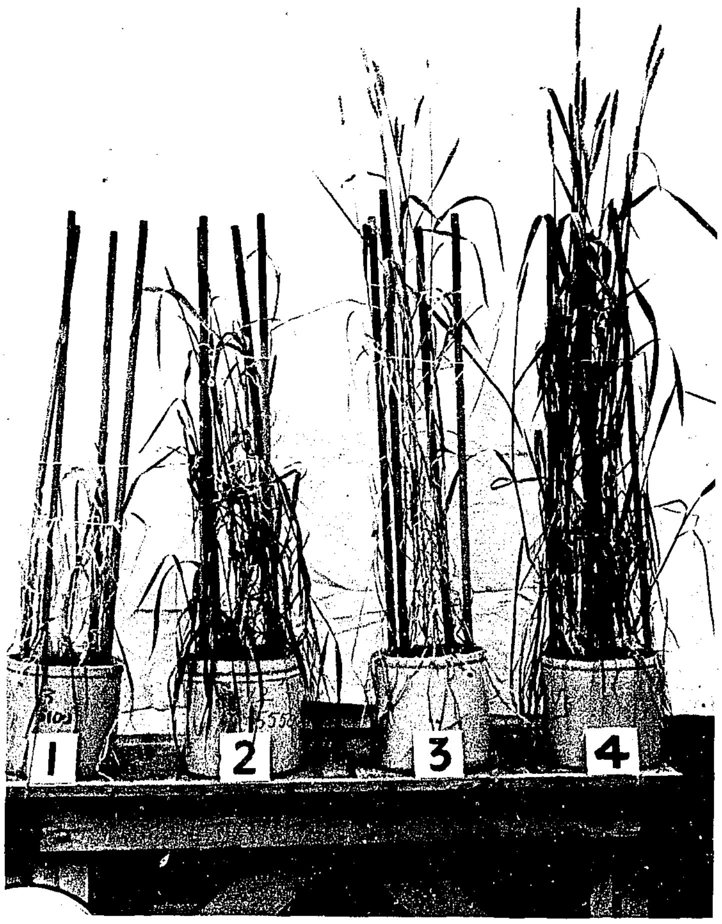

Fig. 18 Response of spring wheat to copper as effected by sterilized and non-sterilized muck soil. Treatments were as follows:
(1) No Cu - Not sterilized, (2) No Cu - Sterilized, (3) Cu - Not sterilized, (4) Cu - Sterilized.

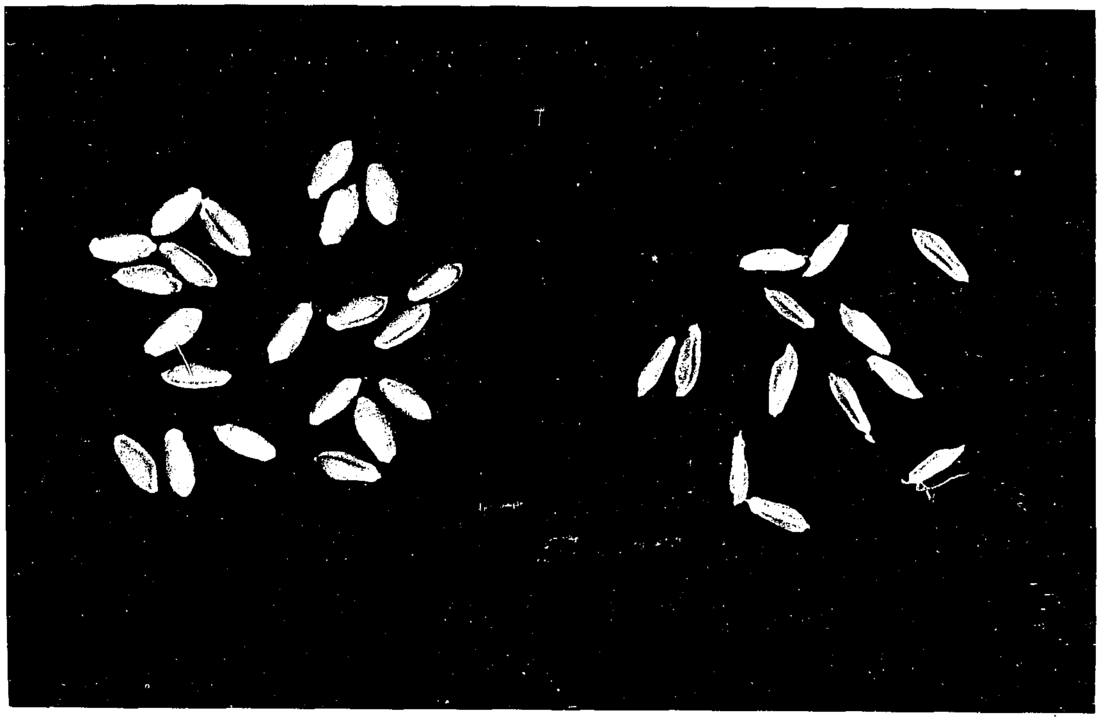

Fig. 19 Comparison of wheat kernels from plants grown on muck soil receiving copper (left) and no copper added (right).

EXPERIMENT 7

The percentage nitrogen was high in all wheat plants to which copper was not applied thus far in these experiments. Kraus and Kraybill (33), and subsequently many other investigators, have sought to give expression to the metabolic roles of carbohydrates and organic nitrogenous compounds in terms of the relative proportions of these compounds in the plant. Working with tomato plants they recognized four different metabolic conditions in terms of the proportionate amounts of these two types of substances present. Each of these conditions are distinguished by a characteristic morphological response on the part of the plants. One of these conditions corresponding to the outward appearances of a copper-deficient wheat plant grown during this investigation, was given as follows:

"A very low proportion of available carbohydrates to available nitrogen. The tomato plants were vigorous vegetatively but unfruitful. Stems were thick, pithy and succulent. Leaves were large, soft and dark green. Chemical analysis showed the plants to be low in reserve carbohydrates, but relatively high in available nitrogenous compounds".

Any interpretation of the results of Kraus and Kraybill, or work of a similar nature by other investigators, must be based on an evaluation of the relative roles of the carbohydrates and nitrogenous foods at different stages in the growth cycle of the plant. Hicks (26) relates that in the wheat plant the proportion of available carbohydrates to available nitrogenous compounds increases progressively throughout the vegetative period, and flowering occurs when this proportion becomes sufficiently high.

A deficiency of carbohydrates has been found by Howlett (28) to induce microspore degeneration and pollen sterility in the tomato.

Yocum (49) believes that if the nitrogen content is kept high, growth may go on at such an abnormal rate, using so much of the food that the plant fails to get a storage supply of carbohydrates sufficient to cause it to become reproductive.

Nickery (38) points out that, of all the biochemical constituents of cells, nitrogenous compounds should be the most closely integrated with vital activity. Through the use of nitrogen (N^{15}) concomitant synthesis and breakdown of nitrogen compounds is now regarded as a characteristic of life. The difficulty and the challenge of nitrogen metabolism lies in the fact that it is not enough to explain the path of synthesis or the reactions of nitrogen compounds; one should know their interrelation with other physiological processes.

Hamilton and Gilbert (22), working with tung trees, found that the higher the level of nitrogen applied, the more severe were the copper deficiency symptoms. Gilbert, Sell, and Drosdoff (21) suggested the existence of a copper/nitrogen ratio. In their work with tung trees they found the rate of photo-synthesis, in copper-deficient tung leaves, to be about one fourth that of the normal leaves. There were no significant differences between leaves from normal and copper-deficient trees in the percentage of non-reducing sugar; but the percentage of reducing sugar in deficient leaves was low and that of starch outstandingly low. This condition indicates either a decrease in formation or an increase in utilization of reserve carbohydrates in such tissues, or both.

Experiment 7 was designed for the following purposes: (1) To note any differences in nitrogen content between a copper-deficient and normal plant, (2) to follow the course of reducing sugar development in these plants, and (3) to observe the growth curve produced.

Seventy-two jars were set up as previously described in the experimental procedure. One half of these jars received a 200 lb./A. application of copper sulfate, the remaining 36 jars did not receive copper. Spring wheat was planted into these jars and the first harvest was made on four jars of each set (copper and no copper treatments) when the plants were 19 days old. The remaining harvest dates were made at 7, then 10 day intervals, using three jars from each set except for the last date of harvest in which only two jars remained. Figure 20 shows the appearance of the plants when they were 116 days old, which was the last harvest date.

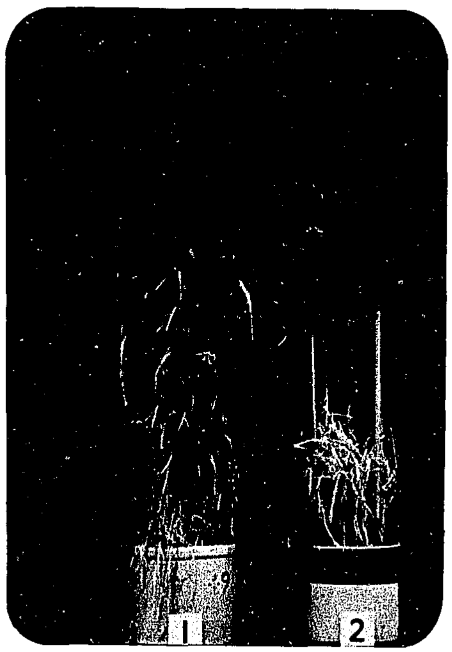

Fig. 20 Response of spring wheat to copper; (1) received copper, (2) copper was not applied.

Reducing sugars and total nitrogen (Kjeldahl) were determined at time of harvest for each set of treatments. Total yield of both green and dry weight was recorded. These data are presented in Figure 21 and Table 9.

During the course of this experiment, a striking difference was noted in the green color of the plants as harvested, or when extracted with ethyl alcohol, between the copper-deficient and normal plants. Thus, when the plants were 47 days old a comparative test for chlorophyll was run on the copper-deficient and normal plants. This test consisted of taking a uniformly chopped 4 gram sample from each harvested treatment. These samples were each thoroughly pulverized by mixing in a Waring blender for nine minutes and the chlorophyll was extracted with ethyl alcohol.

The alcoholic extract was separated from the fibrous remains by use of a suction filter. The residue was washed free of chlorophyll and diluted to 200 cc. An aliquot of this 200 cc. was placed in a colorimeter tube and the light transmitted through the solution was measured by use of the Evelyn colorimeter employing a 440 filter. The results from these comparative chlorophyll determinations are recorded in Figure 22. It is evident that there was less chlorophyll in the copper-deficient plants as compared to the normal plants having received copper. The latter plants progressively increased in chlorophyll content until they were 87 days old, after which the wheat heads began to ripen and the plants began to lose their chlorophyll.

The presence of less chlorophyll in a copper-deficient plant in comparison with that in a normal plant receiving copper is in agreement with the work of Bergman (8), Orth, et al.

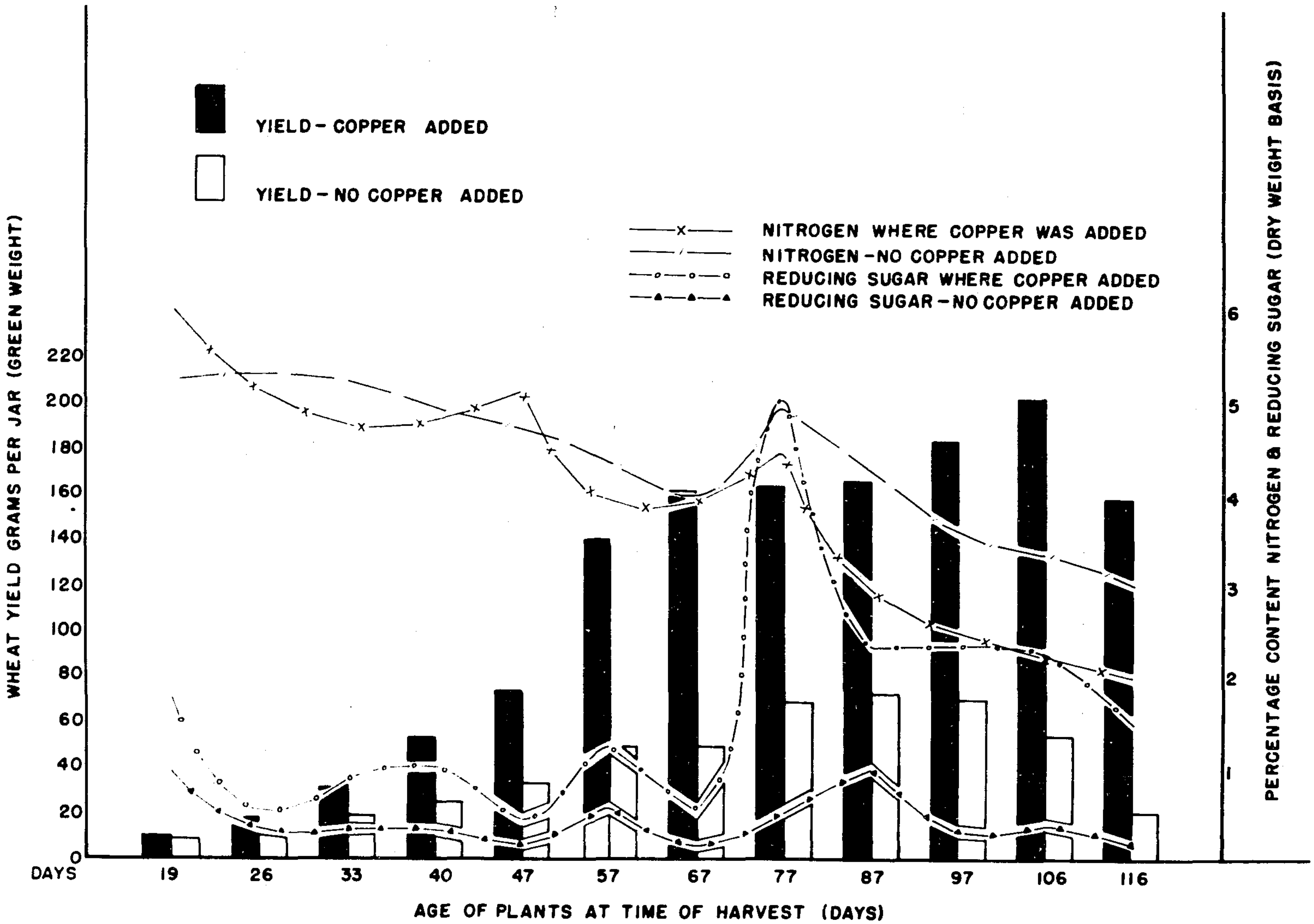

Fig. 21 RESPONSE OF SPRING WHEAT TO COPPER AS MEASURED AT 7 AND 10 DAY INTERVALS TOGETHER WITH PERCENTAGE NITROGEN AND REDUCING SUGAR CONTENT AT THESE INTERVALS

TABLE 9

Yield and composition of spring wheat at different ages as effected by copper applications to muck soil.

Treatment Age of plant harvested	Yield gms. green wt.	dry wt.	Per cent composition in straw Ash	N	K	Reducing sugar
19 days						
1 Cu	9	1	—	6.03	—	1.79
No Cu	9	1		4.21		.94
26 days						
2 Cu	19	2	—	5.14	—	.55
No Cu	15	2		5.30		.35
33 days						
3 Cu	33	4	—	4.75	—	.86
No Cu	21	2		5.26		.38
40 days						
4 Cu	53	6	—	4.81	—	1.02
No Cu	27	3		4.94		.39
47 days						
5 Cu	74	7	—	5.15	—	.45
No Cu	35	3		4.74		.28
57 days						
6 Cu	141	13	12.09	3.85	4.20	1.26
No Cu	50	5	15.54	4.30	4.76	.59
67 days						
7 Cu	163	18	12.16	3.92	4.44	.48
No Cu	50	5	14.18	3.98	5.96	.21
77 days						
8 Cu	164	20	9.80	4.49	4.11	5.13
No Cu	71	7	14.41	5.18	5.20	.53
87 days						
9 Cu	166	31	9.08	2.90	3.21	2.31
No Cu	74	10	13.31	4.24	4.66	1.02
97 days						
10 Cu	184	35	7.62	2.51	2.93	2.33
No Cu	72	11	13.88	3.57	4.92	.30
106 days						
11 Cu	202	47	6.69	2.27	2.05	2.25
No Cu	54	10	15.67	3.34	3.89	.45
116 days						
12 Cu	159	51	6.64	2.04	1.94	1.50
No Cu	20	10	14.97	3.00	3.92	.23

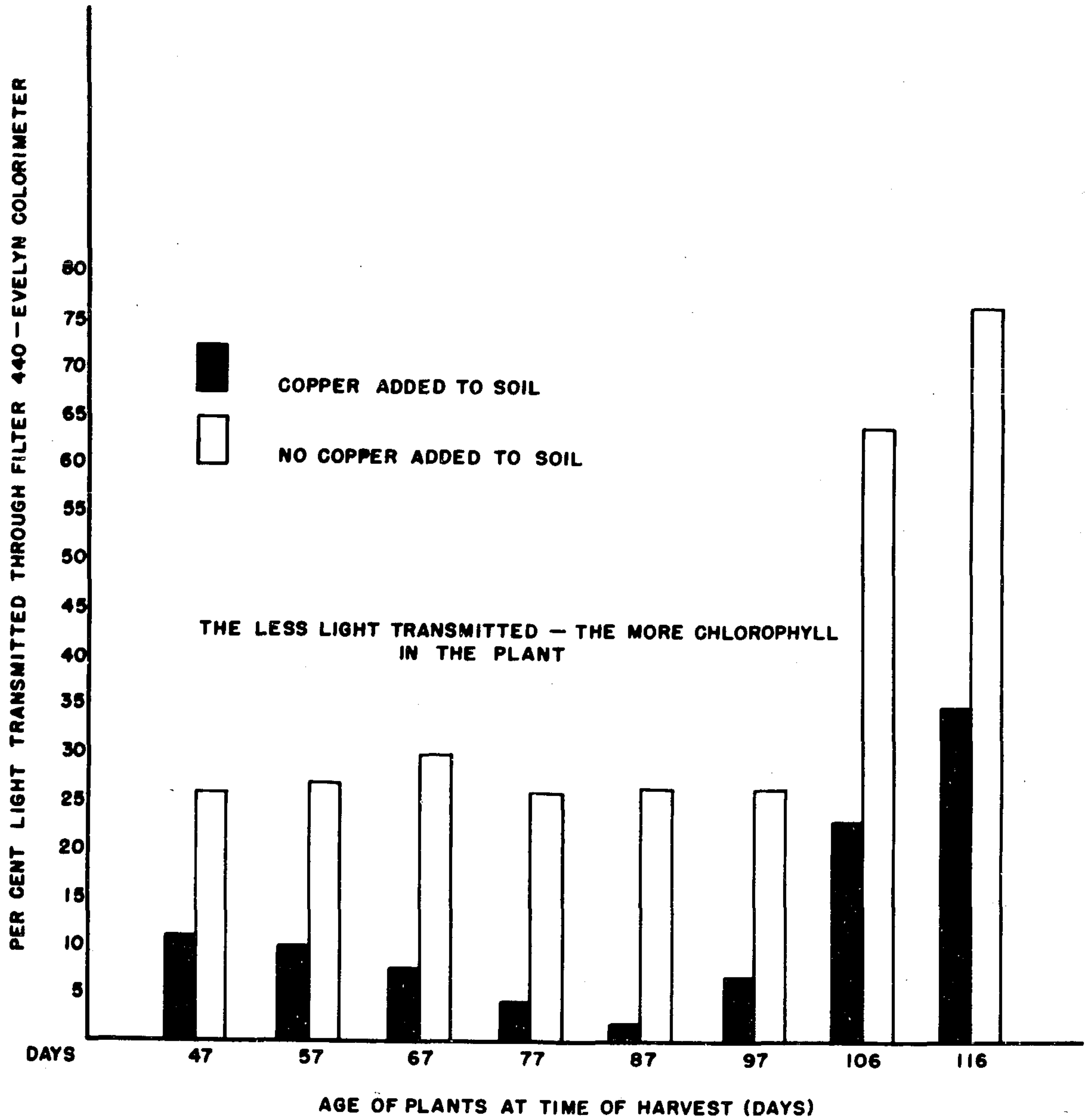

Fig.22 INDIRECT DETERMINATION OF CHLOROPHYLL IN SPRING WHEAT BY MEASUREMENT OF LIGHT TRANSMISSION

(39) and observations made by Harmer (24).

Various suggestions have been offered according to Bennett (7) to explain chlorosis as due to the inactivation of iron, namely, by excesses of other elements in chlorotic leaves. Inactivation has thus been ascribed to an excess of phosphorus, potassium and manganese.

Such element excesses, or high percentage content of the elements, were found present in copper-deficient plants throughout the experiments conducted in this investigation.

Figure 21 shows the yields and relationships of the spring wheat with regard to nitrogen and reducing sugar of the copper-deficient and normal plants. It is of special interest that where copper had been applied to the soil, the reducing sugar content of these plants increased at the approximate time that nodes became evident and head formation apparent. The reducing sugar content of these plants throughout the remaining growth cycle followed approximately the same trend as the total nitrogen content. It was at this stage of growth that differences in the total nitrogen content of the copper-deficient plants and the normal plants became evident, and remained so, throughout the remaining growth cycle of the plants.

SUMMARY

Data from this experiment indicates that the lack of copper, in some way, results in a decrease in chlorophyll formation. This in turn decreases the production of carbohydrates,

and with the very high total nitrogen content existing in the copper-deficient plants, produces a very unfavorable carbohydrate/nitrogen ratio for normal plant development. These plants remained vegetative and did not produce heads. Where copper was applied to the plants, the reducing sugar in the plant advanced approximately in the same order of magnitude as that of nitrogen.

Oats acts similarly to wheat (Table 10 and Figure 23) in its growth habits when its medium for growth is a copper-deficient muck.

TABLE 10

Response of oats to copper grown on a copper-deficient muck soil

Treatment	Yield dry wt. gms.	Per cent composition in straw Ash	Cu	Fe	N	K	P	Ca	Mg
1 No Cu	14	12.5	.0021	.024	4.60	3.19	.86	.81	.51
2 Cu	34	10.0	.0036	.015	2.82	2.49	.67	.46	.52

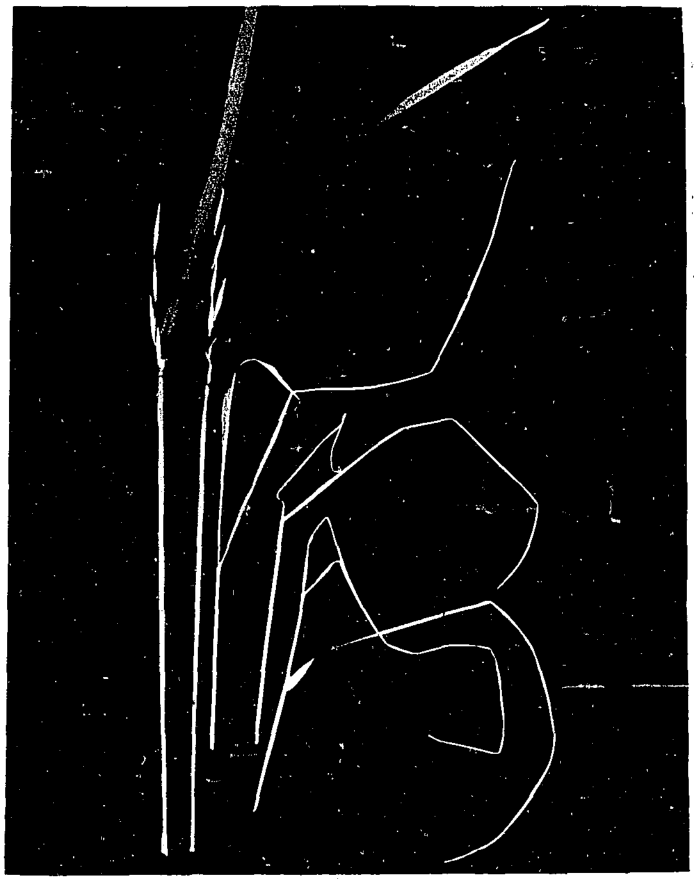

Fig. 23 Comparison between normal oat leaves (left), and copper-deficient oat leaves (right).

EXPERIMENT 8

A corn crop was grown in the greenhouse on a copper-deficient muck soil, with and without copper. When the copper-deficient plants were about two feet high, the leaves failed to unroll at the terminal portion. Disintegration of portions of the leaves and disappearance of chlorophyll appeared at these areas. The plants shown in Figures 24 and 25 are examples of such deficiency symptoms. At approximately a week after these symptoms had appeared, the plants recovered and produced apparently normal plants (Figure 26).

Fig. 24 Copper deficiency symptoms in corn. Note failure of second leaf from left to unroll.

Fig. 25 Copper deficiency symptoms in corn. Note sticking together of three leaves at top.

Fig. 26 Hybrid corn plants grown with and without copper applied to a muck soil. Treatments were: (1) No copper, (2) Copper. The seed planted was raised on mineral soil which showed no copper deficiency.

Why should corn show this recovery and growth without a copper application, whereas the deficiency symptoms were so pronounced in the case of wheat?

Corn and wheat kernels from samples used in previous plantings were analyzed for copper. Four times as much copper was found per kernel in the corn than in the wheat. This indicated the possibility that the larger corn kernel might have sufficient copper to aid the seedling plant in its early growth. Hybrid seed grown on copper-deficient muck (no copper applied) and seed from copper-sufficient mineral soil were planted. The treatments are given in Table 11.

TABLE 11

Variability of corn seed, grown with and without copper, as influencing the production of the succeeding crop grown on a copper-deficient muck soil.

Treatment	Yield gms. dry wt. Corn stalk	Per cent composition in stalks						
		Cu	Fe	N	K	P	Ca	Mg
1* No Cu	19	.0010	.019	2.17	2.04	.66	.46	.35
2** No Cu	33	.0015	.018	2.57	1.91	.59	.58	.33
3*** Cu	53	.0020	.023	1.53	1.31	.46	.46	.30

Seeds planted	Treatment
*1 No Cu grown seed	- Copper not applied to soil
**2 Cu grown seed	- Copper not applied to soil
***3 Cu grown seed	- Copper applied to soil

A progressive difference in the yields between treatments is noted. Figure 27 plainly illustrates the differences which actually existed.

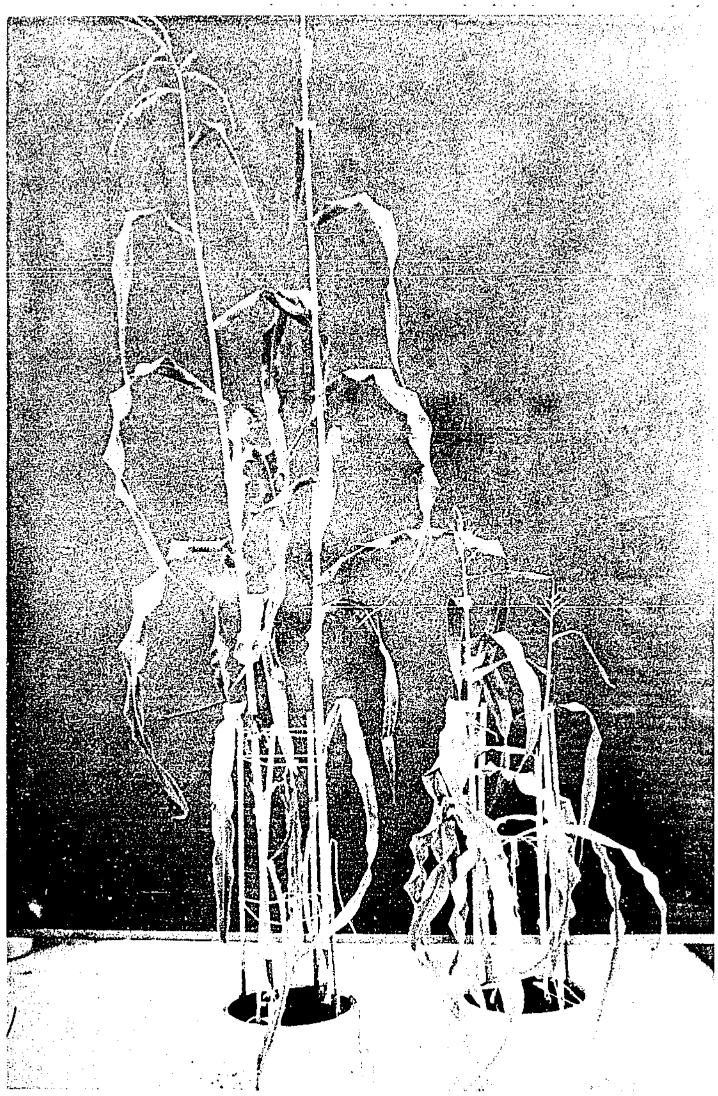

Fig. 27 Response of corn to copper applied to a muck soil. Corn on the left received copper, on the right no copper. The corn used in both treatments for planting was grown on a copper-deficient muck soil, (no copper applied).

Where the seed that was planted had been grown previously on copper-deficient muck soil, stunted growth of the corn plants resulted and very marked copper-deficiency symptoms were noticed.

The original seed grown on mineral soil showing no copper deficiency, (treatment 3, Table 11) received a 200 lb. per acre equivalent application of copper sulfate and it is evident that the plants did respond to this treatment. The total copper and iron contents progressively increased from treatment 1 through and including treatment 3.

Samples of the corn seed planted with the treatments above were analyzed for copper. The results were as follows:

	Number of seed	Wt. grams	% Cu
Original mineral soil grown hybrid corn seed	23	5.52	.00051
Copper-deficient grown hybrid corn seed (muck soil)	23	5.12	.00027

Additional treatments were arranged for planting corn using corn seed grown on sterilized and non-sterilized muck soil, each soil with and without copper. These treatments are given in Table 12. None of the soil from these treatments received copper at the beginning of the experiment. After the plants were one month old and all plants were showing some degree of copper deficiency; one jar from each three of a treatment was given an application of 200 lbs. per acre of copper sulfate broadcast on the surface of the soil. The response was almost immediate. Within four to five days those plants receiving copper recovered from the chlorotic appearance and the symptoms in which the leaves were rolled and stuck together no longer appeared in the new growth.

TABLE 12

Yield and composition of corn stalks as effected by source of seed planted and a copper application to the soil

	Treatment			Corn stalks gms. green wt.	Corn stalks gms. dry wt.	Ash	Cu	Fe	N	K	P	Ca	Mg
1	Copper grown seed	S***	No Cu	197	29	8.78	.0010	.026	1.96	2.31	.72	.38	.23
2			Cu*	253	41	6.67	.0013	.019	1.25	1.70	.45	.50	.23
3		Un-S**	No Cu	199	31	7.51	.0013	.026	1.84	2.40	.52	.46	.27
4			Cu*	246	44	4.91	.0010	.022	.88	1.35	.34	.41	.19
5	No copper grown seed	S***	No Cu	158	20	10.35	.0012	.030	3.11	3.29	.83	.52	.37
6			Cu*	292	57	4.73	.0009	.021	1.06	1.05	.37	.31	.38
7		Un-S**	No Cu	137	16	10.87	.0018	.028	2.40	3.05	.91	.52	.38
8			Cu*	295	51	4.96	.0012	.018	1.14	1.27	.32	.37	.27

* Copper was applied after plants were showing copper deficiency symptoms one month after planting corn.
** Un-S - Unsterilized
*** S - Sterilized

Figures 28, 29 and 30 show corn plants with typical copper deficiency symptoms. Figure 31 shows the difference in growth of plants grown from a copper-deficient seed and a plant grown from the same seed but receiving copper one month after being planted. Approximately the same number of leaves was found on each of these plants. The plant fails to grow at the internodes where intercalary growth takes place. Figure 32 shows the treatments and yields obtained.

The roots from the soil in each jar were screened free of muck soil, washed, dried in an oven at 70°C and then weighed. The treatments, yields, and element composition are given in Table 13.

The yield of roots was extremely low (Figure 32) where copper had not been applied to the soil.

In comparing the copper content of the corn stalks and roots grown with these various treatments (Tables 12 and 13), the percentage copper content of the corn stalks, where copper had not been applied, is in most cases higher than where copper had been applied. The opposite is true in the case of the roots of these same plants. Where copper was applied to the soil the corn roots contained, in most cases, a much larger percentage and total content of copper than roots produced in muck not receiving a copper application.

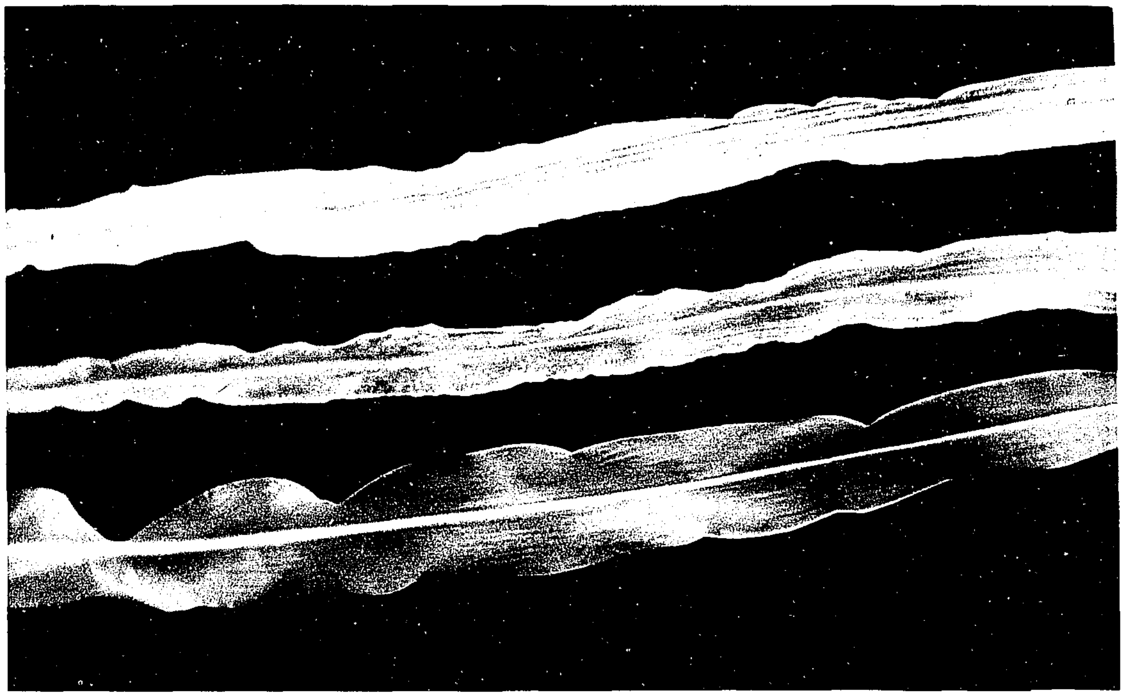

Fig. 28 Corn leaves from normal and copper-deficient plants. (1) Top leaf - from extremely copper-deficient plant in late stages of maturity, (2) middle leaf - showing copper deficiency, (3) bottom leaf - from same plant as middle leaf, one month after copper was applied.

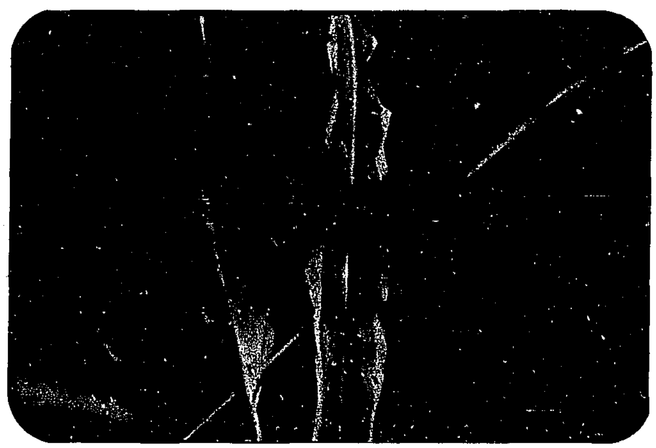

Fig. 29 Comparison of copper-deficient and normal corn leaves. The three copper-deficient leaves show, (left) striped chlorosis appearing, (center) curling of terminal portion of leaf and loss of chlorophyll, (right) striped chlorosis and dying-back from edges; all are at angles to a normal leaf which crosses from bottom left hand corner up into the upper right corner.

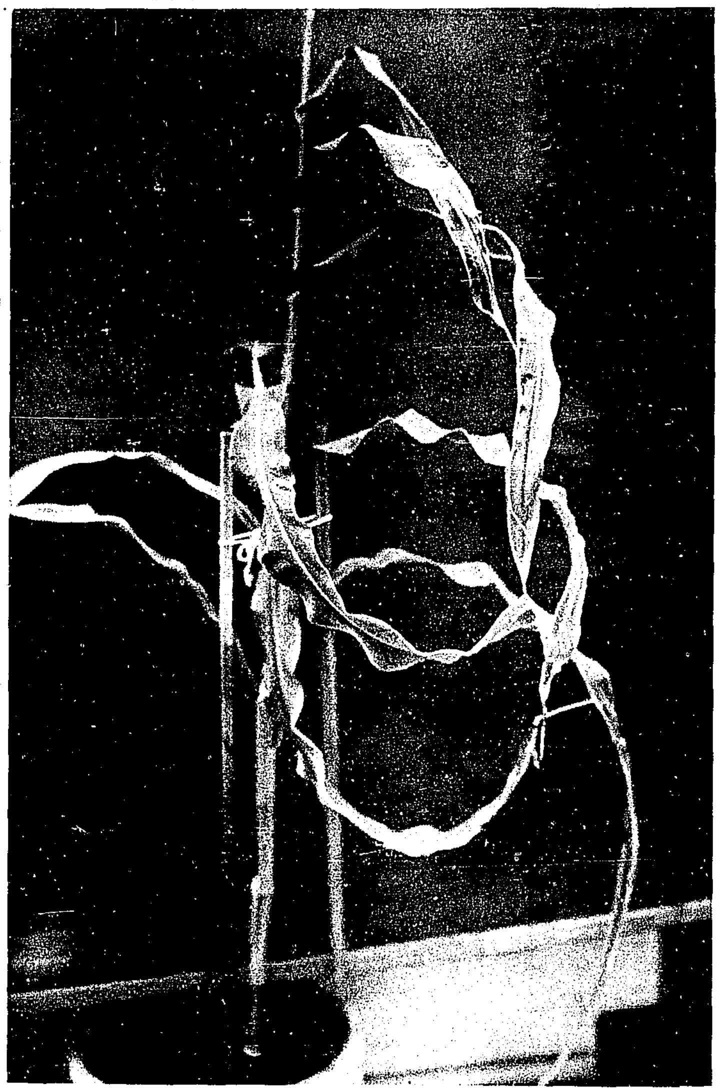

Fig. 30 Corn plant showing characteristic copper deficiency symptoms. Note curling and sticking together of leaves.

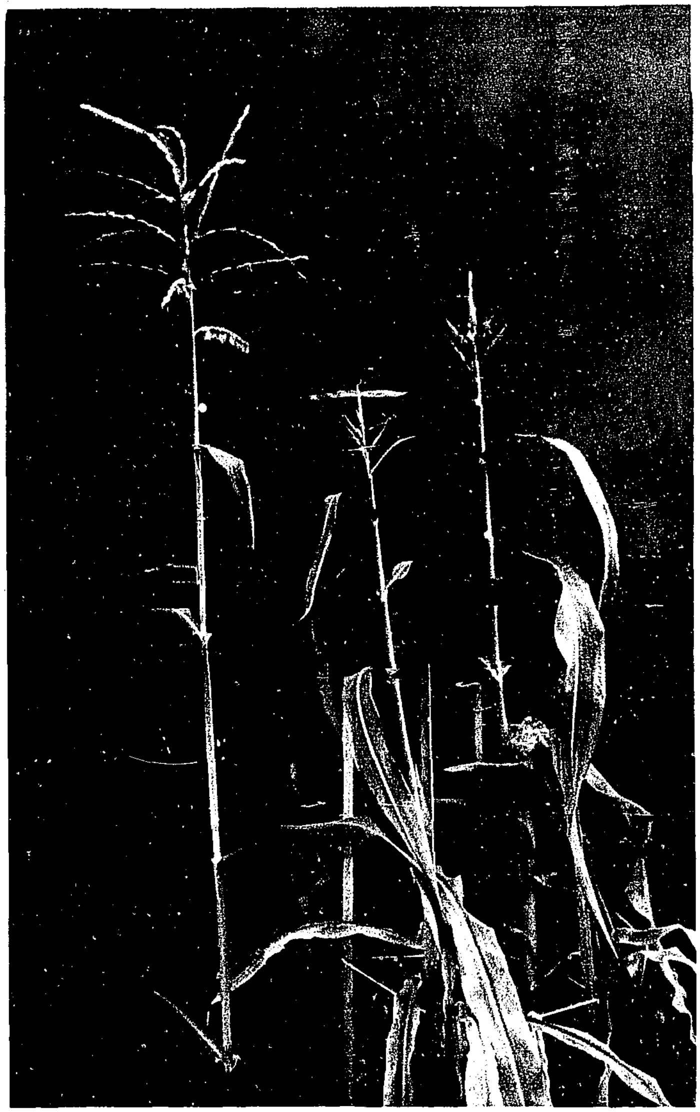

Fig. 31 Comparative differences between normal (left) and copper-deficient corn plants (right) as to length of internodes and development of tassel.

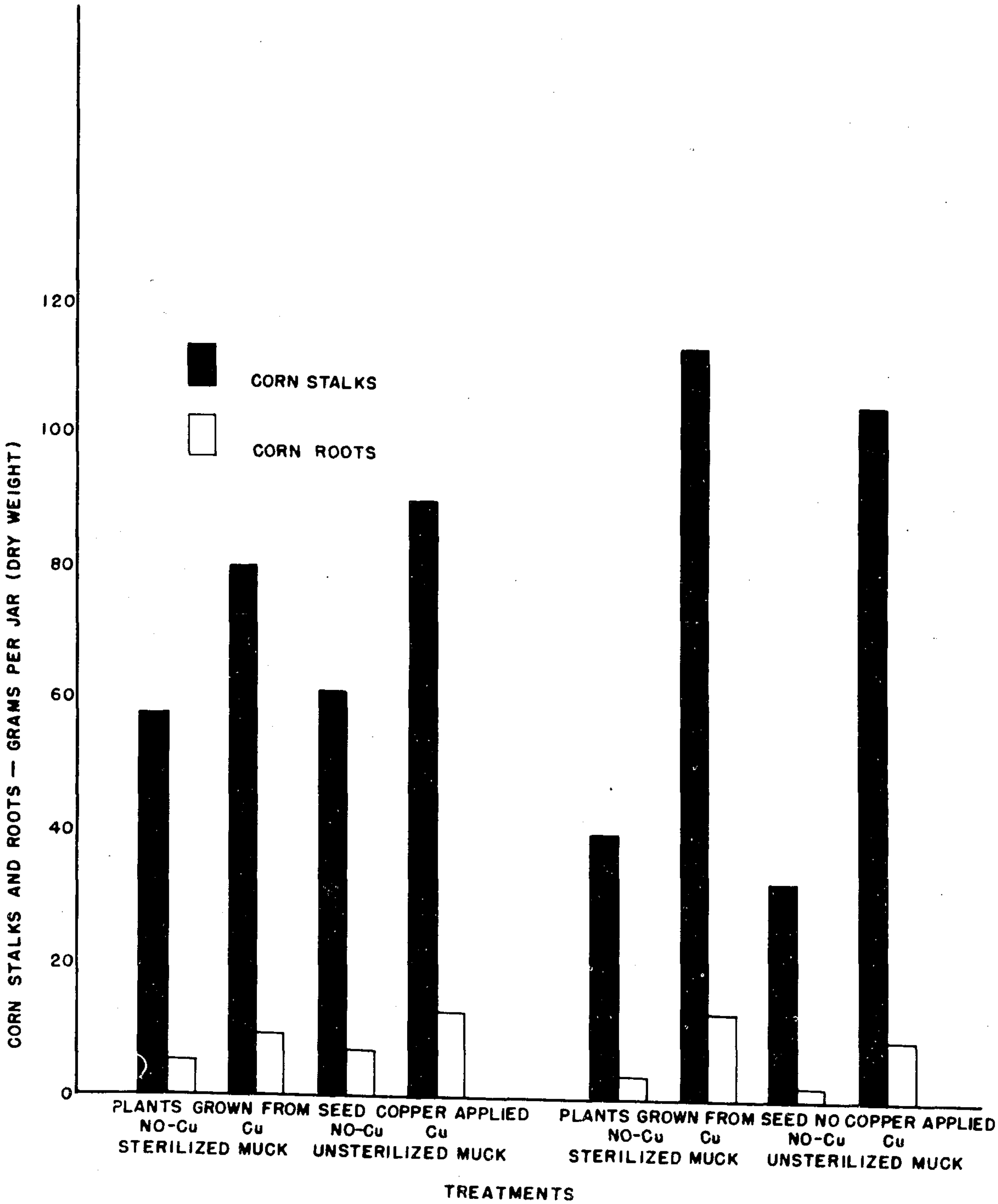

Fig. 32 RESPONSE OF CORN TO COPPER AND VARIATION OF YIELDS RESULTING FROM SOURCE OF SEED GROWN ON MUCK SOIL

TABLE 13

Yield and composition of corn roots as effected by source of seed planted and a copper application to the soil

	Treatment			Corn roots gms. dry weight	Ash%	Per cent composition in roots Cu	Fe	N	K	P	Ca	Mg
1	Copper grown seed	S***	No Cu	6	5.33	.0010	.049	1.83	2.12	.20	.30	.10
2			Cu*	8	3.92	.0022	.055	2.15	1.10	.12	.42	.12
3		Un-S**	No Cu	7	3.96	.0020	.040	1.35	1.61	.15	.24	.11
4			Cu*	11	3.63	.0022	.045	.91	1.10	.10	.28	.14
5	No copper grown seed	S***	No Cu	3	5.79	.0014	.045	2.03	1.85	.24	.38	.27
6			Cu*	13	3.50	.0023	.041	1.10	1.21	.15	.33	.14
7		Un-S**	No Cu	2	5.00	.0010	.044	1.38	2.02	.21	.27	.14
8			Cu*	9	4.00	.0019	.055	1.08	1.13	.11	.48	.16

* Copper was applied after plants were showing copper deficiency symptoms one month after planting corn.

** Un-S - Unsterilized

*** S - Sterilized

SUMMARY

This experiment indicated that there was a minimum, yet sufficient amount of copper in the corn seed grown in a soil containing available copper, to aid in the development of a normal plant. This was not the case for the same variety of corn seed harvested from a copper-deficient muck soil. On the basis of this study it would appear that, when copper-deficient seed is planted on copper-deficient soil, it is likely to produce permanent copper-deficient plants which will not fully mature.

EXPERIMENT 9

It had been observed in some preliminary experiments (not reported here) that tomatoes grown on a copper-deficient soil produced normal blossoms (Figure 33) but these blossoms failed to set fruit to the extent of those to which copper had been applied.

Was this a question of fruit setting? Would the plants maintain and produce more fruit if the fruit were set through hormone treatment?

In an attempt to answer the above questions an experiment was set up using the Spartan Hybrid variety of tomato.

Tomatoes were grown in two series of 18 jars each, each series contained six replications of the following treatments:

(1) No fertilizer applied to the soil.

(2) Complete fertilizer plus copper.

(3) Complete fertilizer, copper not applied.

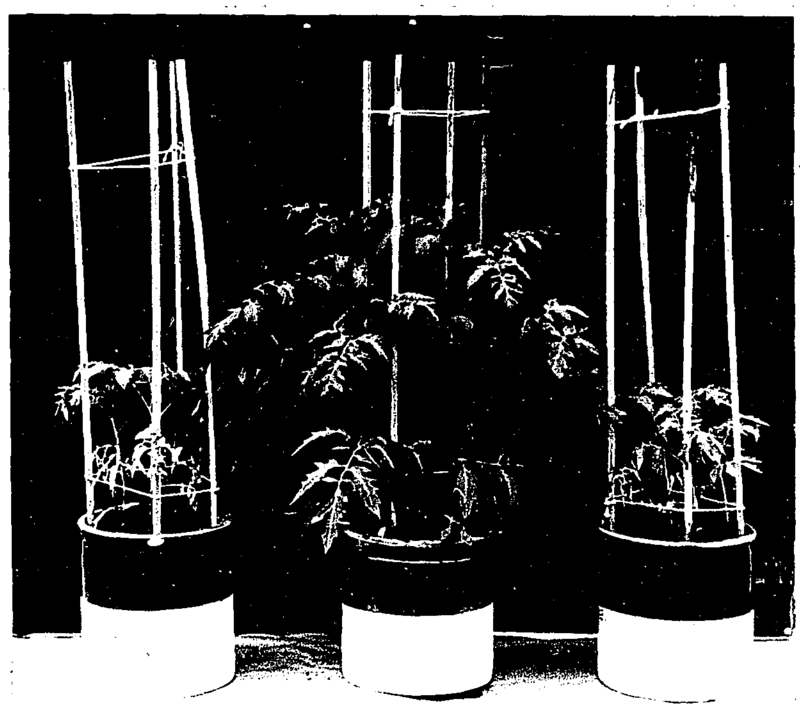

Fig. 33 Response of tomato plants to copper in early stages of growth. Center jar received copper; others did not.

In one series the tomato blossoms were sprayed with hormone to set the fruit, the other series did not receive the hormone spray. The treatments and the tomato fruit yields, by date, are reported in Table 14. The appearance of the tomato foliage of the two series is shown in Figures 34 and 35.

Of special interest in this experiment was the development of necrotic spots which occurred on the tomato fruit where copper had not been added to the soil. Such necrotic areas did not occur on the tomato fruit where copper had been applied to the soil. Figures 36, 37, 38, 39, 40 and 41 show the nature of this tissue breakdown of the tomato fruit.

TABLE 14

Yield and number of Spartan Hybrid tomatoes as effected by application of copper to muck soil and the use of hormone spray in fruit setting.

Hormone Treated

Date harvested	No fertilizer No copper		Fertilizer + Cu		Fertilizer-No Cu	
	Wt. gms.	Number	Wt. gms.	Number	Wt. gms.	Number
3-16-48	182.7	2-0R*	295.7	2-0R*	199.6	3-3R*
3-20-48	725.1	5 "	1709.6	11 "	347.0	4-4R
3-22-48	110.0	1 "	832.0	7 "	550.0	5-1R
3-26-48	87.0	1 "	2163.0	20 "	487.0	4-3R
3-30-48	154.0	2 "	282.0	5 "	533.0	6-4R
4- 3-48	214.0	3 "	520.0	6 "	696.0	7-1R
4- 8-48	145.0	2 "	372.0	4 "	568.0	6-1R
4-13-48	69.0	1 "	119.0	1 "	455.0	5-0R
4-19-48	38.0	1 "	1118.0	12 "	498.0	6-0R
4-24-48**	138.0	8 "	3149.0	63 "	1343.0	34-0R
Totals	1,862.8	26-0R	10,560.3	131-0R	5,676.6	80-17R

Total number harvested - 237
Total weight harvested - 18,099 gms.

No Hormone Used

Date harvested	No fertilizer No copper		Fertilizer + Cu		Fertilizer-No Cu	
	Wt. gms.	Number	Wt. gms.	Number	Wt. gms.	Number
3-16-48	0.0	0-0R*	300.1	5-0R*	46.5	1-1R*
3-20-48	250.5	2-0R	888.8	8 "	361.7	5-4R
3-22-48	192.0	2-1R	1000.0	9 "	526.2	7-3R
3-26-48	515.0	4-1R	1477.0	13 "	394.0	4-4R
3-30-48	115.0	1-0R	481.0	5 "	409.0	6-2R
4- 3-48	382.0	5 "	634.0	7 "	174.0	3-0R
4- 8-48	76.0	1 "	1866.0	22 "	1216.0	17-2R
4-13-48	0.0	0 "	1062.0	12 "	920.0	12-0R
4-19-48	0.0	0 "	384.0	5 "	567.0	8-0R
4-24-48**	568.0	24 "	2362.0	84 "	1508.0	53-0R
Totals	2,098.5	39-2R	10,454.9	170-0R	6,122.4	116-16R

Total number harvested - 325
Total weight harvested - 18,676 gms.

* Number accompanying "R" indicates number of tomatoes with necrotic spots.

** Green fruit from the last harvest.

Fig. 34 Three treatments of tomatoes receiving hormone. Left - no fertilizer, Center - no copper, Right - copper added. Fruit on these tomatoes was set with hormone spray.

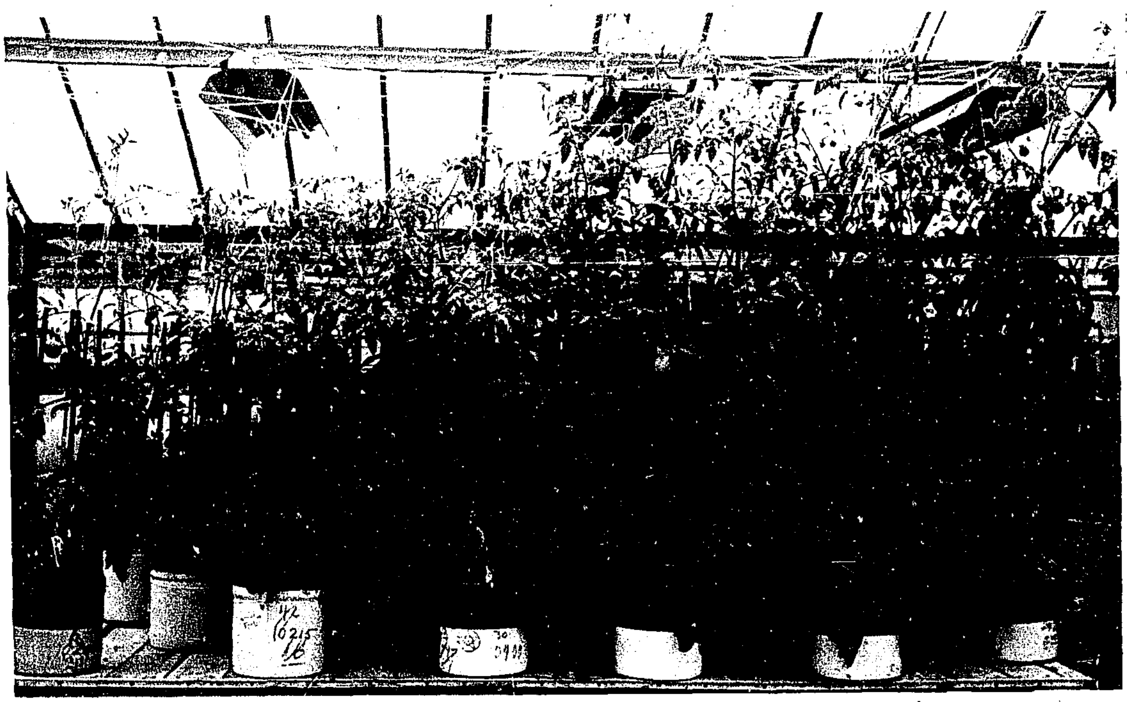

Fig. 35 Three treatments of tomatoes where hormone was not used. Left - no fertilizer, Center - no copper, Right - copper added. Compare difference in height of tomatoes in Fig. 34 and those in this Figure.

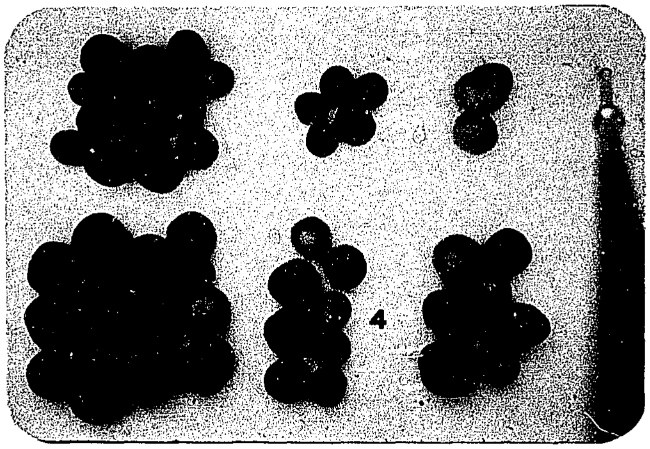

Fig. 36 First harvest of tomatoes with the following treatments:
Top row - hormone not used.
(a) Left - copper added to soil
(b) Center - copper not applied
(c) Right - no fertilizer
Bottom row - fruit set by using hormone spray; same treatments as above.

Note: Necrotic areas in tomatoes where copper was not added to the soil.

Fig. 37 Tomato fruit on copper-deficient plant beginning to show necrotic areas in tissue. Note dark areas around bottom of tomato.

Fig. 38 Tomato fruit on copper-deficient plant showing more advanced stages of necrotic areas in tissue.

Fig. 39 Tomato fruit on a copper-deficient plant showing a tomato on the right with definite necrotic areas in tissue.

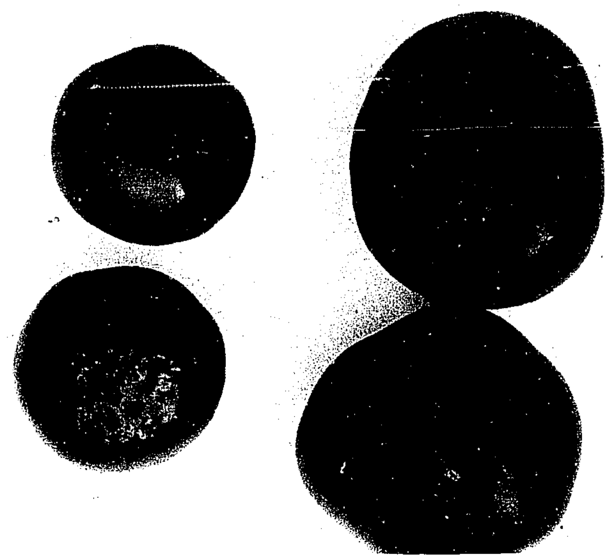

Fig. 40 Tomato fruit from no copper and copper treatments.
Left - No copper applied to soil (note necrotic areas).
Right - Copper applied.

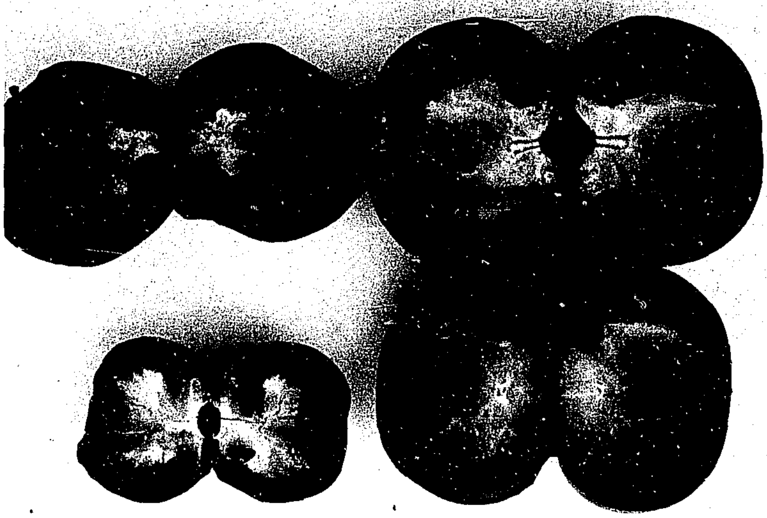

Fig. 41 Longitudinal section of tomato fruit from treatments:
Left - No copper applied to soil (note the nature of necrotic areas).
Right - Copper applied.

Where hormone spray was used to set the fruit of the three treatments, earlier and larger yields were obtained in the first four pickings, as compared to those yields where no hormone was used.

Figure 42 brings out the contrasting difference noted in total weight and total number of tomatoes harvested. Table 15 gives values showing that a greater number of tomatoes were harvested with all treatments where hormone was not used to set the fruit. But, the total weight of harvested tomatoes was slightly more from the hormone treated plants that were grown on soil which had received copper. This same table gives the analysis of the tomato foliage. The amount of copper is higher where hormone and copper were used.

SUMMARY

Hormone spray did not seem to alter the effects of copper upon plant growth. The spray hastened fruit set and enlargement of tomato fruit. This acceleration of growth was maintained by the plants up to a certain point, at which time, vegetative and reproductive growth stopped and the plants only maintained their fruit. The appearance of a copper-deficient tomato leaf is shown in Figure 43.

Where hormone spray was not used, a larger number but smaller fruit was produced. These tomato plants produced greater vegetative growth than where hormone spray was employed.

In either case (hormone or no hormone), the effect of copper on plant growth was reflected in greater number and weight of tomatoes where copper was applied to the soil. Necrotic areas appeared on fruit where copper was not added.

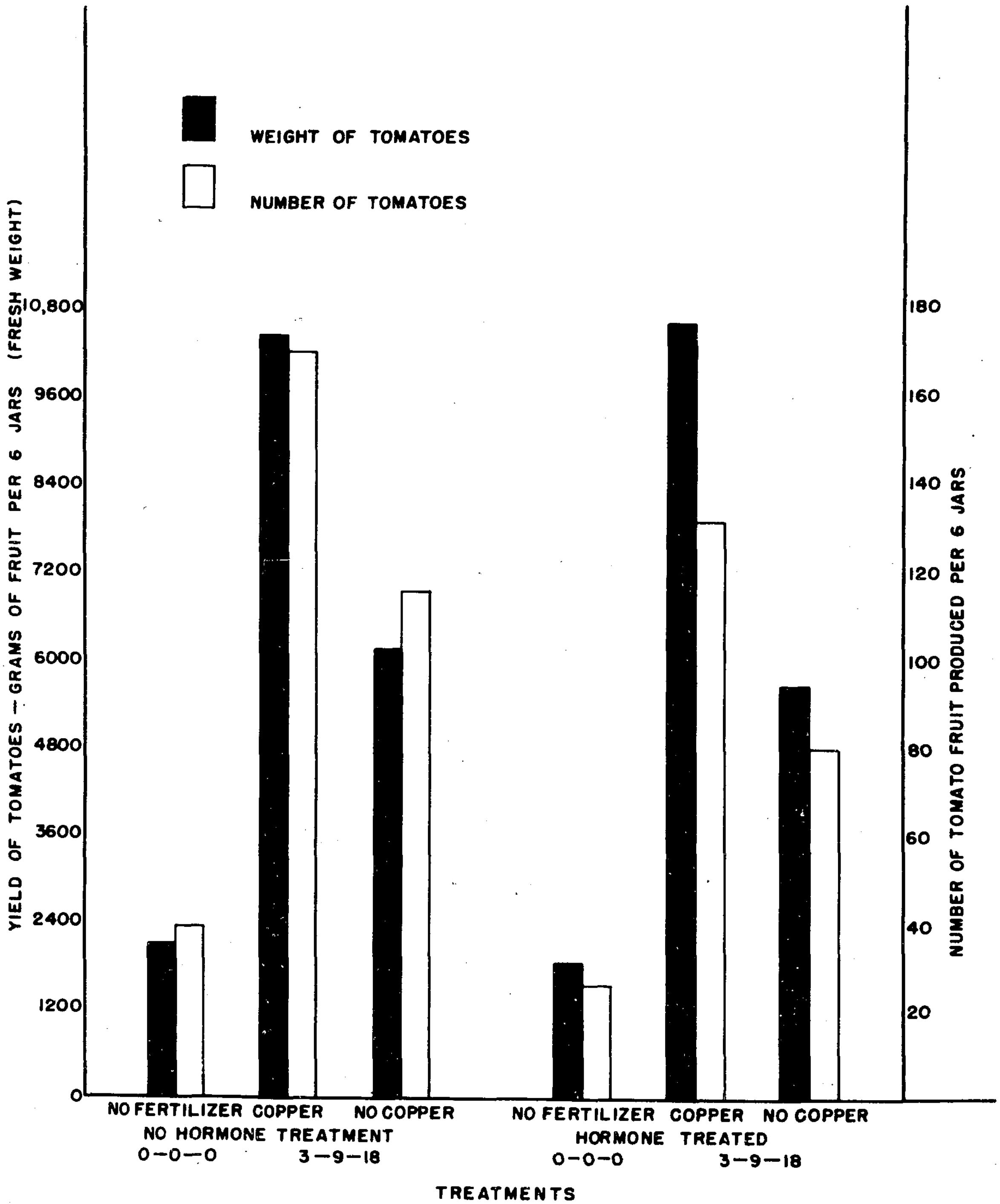

Fig. 42 NUMBER AND WEIGHT OF TOMATOES PRODUCED AS VARIED BY ADDITION OF COPPER AND HORMONE SPRAY TO PLANTS IN JARS OF MUCK SOIL

TABLE 15

Effect of copper, with and without hormone, on foliage growth and composition of tomatoes grown on muck soil

Treatment	Tomato fruit yield gms.	Tomato fruit number	Foliage gms. dry wt.	Ash	Cu	Fe	N	K	P	Ca	Mg
				Per cent composition in tomato foliage							
Hormone used											
1 No Cu*	1,863	26	28	9.87	.0016	.020	1.92	.18	.30	2.68	1.51
2 Cu	10,560	131	88	11.42	.0021	.025	1.17	1.64	.53	2.25	.51
3 No Cu	5,677	80-17R*	68	13.34	.0015	.027	1.97	2.38	.75	2.47	.85
Hormone not used											
1 No Cu*	2,099	39-2R*	36	9.30	.0014	.015	2.15	.15	.29	3.09	1.35
2 Cu	10,455	170	94	11.45	.0013	.023	1.56	2.05	.62	2.26	.55
3 No Cu	6,122	116-16R*	74	12.22	.0014	.021	1.96	1.96	.76	2.77	.77

* No fertilizer used
** Number accompanying "R" indicates number of tomatoes with necrotic spots

Fig. 43 Tomato plant showing copper deficiency in leaves. These spots appeared white or translucent.

EXPERIMENT 10

As pointed out previously in the Review of Literature (reference 6), oxidative enzymes that contain copper may be divided into two groups according to their substrates. Tyrosinase and oxygenase are two of these enzymes.

Steele (44) reports the distribution of tyrosinase in the higher plants as similar to, but not identical with, that of oxygenase. Apple fruit, beet and mangold roots and potato tubers contain both tyrosinase and oxygenase. A difference may exist in this respect between various organs of the same plant, e.g. the leaves of the lac tree contains oxygenase and tyrosinase, but the latex, which contains laccase (31) (a copper enzyme) has no effect on tyrosine.

Steele (44) further relates that in many cases, e.g. potato and mangold, the darkening of cut tissues is due to the oxidation of the hydroxyl-amino-acid tyrosine to a black compound called melanin. An intermediate stage can be distinguished when a red compound is produced temporarily upon exposure to air. Figge (19) claims that melanin formation due to tyrosinase can be regulated by the oxidation-reduction potential of the medium. An enzyme has been isolated which catalyses the formation of the red pigment; the subsequent changes to melanin are independent of enzymes.

Kubowitz (34) has shown that potato oxidase (tyrosinase) contains copper and that the activity of the oxidase toward catechol as the substrate is proportional to the metal content.

Dalton and Nelson (13) show tyrosinase to be a copper protein.

Experiment 10 was set up to see if there was any difference in the activity of the enzyme system of the sugar beet with and without copper applied to the soil. No determination was made of the individual enzymes acting. It was planned to observe any difference in rate of coloration of the cut beet root.

This experiment consisted of three treatments:
(1) No fertilizer used, (2) complete fertilizer plus a 200 lb. per acre application of copper sulfate to the soil and (3) a complete fertilizer but no copper added.

Where copper had been applied, the beet foliage grew faster and maintained a darker green in the early stages of growth.

This difference in size between the plants of the copper and no-copper treatments disappeared as the plants became larger, but the darker green color of the beet leaves on the copper-treated soil was maintained.

These beets were harvested after growing seven months. The yields are shown in Figure 44. Figure 45 shows the relative size of the beets as produced by these three treatments.

After harvesting the beet roots, those from each treatment were separately cut into squares. A red tinge was noted on the beets receiving copper almost immediately after cutting. The beets without additional copper did not develop this red tinge. Figures 46 and 47 show the contrasting differences between the beets receiving these two treatments. Pictures were taken at 2, 5, 6, 9 and 12 minute intervals and differences were very marked in all cases. The red color produced gradually turned black. The differences noted may be attributed either to the lack of an enzyme or to the lack of its substrate in the absence of copper.

The yield of beets and the percentage composition of some of the nutrient elements in the sugar beet leaves are presented in Table 16.

The percentage copper of the beet roots from two fertilized treatments was as follows:

	Per cent	Total gms.
(1) No copper applied to soil	.0011	.0023
(2) Copper applied to soil	.0013	.0039

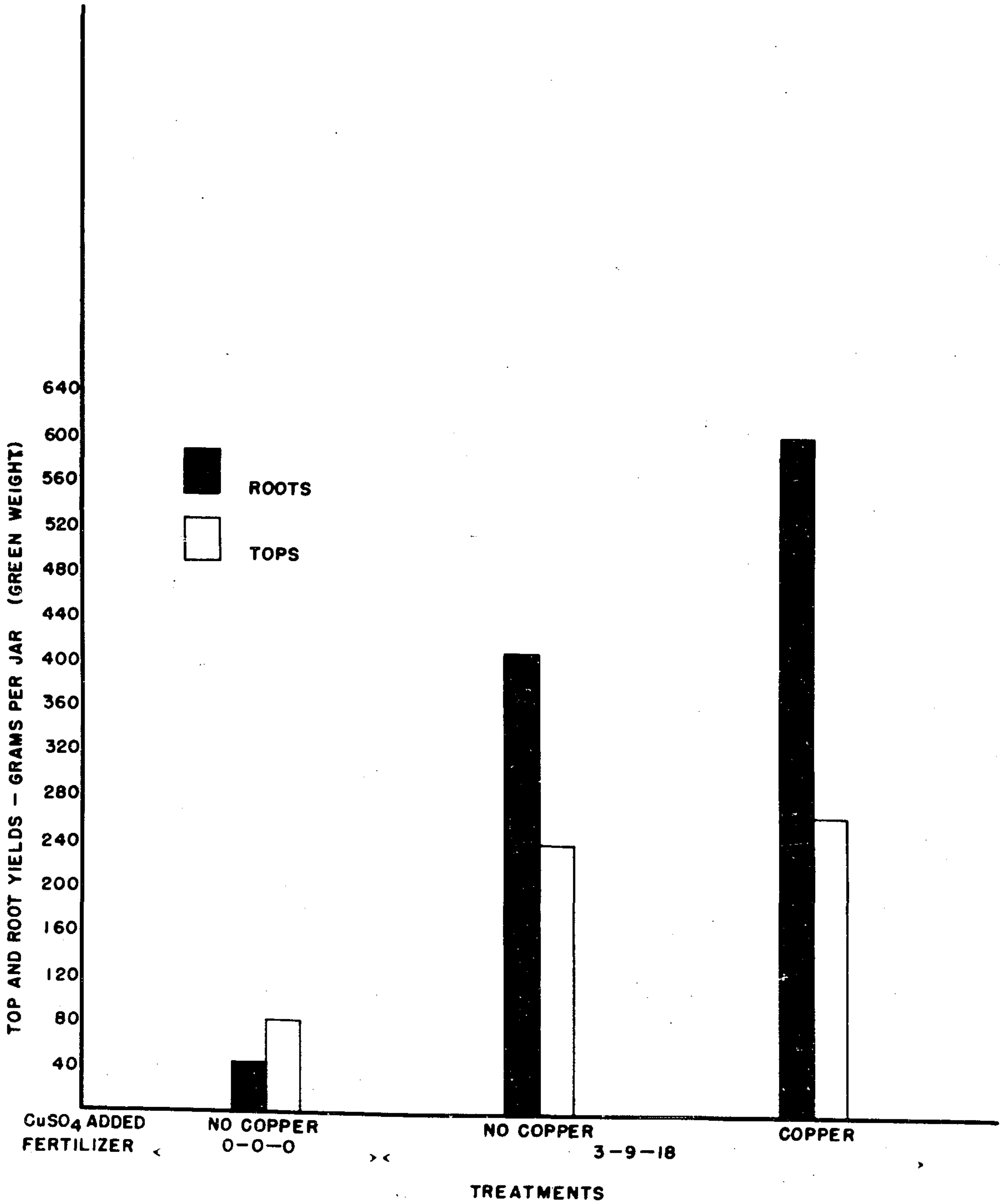

Fig. 44 RESPONSE OF SUGAR BEETS TO COPPER WHEN GROWN ON MUCK SOIL

Fig. 45 Sugar beets grown on fertilized and non-fertilized muck soil with and without copper. Treatments were:
Left - Fertilizer plus Copper
Center - Fertilizer - No copper
Right - No fertilizer - No copper

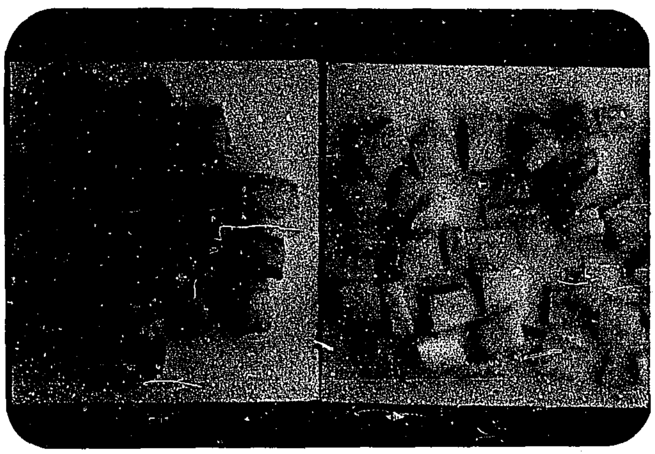

Fig. 46 Sugar beet roots grown with and without copper showing red coloration (left) where copper was applied. The beets on right did not receive copper.

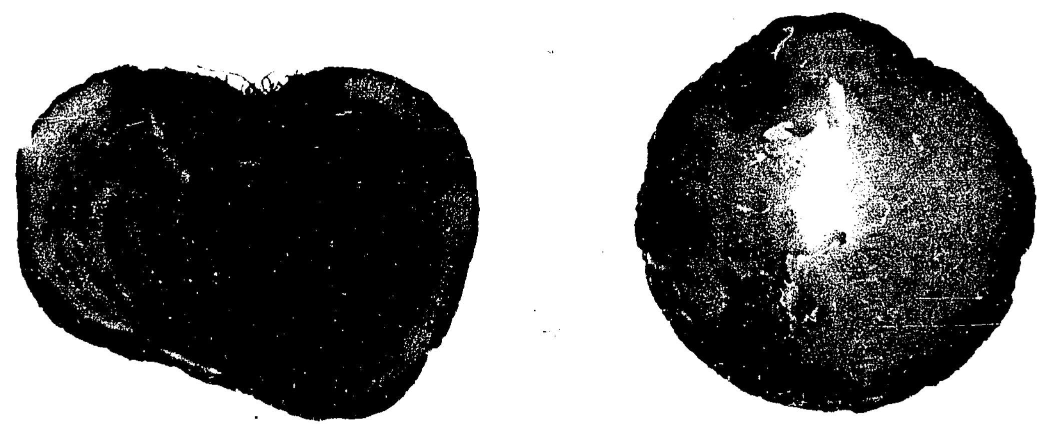

Fig. 47 Cross section of sugar beets from copper and no copper treatments. Left - copper applied to soil. Note dark rings which have developed around beet. Right - copper not added to the soil. Note absence of dark rings.

The black rings noted in Figure 47 (copper applied) approximates each of the secondary vascular systems of the beet root. This is a region of growth and enlargement of cells. This black coloration possibly indicates the presence of an enzyme and its substrate in this region of metabolic activity. These black rings were not produced in the absence of copper.

This may also reflect a varied range of the oxidation-reduction potential as observed in experiments by Figge (19). He found that any system, which would tend to shift the redox-

TABLE 16

The response of sugar beets to copper as applied to a muck soil, and the effect of copper on the chemical composition of sugar beet leaves

Treatment	Yield of beets roots gms. green wt.	Yield of beets leaves gms. green wt.	Per cent composition of beet leaves Ash	Cu	Fe	N	K	P	Ca	Mg
1 No Cu*	22	81	13.30	.0026	.027	3.21	0.21	.27	2.49	3.74
2 200 Cu	302	267	15.96	.0027	.027	1.92	3.35	1.17	1.23	2.31
3 No Cu	205	240	21.78	.0024	.023	2.66	4.10	1.67	2.12	2.56

* No fertilizer applied

potential of a chromogenic granule in a cell or a reaction mixture away from its optimum in either direction, would inhibit the action of the tyrosinase enzyme, while a shift toward the optimum would accelerate it.

GENERAL DISCUSSION

Allison (1) did work to determine if the effect of copper was in the plant itself or in the soil environment of the plant. He found practically normal development followed the working of copper directly into most any part of the plant system without contact of the treatment with the soil itself. Rather marked response was obtained by him, in this way, by using metallic copper placed directly into the seed piece of sugarcane.

King (32) working on soils of varying organic matter content studied the fixation of copper in these soils over a period of one to twelve years. He found the organic matter content of these soils had more influence on the concentrations of soluble copper than did the total quantity applied. Those soils having a higher percentage of organic matter contained a much smaller amount of soluble copper, even though receiving approximately equal amounts of copper.

The growth of cowpeas was retarded in an acid sassafras loam (pH 5.4) when 200 lbs. or more of copper sulfate was used by Daines (12). In peat soil (pH 6.0) he applied copper sulfate at the rate of 6,400 lbs. per acre which did not retard growth.

Jamison (30) states that an ordinary application of copper to soil would doubtless be toxic were it not for the fixation of the copper.

Results obtained in the writer's investigation with corn indicate that the amount of copper in a corn kernel, grown from a soil with sufficient available copper, is at a minimum, but sufficient to aid in the production of a normal plant.

Other investigations on the manner of applying copper to the soil have indicated the importance of the availability of copper to the plant. The copper was less available to the plants when layered "half-way" down in the jar of soil and copper deficiency symptoms developed in wheat until the roots had reached the copper. Copper appears to be needed in the plant for proper metabolic functioning of the plant processes even in the early stages of growth.

Sterilization of the muck soil caused increased growth in the absence of copper, but the wheat plants did show copper deficiency at the heading stage.

The investigations of the varied copper applications to the soil indicate that any beneficial effect on plant growth by copper is not due to the direct effect on the soil itself.

Bailey and Mc Hargue (3), working with tomatoes, found plants or portions of plants showing severe copper starvation contained relatively large amounts of copper and that this copper was evidently in an immobile state.

Willis and Piland (46), working with corn, compared the influence of copper on the intake of iron and considered the accumulation of this element in the nodes as identical with that brought about due to the lack of potassium. Corn plants analyzed by them for iron showed consistantly higher percentage contents of iron, where copper was not applied, as compared to plants produced on a soil which had received copper. These results are in agreement with those of the present investigation.

Iron and copper have been found concentrated in the chloroplasts of leaves by Neish (37). Copper showed an even greater tendency to collect in the chloroplasts than did iron. He points out that iron and copper show the same localization, tending to support the view that some oxidative reactions are centered in the chloroplasts which require their catalytic action. Determinations he made of the inorganic iron and copper showed that, although most of the iron in the chloroplast is present in the inorganic form, there is an appreciable amount which may be in organic combination. Copper is present in the chloroplasts largely in organic combination.

Willis and Piland (47) conducted an experiment in which the roots of iron-deficient cotton plants were divided between solution cultures containing ferric citrate and copper sulfate, alone and in combination, giving evidence that copper sulfate will produce an iron-deficiency chlorosis. It appeared that these effects were due largely to reactions external to the plant, but there was also evidence of the immobilization of iron within the plant under the influence of copper.

Wood and Womersley (48), working with oats, report copper as being immobile in the leaves and present after death in the tissue of the plant. The copper-deficiency symptoms noted in the oats were accounted for by insufficient uptake of copper during spikelet formation, coupled with the immobility of copper in the leaves. The deficiency symptoms reported were in accordance with those observed in this investigation (Figure 23).

Working on the iron and chlorophyll content in the leaves, Jacobson (29) found a proportionality existed between total iron and chlorophyll content. He found that, before chlorophyll can develop, the total iron content of the leaf must exceed a certain minimum level; this is determined by the species and the growth conditions.

In the writer's investigation less chlorophyll was found in the copper-deficient wheat plants than those plants which had received copper. These results are in agreement with Dickey, Drosdoff and Hamilton (14), working with the tung tree, who found the young copper-deficient leaves a lighter green color than were the normal leaves. As the leaves developed, chlorosis was evident over the entire surface of the leaves. Upon analyzing the mid-shoot leaves from untreated one year old copper-deficient trees, they found that these leaves contained 3 p.p.m. of copper. The leaves from the same age trees of the same area, which had recovered from copper deficiency after a soil treatment, contained about 4 p.p.m. of copper. Apparently only minute amounts of copper are necessary to effect recovery from copper deficiency.

Percentage nitrogen was found to be high in the copper-deficient plants of the present investigation. Reducing sugars, on the contrary, were low throughout the life cycle of the wheat plants studied. Where copper was applied to the soil, the reducing sugar content in the plants at the time of wheat heading advanced approximately in the same order of magnitude as that of nitrogen and the chlorophyll content was much higher than that in copper-deficient plants.

Lucas (35) states that the marked response of plants to copper is possibly due to an unbalance of nutrient elements within the plant.

Throughout the present study the difference in nutrient balance is to be noted between the copper-treated and untreated plants. Tomato fruit produced on copper-deficient plants showed necrotic areas on the fruit. These necrotic areas were not produced where copper had been applied to the soil. This necrosis of the tissue, in spots, in addition to fewer number and weight of tomatoes produced on the copper-deficient plants, is believed due to an unbalance of the nutrient elements in the plant and lack of sufficient carbohydrate. The percentage nitrogen and potassium are particularly high in such plants.

Work with copper treated and non-treated sugar beets showed a difference in coloration of the cut beet root upon exposure to air. The development of a red color in the copper-treated beet root and no color where copper was <u>not</u> applied possibly indicates the lack of the enzyme or its substrate in the absence of copper.

Bailey and Mc Hargue (4), working on the enzyme activity of tomato and alfalfa plants, suggest that the amount of polyphenol oxidase present in the plant is a function of the available copper.

SUMMARY

The effect of copper on the growth and composition of wheat, sudan grass, corn, tomatoes, sugar beets, oats and celery (grown on muck soil) was investigated and is summarized as follows:

1. In the acid muck soil studied, copper was not sufficiently available to the plants for normal plant growth.

2. The absorption of copper by the plants investigated effected the uptake of iron, nitrogen, potassium, phosphorus, calcium, magnesium and silica. A relationship between the accumulation of copper and iron in the plants was observed.

3. Copper deficiency in wheat was first evidenced by a retardation in growth and a light green color of the foliage. This was followed by a curling and dying-back of the leaf tips, with subsequent loss of chlorophyll and drying of the leaves. No heads were produced.

4. Corn seed harvested from plants grown on copper-deficient muck soil produced plants showing permanent copper deficiency symptoms. Seed obtained from plants where copper had been applied to the soil contained more copper. The amount of copper in the latter seeds was at a minimum, but apparently sufficient to aid in normal plant growth. The development of roots in the early stages of plant growth are believed to be favorably influenced by the copper contained in the seed.

5. Copper aids in chlorophyll formation. Only where copper was supplied to the soil did the reducing sugar and nitrogen

contents approach equal percentages in growing wheat. Without copper the nitrogen content of the plants remained high and the reducing sugar low.

6. The addition of copper to the soil resulted in a greater number and weight of fruit harvested from the tomato plants. Necrotic spots appeared on some of the tomato fruit where copper had not been included in the fertilizer. These necrotic areas were not found on the fruit harvested from plants where copper had been applied.

7. Sugar beet yields were higher where copper was applied to the soil and a red coloration appeared on the cut surfaces of the root. The red color did not appear when copper was not used.

A cross section of the beet showed the coloration to be concentrated in concentric rings. These rings corresponded to the secondary vascular tissue. The coloration is believed to be due to the presence of a copper enzyme and a suitable substrate.

8. Copper is believed necessary for the normal metabolic activity of the plant processes.

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