THE EFFECTS OF COPPER IN THE NUTRITION OF PLANTS WITH SPECIAL REFERENCE TO YIELD AND PLANT COMPOSITION

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By

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Thesis submitted to the Faculty of the Graduate School
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fulfillment of the requirements for the
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Joseph Rhodes Haun, Doctor of Philosophy, 1951

THE EFFECTS OF COPPER IN THE NUTRITION OF PLANTS WITH SPECIAL REFERENCE TO YIELD AND PLANT COMPOSITION

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INTRODUCTION

The significance of copper in plant nutrition may be recognized by its designation as an essential microelement or micronutrient for the growth of plants. These terms are appropriate for copper when distinguishing it from other nutrient elements such as nitrogen, phosphorus, or potassium which are required by plants in quantities of a much greater magnitude. The term micronutrient will be used in this discussion.

As an essential element for the growth of plants and animals, copper has received a great amount of attention. This is partially explained by the fact that it was one of the first of the micronutrients to be established as an essential for plant growth. In the past twenty-five years a great amount of work has been done in the field of copper nutrition of animals as well as that of plant nutrition. The accumulation of information on this subject is, in a large measure, due to improvements in techniques of biochemical analyses and in chemical purification procedures. Inasmuch as copper is present in sufficient amounts for plant growth in ordinary sources of unpurified water and chemical compounds it is necessary to adopt special methods for removing it or at least greatly lowering its concentration so that adequate control of any supplied copper may be obtained for experimentation.

Since the discovery of copper as an essential element for plants and animals, the cause of several important nutritional disorders has been explained. In this regard, exanthema of fruit trees (43), "reclamation disease" of cereals (35), and chlorosis of plants in certain areas are examples of nutritional diseases of plants which are the result of insufficient copper. Among animals, enzoötic ataxia (9), "stringy wool" (8) of sheep, and "falling disease" (10) of cattle are disorders which have been attributed to copper deficiency. Animals may obtain sufficient copper from their forage diet, but in areas where pasture plants are characteristically copper-deficient nutritional disorders are likely to develop. Animals of these areas may be subnormal not only from a deficiency of copper, but also because of a decreased supply of forage caused by copper-deficient soils.

Two problems are presented by these considerations; namely, detecting copper-deficient soils and determining an adequate method for supplementing copper so that it may be utilized by plants.

Copper deficient soils may be grouped into three general categories: the muck or peat soils which may contain relatively large amounts of copper but in which the copper is unavailable for plants; the extremely sandy soils which actually contain so little copper as to be insufficient for plant growth; and the last category includes soils which, although they may contain sufficient copper _per se_ for plants, show a beneficial response to additional copper apparently by virtue of making other elements more available. The copper requirement for the third group of soils is the most difficult to evaluate because the factors which regulate the effects of copper in the soil are incompletely understood.

With respect to its role in plants certain functions have been assigned to copper. It is now well established that copper is a constituent of certain enzymes (4, 6, 30) although the physiochemical relationship of copper to their molecules is not understood.

Copper may be considered as one of the two most important elements used as fungicides. It is extremely toxic to higher plants as well as to fungi in concentrations which, by comparison with those of macronutrients, are very minute. Beyond the classic explanation for the effect of heavy metals on protoplasm very little is known of the effects of copper on plants in concentrations ranging from the point of sufficiency to excess.

The investigations reported herein are concerned with several aspects of copper nutrition. A primary objective was the assessment of the available copper for plants on two soil types, combined with a study of the type of copper compound which would most readily fulfill the requirements of a variety of plant species. Certain measurements have been made of plant constituents with the aim of evaluating the direct or indirect influences of copper on biochemical processes. Particular emphasis was placed on an examination of the plant responses to copper in the range of concentration from sufficiency to excess.

REVIEW OF LITERATURE

The literature on copper nutrition up to 1948 has been extensively reviewed in the fourth edition of the Bibliography of the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition (14). The literature relating to the biological significance of copper in foods has been reviewed by Monier-Williams (31). More recently a series of papers and discussions from a symposium on copper metabolism held at the Johns Hopkins University (30) have comprehensively summarized the advances in the fields of soil science, animal and plant nutrition, and biochemistry.

It is now generally agreed that Bortels (11) in 1927 was the first to demonstrate that copper is an essential nutrient for the growth of fungi. Working with *Aspergillus niger* he found that the yield and color of spores were influenced by the quantity of copper present. In the absence of copper the growth of this organism was decreased and its spores light brown in color, but with the addition of copper the yield was increased and normal black pigmentation developed.

Conclusive evidence for the essentiality of copper in the nutrition of higher plants was initially presented in 1931 by Anna L. Sommer using sunflowers, tomatoes, and flax (45). Prior to and since this discovery copper has been recommended as a remedy for dieback (or exanthema attributed to copper deficiency) in many species of fruit trees (2, 12, 16, 17 and 19). The beneficial response of copper additions to muck and peat soils was revealed by the research of Felix (18) in New York, and Allison, Bryan, and Hunter (1) in Florida.

Russell and Manns (39, 40) obtained increases in yield on several truck and field crops in Delaware, Maryland, New Jersey, Virginia, North Carolina, South Carolina and Georgia by the use of ten to fifty pounds of copper sulfate per acre.

With respect to the unavailability of copper in organic soils Mulder (33) has made the following observations:

> Although the copper content of most soils is very low, deficiency symptoms of the plants as a result of an absolute lack of copper are found only sporadically. Presence of certain types of peaty substances will often reduce the availability of soil copper, as a result of which deficiency symptoms in the plants occur. In his earlier work the writer has shown (28) that such substances are able to fix relatively large amounts of copper in such a way that it was unavailable for Aspergillus niger. Furthermore it was demonstrated that hydrogen-sulphide producing bacteria are able to fix copper in an unavailable form. This was not simply copper sulphide, since copper supplied in this form was found to be readily available for plants and microorganisms like A. niger. . . . . The behavior of copper in organic soils is different from that of other cations. Lucas(31) observed that copper sulphate was precipitated, probably as the hydroxide, when the pH of the soil-water suspension was greater than 4.7. When a hydrogen-soil was treated with copper acetate, copper was adsorbed as the divalent cation Cu^{++} and as the monovalent cation complex $(CuCH_3COO)^+$. Hurwitz (32) found that addition of oat straw and alfalfa meal with a carbon-nitrogen ratio of 30:1 gave a rise in exchangeable copper of a sandy loam from 8.4 to 22.0 micrograms per 100 gm. of dry soil. This increase was not due to the presence of copper in the organic matter. When the amended soil was incubated at 29°, 37°, and 45° C. for 14 days, available copper had again reached the initial low level. At 2° no change was found, indicating that the organic compounds which were responsible for the increase of exchangeable copper were decomposed by microbiological activity.

The literature pertaining to copper deficiency symptoms for many plants has been summarized in Diagnostic Techniques for Soils and Crops (25). There is a marked similarity of the most obvious symptoms for most herbaceous plants, viz., stunting of growth, all leaves chlorotic or blue green in color, and certain derangements of terminal growth characterized by cupping, withering and death of terminal leaflets, and the failure to form seed.

The toxicity for plants of several copper compounds has been determined. Coupin (15) found that for wheat plants the following percentages in a water culture solution were lethal: copper bibromide, 0.0048; copper bichlorate, 0.005; copper sulfate, 0.0055; copper acetate, 0.0057; and copper nitrate, 0.006. He concluded that there is little difference in the toxicity of the different copper compounds since their effect is due to the copper ions. Isizuka (24), working with rice plants, found that this species could withstand concentrations of 100 p.p.m. Cu if only one-half of the roots were subjected to it while similar plants died when all of the roots were subjected to 50 p.p.m.

The copper content of healthy plants varies widely among species (7, 22) and within species grown on different soils (23). Beeson (7) and Goodall and Gregory (22), in their reviews on mineral composition of plants, have copiously cited research dealing with the copper content of many species of plants. The copper content of plant material is not necessarily an indication of the adequacy of copper supply. Bailey and McHargue (5), using the tomato, found that the copper content of copper-starved plants was slightly higher than that of plants receiving copper treatments, on the basis of unit dry weight; furthermore, the accumulations of copper in plants with various copper treatments were essentially the same.

Mulder (34), using Aspergillus niger as an indicator plant, has considered the following copper concentrations as criteria of probable plant response: In micrograms per gram, < 0.4, very deficient; 1 to 1.5, slightly deficient; and > 2.0, not deficient.

Most experiments on the effects of copper on plants growing in the greenhouse and in the field have utilized copper sulfate; however, some research has been done using other compounds. Manns (29) found that $CuCO_3$ and $Cu_3(PO_4)_2$ gave as good response to crops as $CuSO_4$. On marsh soils in Russia the nitrate, oxide, chloride, acetate, carbonate, and malachite forms of copper gave beneficial responses (47).

It has been found that the copper content (of substrates and within the plant) influences various chemical constituents of plants. As would be inferred from the chlorotic symptom associated with copper deficiency the chlorophyll content varies directly with the copper content of the plant (41). In accord with their concept of nutrient-element balance Shear, Crane, and Meyers (42) have cited evidence that aluminum may exert a competitive effect on copper and, when absence of aluminum is approximated, copper may become toxic. Riceman and Donald (38), growing rye on copper deficient soils in Australia, found that additions of potassium and copper reduced the amount of iron in the plant ash, and additions of copper increased the copper content and reduced the manganese in the ash. The research of Lipman and Gericke (27) indicated that copper antagonised sodium chloride, sodium sulphate and sodium carbonate in Berkeley adobe soil, the antagonism being evident with three successive barley crops. Arnon (3) found that the growth of barley plants supplied with ammonium nitrogen was favorably affected by the addition of copper (in contrast to a lack of response when the plants were supplied with nitrate nitrogen). Gilbert, et al. (20, 21), in comparing the composition of leaves and fruit of copper deficient tung trees with that of normal trees, found no difference in per cent of non-reducing sugars, a lower per cent of reducing sugars and starch in deficient leaves, and water insoluble nitrogen higher in deficient leaves. In making a similar comparison Lucas (28) reported that the addition of copper to deficient muck soils in the greenhouse increased the ascorbic acid content of barley, oats, and fall-grown spinach. Also the application of copper increased the carotene content of greenhouse-grown wheat, spinach, barley, carrots, and oats. The protein content of plants deficient in copper was found to be abnormally high, indicating that copper is not directly involved in the formation of proteins.

MATERIALS AND METHODS

Certain phases of the copper nutrition of plants were concentrated upon in the research reported herein. The first objective was the quantitative determination of copper in the soils of several locations in the state of Maryland. The second objective included a study of the type of copper compound from which copper would be most efficiently supplied to plants. The third objective was directed toward the accumulation of further information on the role of copper in certain species of plants, with the hope that generalizations might be possible.

Field Experiments:

The problem of determining the quantity and availability of copper for plants in certain areas of Maryland was studied by the use of several different techniques. The first to be described is that of measurement of copper in the soil.

Since the soils of Maryland are relatively low in native organic matter, the actual copper content may be expected to more closely parallel the amount which is available to plants. This is in contrast to the muck and peat soils of Florida, Michigan and New York in which the copper is rendered unavailable to plants by its association with the organic matter.

Two areas on the University of Maryland Experiment Station farms (College Park and Salisbury) were selected for field scale experimentation. The soils at these locations differed in certain respects. At College Park the soil was a comparatively infertile sandy loam (Sassafras) although, by comparison, it was much more productive than the soil at the Salisbury location. The plots at Salisbury were situated on a very sandy, well-drained soil (Evesboro) of low fertility which is characteristic of the "eastern shore" or peninsula soils. Soil samples were taken at uniform intervals and the copper content determined colorimetrically, after extraction with N NaCl (37) by the use of rubeanic acid (13) and also bioassayed by means of the Mulder _Aspergillus niger_ Method (34).

Observations of plants grown in the areas mentioned above with a variety of supplemental copper treatments constituted the second phase of the investigations and may be considered as another method for identifying the quantity of copper in the soil. In order to determine the response of crop plants in general to additions of copper, a wide variety of plants were selected for growth on these soils. In this regard the species were selected with as much diversity of type and growth habit as possible, i.e., root crops, foliage crops, monocotyledonous and dicotyledonous plants. The horticultural and agronomic crops used in these tests were, furthermore, selected in such a way as to represent those of importance in Maryland both from the standpoint of acreage and of cash value. The selection was limited to row crops in order to include a greater number of crops with a minimum of complexity in cultural procedures. The following species and varieties were used in 1949: Cucumber (_Cucumis sativus_ L. c. A & C), Beet (_Beta vulgaris_ L. c. Detroit Dark Red), Snap Bean (_Phaseolus vulgaris_ L. var. _humilis_ Alef. c Stringless Green Pod), Bush Lima Bean (_Phaseolus limensis_ Macfad. c. Henderson), Irish Potato (_Solanum tuberosum_ L. c. Katahdin), Sweet Potato (_Ipomoea Batatas_ L. Poir. c. Nancy Hall), Tomato (_Lycopersicum esculentum_ Mill. c. Rutgers), Tobacco (_Nicotiana Tabacum_ L. c. Maryland Broad Leaf), Sweet Corn (_Zea Mays_ L.

var. rugosa Bonaf. c. Golden Cross Bantam), and Field Corn (Zea Mays L. var. indentata Bailey c. Southern States #362).

With ten species being used it was necessary to limit the number of treatments to a minimum so as to have sufficient replication. Consequently a control and only two treatments were used in the 1949 season, namely, copper sulfate as a soil application and as a foliage application. This salt of copper was selected for a variety of reasons. First, it is one of the most soluble salts of copper and, therefore, would be expected to be readily available to plants. Second, copper sulfate as a fungicide has been the subject of many investigations not only in view of its primary effect as a fungicide but also because of a stimulatory effect on the plant itself which has been suspected. Third, copper sulfate has been the most commonly used salt for supplying this micronutrient to commercial fertilizer mixtures. However, in spite of these considerations, it will be shown later that this salt is probably not the ideal way of supplying copper to deficient soils.

In the 1949 tests all plots were treated with the remaining essential micronutrients (with the exception of iron) so that a deficiency of any one of them would not obscure the observation of a response to copper if this element were actually deficient.

Experiment at College Park, Maryland, 1949: The design was composed of nine blocks, each of which was thirty by fifty feet in area. These blocks were arranged in a 3 x 3 Latin square and each block contained one fifty-foot row of each of the ten crops mentioned earlier (Plate 1). The sequence of crops was the same within all blocks so that any shading

PLATE 1

View of five of the nine blocks in the test plots on the University of Maryland Experiment Station Farm at College Park, Maryland, August 1949. The crops from left to right, field corn, sweet corn, tobacco, tomatoes, sweet potatoes, Irish potatoes, lima beans, snap beans, beets, and cucumbers.

or border effect on any particular crop would, therefore, be a uniform effect throughout the experiment. On June 14, 1949 the crops were planted. Prior to planting, the soil received 5-10-5 fertilizer applied at the rate of 500 pounds per acre. At planting time additional 5-10-5 fertilizer was applied at the rate of 500 pounds per acre as a band six inches from the plants on both sides of each row, with the exception of sweet corn and field corn. To the band application of fertilizer zinc sulfate and borax, each at the rate of 10 pounds per acre, were added. Copper sulfate at the rate of 10 pounds per acre was added to the band application for three of the blocks in an arrangement consistent with the Latin square. Sand was used to distribute these micronutrients on the corn since no additional 5-10-5 fertilizer was added. Elemental sulfur in an amount equivalent to that applied as copper sulfate was added to the band applications on the blocks not receiving this compound. Manganese chloride (500 p.p.m.) was sprayed on plants of all blocks on July 23 at the rate of 4 gallons per block. On the same day copper sulfate was sprayed on the plants of three blocks at the same concentration and rate as that of manganese chloride. This amount of copper is equivalent to approximately 2 grams of elemental copper per block. The remaining three blocks received no copper and thus served as controls. Yield data were taken at appropriate intervals for the various crops and by means of analysis of variance the treatments were compared.

Experiment at Salisbury, Maryland, 1949: At this location the experiment consisted of the same features as those of the College Park location with the exception of planting dates, which were approximately one week earlier, and arrangement of blocks. Owing to the shape of the area it was impossible to use a Latin square, so the blocks were randomized in a manner which prevented any adjacent repetition of treatments. The data at this location were statistically analyzed on the basis of three replications.

The results of these first field experiments (1949) indicated that the application of copper sulfate was not beneficial to yield at either location. The foliage application at College Park was actually detrimental to yield. Inasmuch as the concentration of treatment was relatively low the possibility that available copper was not deficient was suggested. Therefore, the following field experiment in 1950 was limited to and its scope increased at the Salisbury location where copper insufficiency would be more likely.

An additional factor in the 1950 field experiment was the use of several different copper compounds. In order to facilitate this increase in treatments it was necessary to decrease the number of crops for investigation. This revision of design allowed the statistical evaluation of treatments on individual crops. The following species and varieties were used in 1950: Tomato (Lycopersicum esculentum Mill. c. Rutgers), and Tobacco (Nicotiana Tabacum L. c. Maryland Broad Leaf). In order to determine any possible effect of other micronutrients on the availability of copper the treatments consisting of the various copper compounds were split so that only one-half of each would receive a mixture of compounds supplying boron, manganese, zinc, magnesium and iron.

Experiment at Salisbury, Maryland, 1950: Figure 1 shows the modified Latin-square arrangement of treatments in this experiment. Each column consisted of four rows of tomatoes and four rows of tobacco (Plate 2), the rows being five feet apart and 150 feet long. An additional space of ten feet was left between the nine major blocks of the Latin square. Each area to which a single treatment (e.g., 1*) was applied, within any major block of the Latin square, contained four 10-foot rows or twelve plants of each species. Thus, each of the seven major treatments was applied to 72 plants of each species, half of which received all micronutrients except copper. The tomato plants were set in the field on May 17 and the tobacco plants on June 7. Fertilizer (5-10-5) was applied in the same manner and at the same rate as that of the 1949 experiments, the soil treatments with the various copper compounds being added to the band application. The concentration of the copper sulfate soil treatment was increased over the 1949 level tc a rate of 15 pounds per acre. The remaining copper compounds were supplied in amounts which contained weights of elemental copper equivalent to that contained in the copper sulfate treatment. The foliage application of copper sulfate was the same concentration (500 p.p.m.) and rate as in 1949; however, it was applied at three times (July 11, July 20 and August 1, 1950) instead of only once. Yield data on tomatoes were taken on six dates of picking,

FIGURE 1. ARRANGEMENT OF TREATMENTS IN THE SALISBURY, MARYLAND, FIELD EXPERIMENT, 1950.

	Column I	Column II	Column III
Row 1	1* 2 3* 4 5*	6 7* 1 2* 3	4* 5 6* 7 --
Row 2	4* 5 6* 7 --	1* 2 3* 4 5*	6 7* 1 2* 3
Row 3	6 7* 1 2* 3	4* 5 6* 7 --	1* 2 3* 4 5*

Composition of Treatments:

1 Check
2 $CuSO_4 \cdot 5 H_2O$
3 CuO
4 Cu_2O
5 CuS (Concentrates)
6 Cu (Flake)
7 Foliage Spray $CuSO_4 \cdot 5 H_2O$

Treatments 2 through 6 were soil applications.

*Indicates the following compounds, each at the rate of 5 lbs./A., were added to the regular fertilization.

Borax
$ZnSO_4 \cdot H_2O$
$MgSO_4 \cdot 7 H_2O$
$MnSO_4 \cdot H_2O$
$Fe_2(SO_4)_3 \cdot X H_2O$

whereas the data on tobacco were tabulated at harvest and at the time of stripping and grading of leaves. It should be noted that extreme care was taken to have perfectly symmetrical spacing of plants in all treatments and to have a complete stand of as uniform plants as possible. In order to accomplish the latter, certain plants were removed and replaced at an early date. In view of these precautions and with the aim of increasing statistical precision, yield data were taken on the individual rows of plants within each treatment (e.g., 1*). Thus 168 observations were tabulated for each crop for any particular growth measurement.

Greenhouse Experiments:

Copper Deficiency: In order to observe deficiency symptoms in several species of plants, a water culture experiment was instituted. The method of Stout and Arnon (46) was used, with certain minor variations (Plate 3). Two-liter pyrex beakers, used as culture containers, were painted on the outside, first with black paint and then with white paint. Covers with holes to accommodate plants and aerators were cast from plaster of Paris and soaked in paraffin. Immersion pyrex fritted filter tubes of fine porosity (30 mm. diam. disc.) were used for aeration of the cultures. Air pressure at 8-10 pounds per square inch was provided by an air compressor. Five 150-watt incandescent light bulbs approximately eighteen inches above the tops of the cultures provided additional illumination in the morning from 4:00 a.m. until 9:00 a.m. and in the evening from 4:00 p.m. until 8:00 p.m.

PLATE 2

Experiment at Salisbury, Maryland, 1950

The species used included Kale (Brassica oleracea L. var. acephala DC. c. Hardy Winter), Cauliflower (Brassica oleracea L. var. botrytis L. c. Giza 6-1) and Romaine Lettuce (Lactura sativa L. var. longifolia Lam.)

Observations and photographs were made at various intervals of growth and finally the plant material was analyzed for copper.

Copper Excess: Another experiment was performed in the greenhouse with plants growing in soil. It was designed to determine some of the effects of excessive amounts of copper on two species of higher plants.

One-year-old hydrangeas (Hydrangea macrophylla (Thunb.) DC.) growing in six-inch pots of sandy loam soil and tomatoes (Lycopersicum esculentum Mill. c. Rutgers), approximately 2½ feet tall, growing in a ground bed containing a similar mixture of soil. The composition of these soils with respect to copper was within the range for normal plant growth. Since excessive amounts of copper were applied with the aim of producing detrimental effects, the initial amount of copper in the soil was not a consideration.

Forty plants of each species were divided into ten groups of four plants each. Each of the five treatments was applied to two of the groups, or to a total of eight plants, of each species. The treatments were spaced in such a way as to have the maximal distance between replicate groups. The groups of tomatoes in the ground bed were divided by means of panes of greenhouse glass vertically sunk in the ground to a depth of ten inches, thereby, enclosing an area of approximately 12.8

PLATE 3

Water cultures used in the copper deficiency experiment.


19
20
21
22

square feet for the four plants within (Plate 4). Copper sulfate, the only compound used in this experiment, was applied to the soil in the following amounts per plant of each species: .165, .5, 1.5, 4.5 and 13.5 grams. The copper sulfate for each group of four plants was dissolved in a uniform volume of water and applied in this form.

After treatments were applied the soil in which the hydrangeas were growing contained, on a dry weight basis, approximately 30, 90, 270, 810 and 2430 p.p.m. of added elemental copper, respectively, in the above sequence of treatments. The hydrangeas received only one application, on March 4, 1951, whereas the tomatoes received two applications, on March 4 and on March 15. The copper concentration of the soil in which the tomatoes were growing could not be estimated with any degree of accuracy; however, if compared to field scale applications the rates in pounds per acre would have been 10, 30, 90, 270 and 810, respectively. Several weeks after the application of treatments, observations and photographs were taken and leaf samples were preserved for chemical analysis.

Chemical Determinations:

Unless otherwise stated samples representing the youngest fully mature leaves were dried immediately in a forced-draft oven at 65° C. All samples except those of the 1949 field experiment were ground with a mortar and pestle to prevent copper contamination.

For copper determinations three- to five-gram samples of dried leaf material (unless the size of the sample was limited) were ashed for twelve hours at 500° C. and the ash taken up with 10 ml. of

PLATE 4

Tomato plants used in the copper excess experiment.

glacial acetic acid. The ash solution was reduced to approximately 5 ml. by boiling, then filtered through a "Whatman 40" filter paper and brought to a volume of 50 ml. with doubly distilled water. This solution was then analyzed for copper by the rubeanic acid method (13).

For the analysis of potassium, calcium, and magnesium, samples of leaf material were ashed in the same manner as above. However, the ash was taken up with 10 ml. of 1:1 hydrochloric acid, evaporated to dryness, placed on a steam bath for two hours and the residue then taken up in an additional 5 ml. of 1:1 hydrochloric acid.

Potassium and calcium were determined by the use of a Beckman Spectrophotometer with a flame attachment.*

Magnesium was determined colorimetrically by the use of titan yellow (37).

Phosphorus was determined by an adaptation of standard colorimetric methods based upon the formation of molybdenum blue (26).

Statistical Treatment of Data:

The analyses of variance were made according to the method outlined by Snedecor (44).

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*Courtesy of Dr. L. E. Scott, Department of Horticulture, University of Maryland.

RESULTS

Field Experiments 1949:

The results of the rubeanic acid method and the Mulder *Aspergillus niger* method, for the quantitative determination of copper in the soil samples from College Park and Salisbury, were not in agreement. By the first method concentrations of 0.41 p.p.m. at College Park and 0.34 p.p.m. at Salisbury were indicated. The second method indicated that 1.4 and 0.4 p.p.m. copper were present in the College Park and Salisbury soils, respectively (Plate 5).

Concerning yield data in 1949 only nine observations were possible per location for each crop (for any particular growth measurement); therefore it was impossible to test for statistical significance, by analysis of variance, on each crop. However, data on each crop, representing a definite criteria of development, were selected and analyzed simultaneously. The data which were used in this analysis (Appendix tables 1, 2, 3, 4, 5, 6, and 7) are shown graphically in figures 2 and 3.

The growth of Irish potatoes and the two types of corn was extremely irregular within blocks and, therefore, the data on these crops was not included in the statistical analyses.

Table I shows the simultaneous analysis of variance of the raw data from both locations. As would be expected in an analysis of several crops with great differences in magnitude of yield, the variance due to crops was highly significant. The high significance of locations may be largely attributed to the variation in crop response to the different soils.

0.0 0.4 0.6 1.2 1.6 2.0

Standard Cultures (p.p.m. Cu^{++})

Salisbury, Md.
(0.4 p.p.m.)

College Park, Md.
(1.4 p.p.m.)

PLATE 5

Mulder <u>Aspergillus niger</u> determination of copper in soil samples from Salisbury and College Park, Maryland.

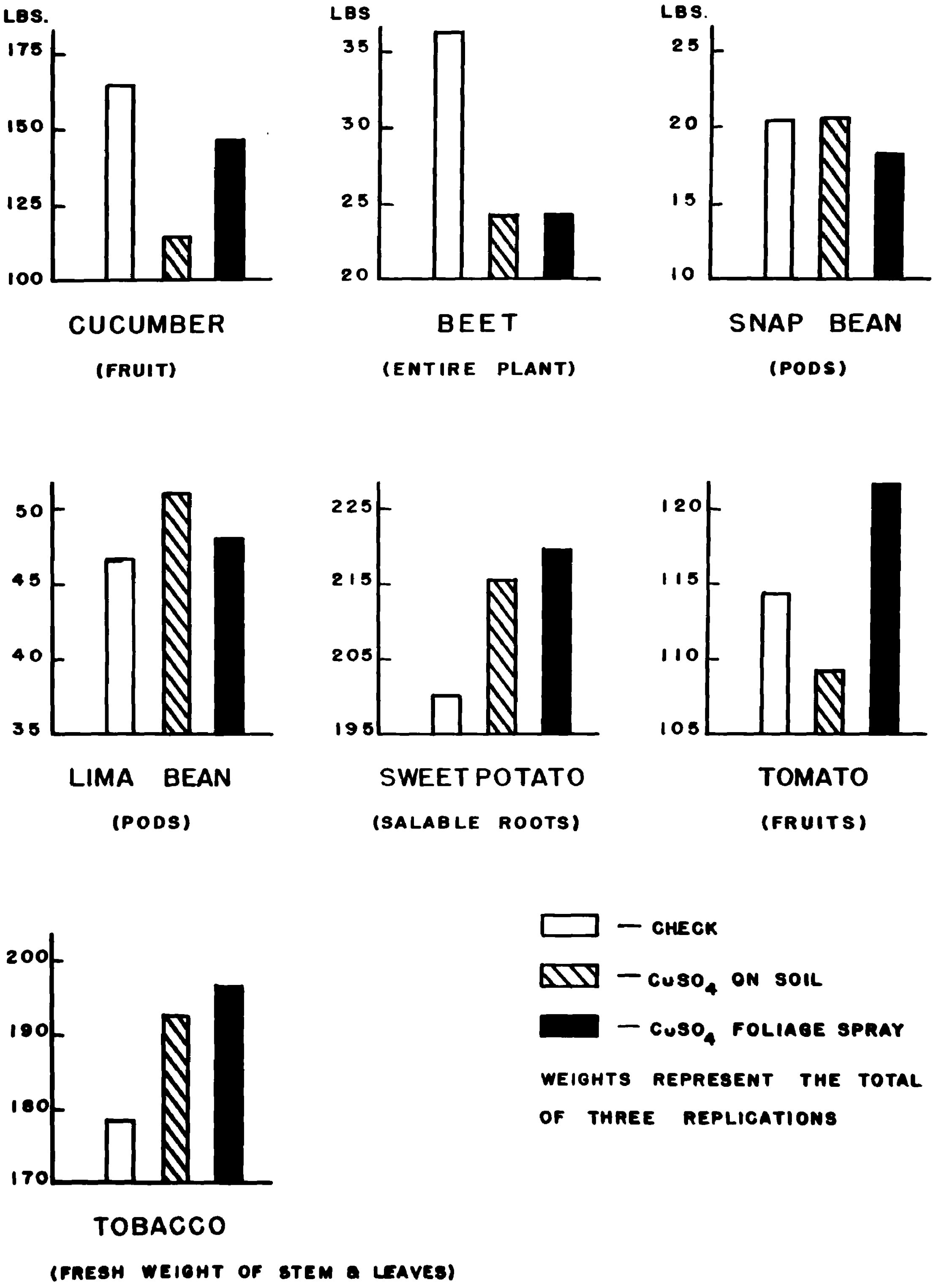

FIGURE 2. A COMPARISON OF CROP YIELDS
1949 FIELD EXPERIMENT
SALISBURY, MARYLAND

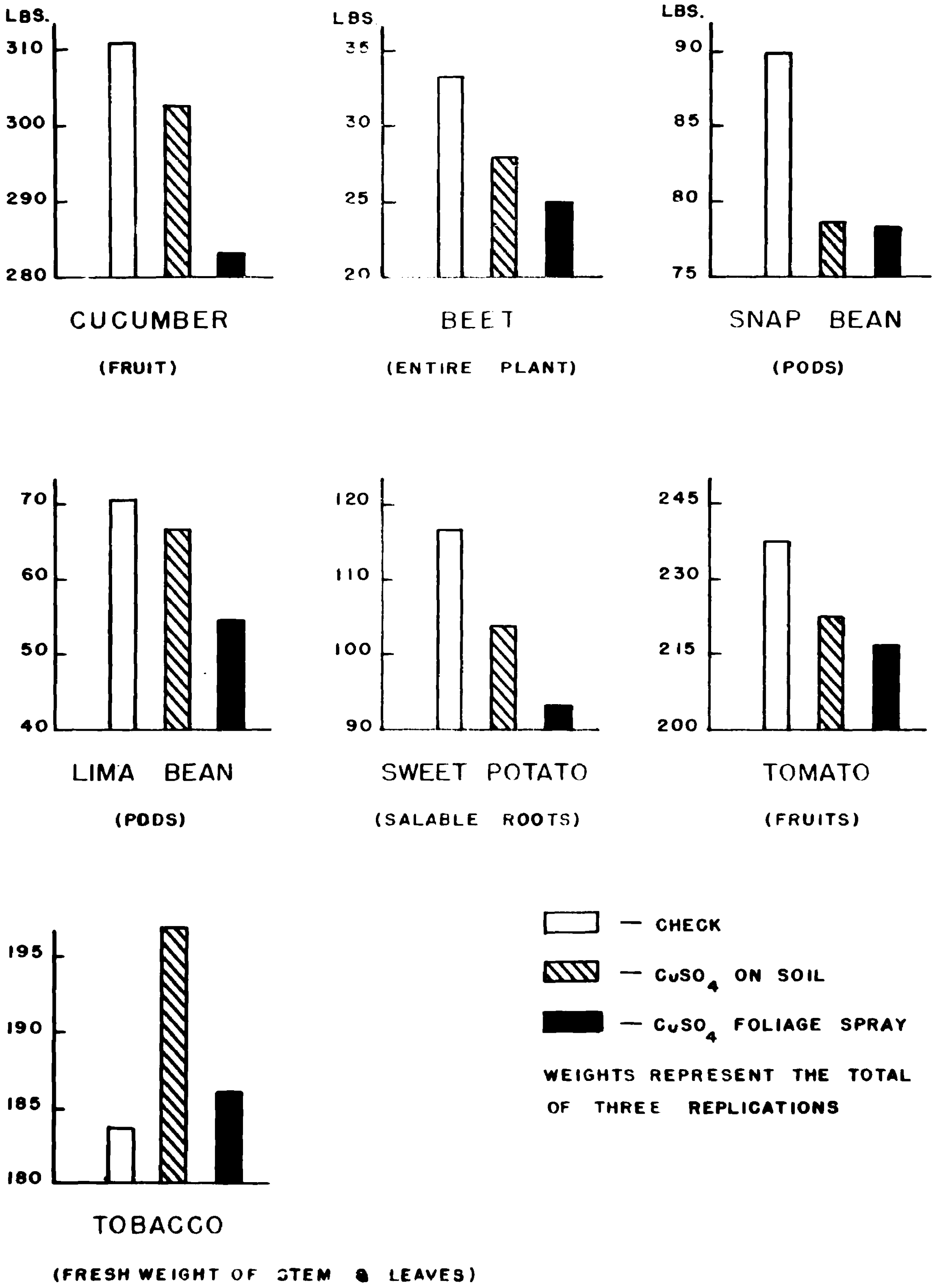

FIGURE 3. A COMPARISON OF CROP YIELDS
1949 FIELD EXPERIMENT
COLLEGE PARK, MARYLAND

TABLE I

ANALYSIS OF VARIANCE OF RAW DATA FROM THE COLLEGE PARK AND SALISBURY FIELD EXPERIMENTS — 1949

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Total	125	106020.84		
C	6	71557.02	11926.17	168.16**
T	2	91.26	45.63	0.64
R'	5	4499.19	899.38	12.68**
(L)	(1)	(4193.52)	4193.52	59.13**
(R)	(4)	(305.67)	76.41	1.07
C x T	12	429.74	35.81	0.50
C x R'	30	23657.28	788.57	11.11**
(C x L)	(6)	(21793.26)	3632.21	51.21**
(C x R)	(24)	(1864.02)	77.66	1.09
T x R'	10	1531.01	153.10	2.15*
(T x L)	(2)	(221.24)	110.62	1.55
(T x R)	(8)	(1309.77)	163.72	2.30*
C x T x R'	60	4255.34	70.92	
(C x T x L)	(12)	(405.84)	33.82	
(C x T x R)	(48)	(3849.50)	80.19	

C -- Crops
T -- Treatments
R & R' -- Replications
R' -- Indicates that the two sources immediately below have contributed to its variation.
L -- Locations
*Significant at 5 per cent.
**Significant at 1 per cent.

A comparison of the treatments on various crops at Salisbury reveals no systematic trends (fig. 2). However, the same comparison at College Park (fig. 3) indicates that in six of the seven crops there was a downward trend in yield in the following order: check $>$ $CuSO_4$-on-soil $>$ $CuSO_4$-foliage spray. Thus, further statistical analyses of the data from individual locations were made. An analysis of variance of the raw data from College Park (table II) failed to show significance in this trend. The high significance in crops suggested that crop variability was obscuring any possible significance of treatments. It is apparent that the analysis of raw data does not make allowance for the wide differences in yield and in spread of yield among the various crops. Likewise the same least significant difference cannot be applied to all crops. Consequently, two methods of coding were used (tables III and IV) and although one was slightly more striking in effect, both methods removed the variation due to crops. The analysis by the second method is probably more affective in reducing all crop yields to comparable terms for analysis. Inasmuch as we are not interested in the comparison of absolute yields, coding methods may be justified.

The interactions were combined with the original error term since no significance was shown when they were taken out.

The copper sulfate foliage application was the only treatment that produced a significantly different yield (lower) from that of the control. Only general conclusions may be drawn regarding the effects of treatments in this experiment since significant differences due to treatment may only be found in the treatment totals. The total yields for the copper sulfate foliage spray indicate a

TABLE II

ANALYSIS OF VARIANCE OF RAW DATA FROM THE COLLEGE PARK FIELD EXPERIMENT -- 1949

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Total	62	62208.60		
Treatments	2	262.61	131.30	2.08
Crops	6	57657.58	9609.59	152.55**
Rows	2	297.38	148.69	2.36
Columns	2	841.08	420.54	6.67**
Error	50	3149.95	62.99	

Treatment Means for Crops

	CuSO$_4$ with fertilizer	CuSO$_4$ as foliage spray	Control	L. S. D.	
				5%	1%
Cucumber	100.9	94.4	103.5		
Beet	9.3	8.3	11.0		
Snap Bean	26.2	26.2	30.3		
Lima Bean	22.4	18.2	23.3		
Sweet Potato	34.7	31.1	38.9		
Tomato	74.2	72.3	79.2		
Tobacco	65.9	62.2	61.2		
Treatment Totals					
	1001.4	938.6	1042.9	103.29	137.75

TABLE III

ANALYSIS OF VARIANCE OF RAW DATA CODED AS PER CENT OF MEAN FOR EACH CROP IN THE COLLEGE PARK FIELD EXPERIMENT -- 1949

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Total	62	24266.46		
Treatments	2	2428.62	1214.31	3.36*
Crops	6	00.00	00.00	0.00
Rows	2	2305.05	1152.52	3.19*
Columns	2	1492.10	746.05	2.06
Error	50	18040.69	360.81	

Treatment Means for Crops

	CuSO$_4$ with fertilizer	CuSO$_4$ as foliage spray	Control	L. S. D. 5%	L. S. D. 1%
Cucumber	101.2	94.7	103.8	32.32	43.11
Beet	97.4	87.3	115.4		
Snap Bean	95.0	94.8	109.9		
Lima Bean	105.0	85.3	109.5		
Sweet Potato	99.3	89.1	111.4		
Tomato	98.6	96.0	105.1		
Tobacco	104.3	98.5	96.9		

Treatment Totals

	CuSO$_4$ with fertilizer	CuSO$_4$ as foliage spray	Control	L. S. D. 5%	L. S. D. 1%
	2103.3	1937.7	2257.0	247.18	329.66

TABLE IV

ANALYSIS OF VARIANCE OF RAW DATA CODED BY ADDING 10 TO EACH DATUM AND EXPRESSING IT AS PER CENT OF MEAN[1] FOR EACH CROP IN THE COLLEGE PARK FIELD EXPERIMENT -- 1949

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Total	62	11632.39		
Treatments	2	1165.33	582.66	3.59*
Crops	6	00.00	00.00	2.29
Rows	2	1087.53	543.76	3.35*
Columns	2	1280.61	640.30	3.95*
Error	50	8098.92	161.97	

Treatment Means for Crops

	CuSO$_4$ with fertilizer	CuSO$_4$ as foliage spray	Control	L. S. D. 5%	L. S. D. 1%
Cucumber	101.2	95.2	103.5	20.86	27.82
Beet	98.7	93.7	107.5		
Snap Bean	96.4	96.2	107.3		
Lima Bean	103.4	90.0	106.5		
Sweet Potato	99.5	91.5	108.9		
Tomato	98.8	96.5	104.6		
Tobacco	103.8	98.7	97.3		

Treatment Totals

2105.8	1986.7	2207.7	165.59	220.85

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[1] New mean is equivalent to original mean plus 10.

detrimental effect of this treatment when compared to the yield of the control.

At Salisbury there were no statistically-significant differences as a result of treatment. However, it may be that the insufficiency of soil moisture, during most of the season, precluded a beneficial response to copper. The copper content of leaves (fig. 4 and Appendix table 8) was not influenced by the treatments.

Field Experiments 1950:

After the tobacco plants were cured (air-dry) the leaves were stripped and placed in three grades; those from the top in one grade, from the middle portion of the plant in a second grade, and those from the base in a third grade. The leaves from the middle portion of the plant were selected for best quality as indicated by color and size and the remaining leaves were automatically placed in their respective grades. The analysis of variance of these data (table V) therefore takes into account the great differences in weight among grades. (A much greater quantity of leaves went into the grade from the middle portion of the plant than into the other grades.)

The data were analysed on the basis of replications, each row of plants being considered a replication (Note — twelve rows of plants extended in the same direction as columns — see fig. 1), since a greater variation due to soil differences and to the proximity of rows of tomatoes to tobacco could be accounted for than when these differences were calculated on the basis of the Latin-square arrangement. One object for the analysis of data, with grades as a source of variation, was to evaluate the relation of copper treatments to quality, i.e., the interaction of treatment x grades. Since this

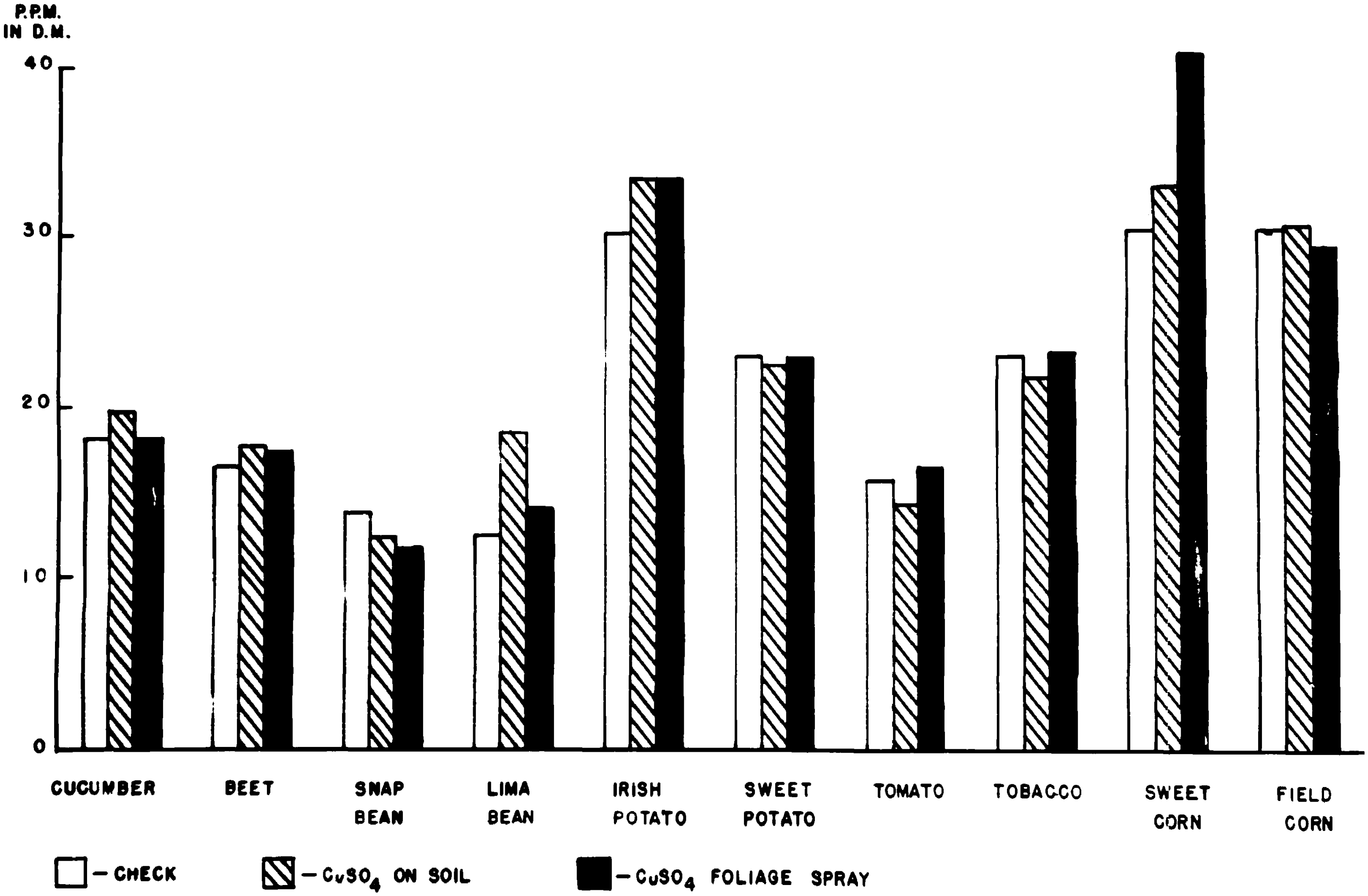

FIGURE 4. <u>COPPER CONTENT OF LEAVES</u>, 1949 FIELD EXPERIMENT, SALISBURY, MD.

TABLE V

ANALYSIS OF VARIANCE ON AIR-DRY WEIGHT OF TOBACCO LEAVES

SALISBURY, MARYLAND, 1950

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Treatments	6	7827	1304.5	.67
Trace Elements	1	122	122.0	.07
Grades	2	7308207	3654103.5	1884.82**
Replications	11	44440	4040.0	2.08*
Tr. x Trace El.	6	2124	354.0	.18
Tr. x Gr.	12	16593	1382.7	.71
Tr. x Reps.	66	102186	1548.3	.80
Trace El. x Gr.	2	4158	2079.0	1.07
Trace El. x Reps.	11	13906	1264.2	.65
Gr. x Reps.	22	164305	7468.4	3.85**
Tr. x Trace El. x Gr.	12	8743	728.6	.38
Tr. x Trace El. x Reps.	66	87889	1331.7	.69
Tr. x Gr. x Reps.	132	364668	2762.6	1.43*
Trace El. x Gr. x Reps.	22	69590	3163.2	1.63*
Error	132	255902	1938.7	
Total	503	8450660		

*Significant at 5 per cent.
**Significant at 1 per cent.

interaction was insignificant another analysis of variance was made on the summation of grades (table VI). In this manner the variance due to grades was completely removed and the precision for estimating other variables was increased. Both methods of analysis indicated a lack of significant differences due to treatments. The significance of replications may be attributed in part to soil variations and also to the border effect of one crop upon the other.

Tomato yields were recorded on six dates of picking and analyzed on the basis of twelve replications, as were the tobacco data. In the analysis of variance of these data (table VII) the second order interactions were included in the error term since none was significant when considered as a distinct source of variation. The significant differences in yield among treatments is graphically presented in figure 5 A. It is evident that the yields of cuprous and cupric oxide treated plants are significantly greater than those of the check, copper sulfate soil application, and copper sulfate foliage application.

The increasing yield of all treatments on successive dates accounts for the significance in this source of variation. Variations in yields among replications may be attributed to the same factors causing variation among replicates of treatments on tobacco.

The significant interaction of treatment x trace element is largely due to the lower yield when the additional trace elements were added to cuprous oxide, cupric oxide, and to the copper sulfate foliage treatments, whereas the trace element addition increased yields of the remaining treatments (fig. 5 B -- the same data were used in 5 A).

TABLE VI

ANALYSIS OF VARIANCE ON SUMMATION OF GRADE WEIGHTS OF AIR-DRY TOBACCO LEAVES

SALISBURY, MARYLAND 1950

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Treatments	6	23480	3913	.98
Trace Elements	1	366	366	.07
Replications	11	133320	12120	3.03**
Tr. x Trace El.	6	6373	1062	.27
Trace El. x Reps.	11	41717	3792	.95
Tr. x Reps.	66	306558	4644	1.16
Error	66	263667	3994	
Total	167	775481		

*Significant at 5 per cent.
**Significant at 1 per cent.

TABLE VII

ANALYSIS OF VARIANCE ON TOMATO YIELD

SALISBURY, MARYLAND, 1950

Source of variation	Degrees of freedom	Sum of squares	Variance	F
Treatments	6	13374	2229	2.25*
Trace Elements	1	3013	3013	3.05
Dates	5	2507362	501472	506.80**
Replications	11	55901	5081	5.14**
Tr. x Trace El.	6	19470	3245	3.28**
Tr. x Dates	30	20437	681	.69
Tr. x Reps.	66	103163	1563	1.58**
Trace El. x Dates	5	8303	1660	1.68
Trace El. x Reps.	11	18735	1703	1.72
Dates x Reps.	55	228801	4160	4.20**
Error	811	802469	989	
Total	1007	3781034		

*Significant at 5 per cent.
**Significant at 1 per cent.

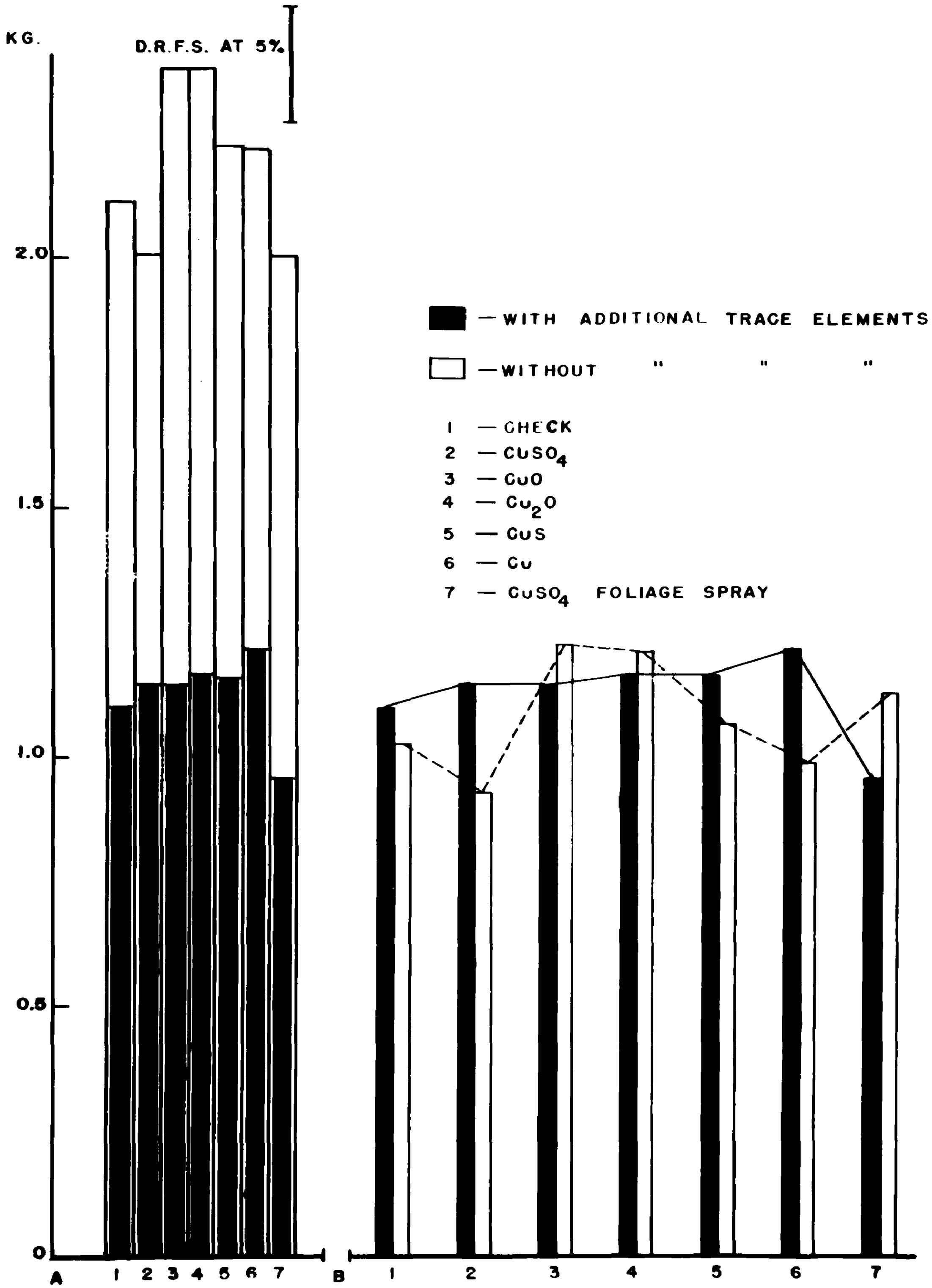

FIGURE 5. YIELD OF TOMATO, 1950 FIELD EXPERIMENT, SALISBURY, MARYLAND (VALUES REPRESENT MEANS OF 144 OBSERVATIONS FOR EACH TREATMENT)

Greenhouse Experiments:

Copper deficiency: Of the three species of plants grown in the water culture experiment, cauliflower and kale developed visual symptoms of copper deficiency. In both species the only variation in foliage color from that of the control plants was one of a decrease in the intensity of greenness (Plates 6 and 7). The cauliflower plants in the copper deficient culture showed a marked stunting of growth three weeks after germination while the kale plants showed no reduction in size.

Table VIII shows the variations in copper content of plants which were grown in the deficiency experiment.

Copper excess: Since the hydrangea plants were growing in a much smaller volume of soil than the tomatoes, relative to the size of copper sulfate applications, a much more drastic effect of the treatments was noted. Within 24 hours after the treatments were applied the leaves of the hydrangea plants which received 13.5 grams per pot showed a water soaked appearance and were reddish brown in color. Plate 8 illustrates the effect of the higher rates of copper treatment. (Note -- one-half of the leaves had been removed as an "initial sample" prior to treatment.)

When the leaves were killed on the plants of the two highest treatments (four days after treatment) they were removed for chemical analysis. Leaves of the remaining three treatments were not killed at this time and were sampled for analysis 15 days after the treatments were applied.

Injury appeared only on the foliage of the tomato plants which had received the highest level of treatment and on these plants only

Check Minus Copper

PLATE 6

Symptoms of copper deficiency on cauliflower.

Check Minus Copper

PLATE 7

Symptoms of copper deficiency on kale.

TABLE VIII

COPPER CONTENT OF PLANTS

COPPER DEFICIENCY EXPERIMENT -- 1951

Species	Age of plants when sampled (days)	Treatment	Number of plants in each composite sample*	Number of determinations averaged	Copper content in p.p.m. of D.M.
Cauliflower	80	Control	5	4	24.3
		Minus Copper	5	2	18.1
Romaine Lettuce	56	Control	15	4	30.4
		Minus Copper	15	2	26.7
Kale	45	Control	3	2	11.2
		Minus Copper	3	1	6.9
Kale	75	Control	2	2	10.5
		Minus Copper	2	1	1.0

*The stem and all leaves of each plant were included.

PLATE 8

Copper excess on hydrangea leaves. (Taken two days after 4.5 grams of copper sulfate had been applied to the plant.)

PLATE 9

Copper excess on tomato leaves. (Taken six days after the second application of 13.5 grams of copper sulfate to the plant.)

the older leaves were affected (Plate 9). Leaf samples were taken for analysis 26 days after the first application of copper sulfate.

The results of the chemical determinations on leaf samples from the excess experiment are shown in table IX and figure 6. Both species receiving the two lowest levels of treatment were the same with respect to the quantities of elements determined despite the fact that the second level of treatment was three times as large as the first. The differences in copper content as a function of treatment are shown graphically on a log-log scale. Inasmuch as this experiment was of relatively short duration, inorganic constituents would not be expected to show extreme quantitative variations. This lack of variation is evidenced by the data of table IX and only those elements showing systematic trends are shown graphically in figure 6, namely, copper, total nitrogen, and potassium.

TABLE IX

INORGANIC COMPOSITION OF LEAVES

COPPER EXCESS EXPERIMENT -- 1951.

	g. $CuSO_4$ added per plant	Cu p.p.m. in D. M.*	N % total in D. M.**	m.e./g. D. M.**			
				PO_4	K	Ca	Mg
Hydrangea	.165	12.3	1.88	.55	.74	.67	.26
	.5	11.0	1.89	.59	.73	.68	.24
	1.5	20.2	1.99	.57	.75	.61	.26
	4.5	285.1	2.36	.58	.77	.67	.27
	13.5	3142.6	2.37	.61	.90	.60	.26
Tomato	.33	16.8	4.37	.71	.55	2.52	.45
	1.0	16.8	4.44	.72	.62	2.32	.47
	3.0	22.6	4.45	.73	.72	2.07	.39
	9.0	34.4	4.56	.69	.72	2.20	.45
	27.0	141.8	4.43	.69	.69	2.24	.48

*Average of eight replicates.

**Average of duplicates taken from composite samples of eight replicates each.

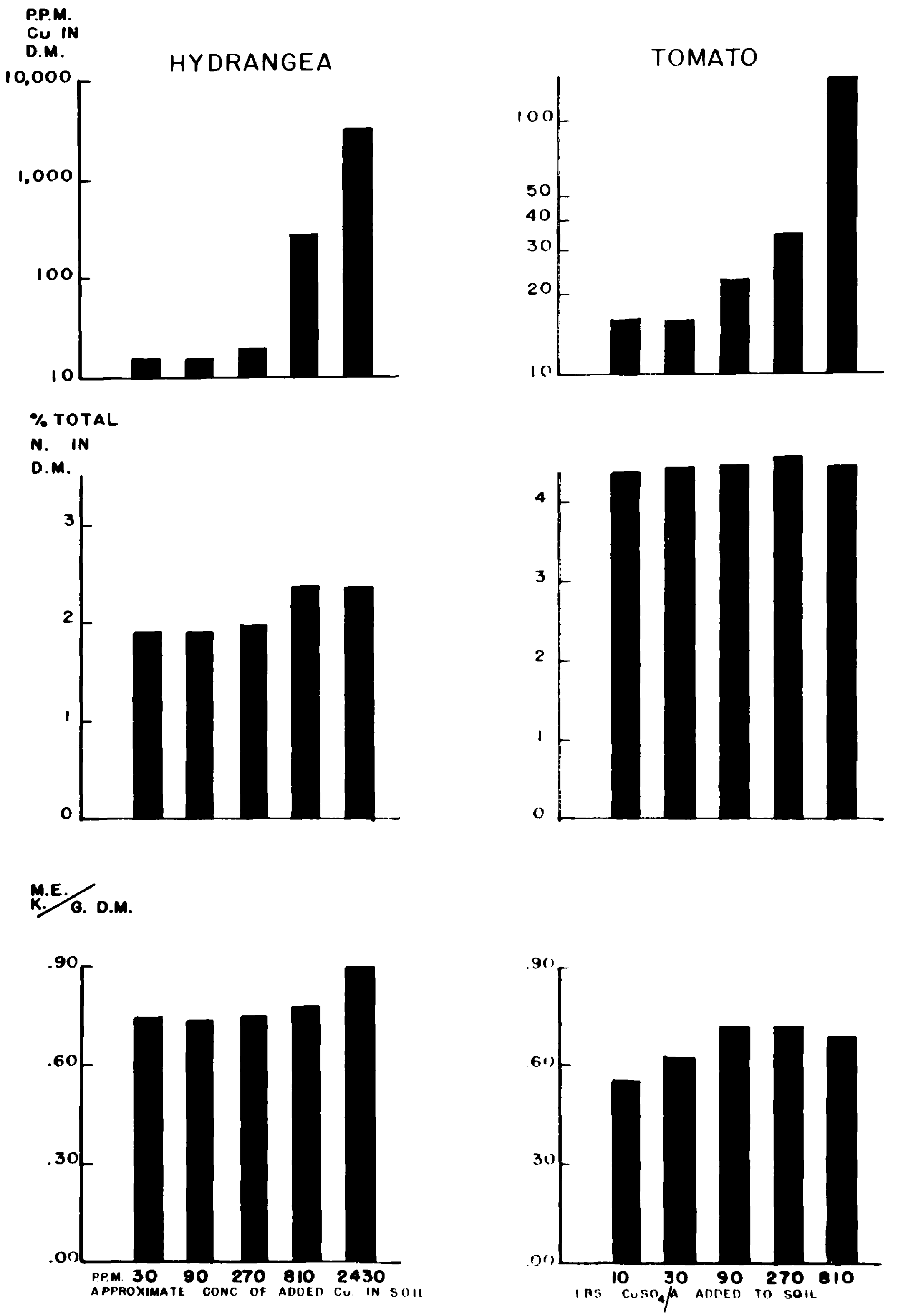

FIGURE 6. COPPER, TOTAL NITROGEN, & POTASSIUM CONTENT OF LEAVES, COPPER EXCESS EXPERIMENT

DISCUSSION

In this investigation a variety of methods have been used for determining the amount of copper available to plants. A comparison of the chemical and biological assay methods indicates the latter as being the more reliable.

There are several reasons for the undesirability of chemical methods. Different chemical methods than those which were used might be more sensitive or might be better adapted for determining the utilizable fraction of total copper present in a soil, but the final conclusion might be applicable only to a limited number of soil types and species of plants. A reliable chemical method for soil analysis should make allowance for a consideration of the organic matter present in the soil, inasmuch as this factor profoundly influences the availability of copper (33). Chemical analysis of plant material may be even less adequate than chemical soil analysis since the copper content of tissues may not vary on a unit dry weight basis with variations in copper supply (5).

The outstanding advantage of biological methods for the analysis of copper in soils is that they more accurately determine the portion of total copper which is available to plants. The Mulder _Aspergillus niger_ Method was found quite reliable in this respect. A final evaluation of results in this method is possibly less critical than in chemical methods; however, the error involved should be insignificant in experimental and practical commercial application. The growth of higher plants on a variety of copper treatments, particularly the species for which a soil is to be used, represents the ultimate criterion for the identification of copper deficient soils.

The results of these experiments suggest that the form of copper, i.e., compound, used for the correction of deficiencies has received too little attention in the past. Most experiments and commercial applications have utilized copper sulfate, and where soils were actually deficient a beneficial response to this compound has been obtained. With the insignificant effect of the single concentration of copper sulfate, when compared to the check in the field experiments at Salisbury, Maryland, it is impossible to state whether higher concentrations would have produced beneficial responses. The prominent feature of this experiment may be observed in a comparison of the treatments involving various copper compounds. Considering the fact that all compounds were supplied to the soil in a manner which would provide chemically equivalent amounts of copper it is noteworthy that the oxides produced a significantly greater yield than the check or copper sulfate treatment. An explanation for the difference in response cannot be advanced on the basis of these experiments; however, a theoretical interpretation could be based on differences in solubility of the compounds. Inasmuch as the oxides are far less soluble than the sulfate they might remain in contact with roots for longer periods of time while the sulfate, by comparison, would be leached from the soil relatively soon, particularly on the very sandy coastal soils with which this investigation is concerned.

The behavior of plants in the copper excess experiments furnishes certain information on the toxicity effects of copper. The results indicate that copper, within the range of concentrations used in the soil, was not absorbed by the plant in direct proportion to its concentration in the substrate (table IX, fig. 6). At the two lowest concentrations of treatment the plants contained essentially the same quantities of copper despite the fact that the second concentration was three times as large as the first. While treatment concentrations were increasing in geometric sequence the hydrangea plants of the three highest treatments accumulated copper in proportionately greater increments. The same effect was in evidence, to a lesser extent, in the two highest levels of copper treatment applied to the tomato plants. There were no evidences of injury to the hydrangea plants in the first three levels nor to the first four levels on tomato plants. From these observations it is apparent that hydrangea and tomato plants have wide tolerances with respect to copper concentrations in the soil as long as the concentrations remain below a critical level (a level between the third and fourth treatment in hydrangeas; and between the fourth and fifth in tomatoes). Above this critical level the mechanism for regulating absorption is incapacitated, which in turn results in death of the plant.

Impressive differences in the accumulation or decrease of other elements as a result of excessive copper may not be observed from this experiment. This is in part due to the limited sequence of treatments and in part due to the short duration of the experiment. However, in view of the relatively short length of time in which the treatments were in effect the differences in chemical composition, though small, may be considered as substantial evidence of real effects. Of the elements analyzed only total nitrogen and potassium concentrations showed uniform trends in successive treatments and though the trends are not identical in the two species of plants there is the indication that both constituents may accumulate to a greater extent as the copper concentration increases.

SUMMARY AND CONCLUSIONS

Field experiments were carried out on two contrasting soil types in Maryland to determine whether copper dificiencies were present. The results of experiments in 1949 indicated no significant effect of copper sulfate treatments on the Salisbury soil, while at College Park a detrimental effect was noted when this compound was applied as a foliage spray. A bio-assay for copper in the soil at the College Park location confirmed the assumption that it already contained a quantity of copper which would not be limiting in the growth of plants. The results of the 1950 experiments substantiated those of 1949 inasmuch as the copper sulfate again gave no significant effect at Salisbury. However, the effects of supplementary copper in several other chemical forms were investigated.

In the greenhouse, copper deficiency symptoms were produced in cauliflower and kale growing in water cultures. The effects of copper excess in soils, with respect to symptoms and several chemical constituents, were investigated using hydrangea and tomato plants.

The copper content was determined for a number of species of plants.

Under the conditions of these experiments the following conclusions may be made:

1. In agreement with other copper determinations of coastal soils, the soil involved in the experiments at Salisbury, Maryland, is deficient in copper, insofar as the yield of the tomato and the bio-assay for copper using *Aspergillus niger* is concerned.

2. Cupric and cuprous oxides result in significant increases in yield of tomatoes whereas an equivalent amount of copper, as copper

sulfate, does not.

3. Tomato and hydrangea plants maintain relatively uniform amounts of copper in their leaves over a wide range of substrate concentrations until a maximum or critical concentration is reached, at which the entry of copper is no longer regulated and is accumulated in great quantities.

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APPENDIX TABLE 1

CUCUMBER (WEIGHT YIELD -- FRUIT)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	66.9	98.8	165.7
	2	51.6	99.8	151.4
	3	46.6	111.9	158.5
	Total	165.1	310.5	475.6
$CuSO_4$ Foliage Spray	1	49.7	68.8	118.5
	2	21.0	117.8	138.8
	3	75.6	96.6	172.2
	Total	146.3	283.2	429.5
$CuSO_4$ on Soil	1	37.4	98.8	136.2
	2	47.1	96.2	143.3
	3	30.0	107.8	137.8
	Total	114.5	302.8	417.3

APPENDIX TABLE 2

BEET (WEIGHT OF ENTIRE PLANT)

FIELD EXPERIMENTS — 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	13.4	9.5	22.9
	2	11.1	8.0	19.1
	3	12.1	15.7	27.8
	Total	36.6	33.2	69.8
$CuSO_4$ Foliage Spray	1	6.8	10.2	17.0
	2	6.5	4.8	11.3
	3	11.0	10.1	21.1
	Total	24.3	25.1	49.4
$CuSO_4$ on Soil	1	11.0	5.1	16.1
	2	5.8	11.7	17.5
	3	7.4	11.2	18.6
	Total	24.2	28.0	52.2

APPENDIX TABLE 3

SNAP BEAN (WEIGHT YIELD -- PODS)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	6.2	22.7	28.9
	2	5.4	32.2	37.6
	3	8.9	36.2	45.1
	Total	20.5	91.1	111.6
$CuSO_4$ Foliage Spray	1	5.4	19.3	24.7
	2	5.6	32.6	38.2
	3	7.3	26.7	34.0
	Total	18.3	78.6	96.9
$CuSO_4$ on Soil	1	7.8	17.8	25.6
	2	3.7	31.3	35.0
	3	9.4	29.7	39.1
	Total	20.9	78.8	99.7

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APPENDIX TABLE 4

LIMA BEAN (WEIGHT YIELD -- PODS)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	18.3	26.0	44.3
	2	10.8	21.3	32.1
	3	17.6	22.8	40.4
	Total	46.7	70.1	116.8
$CuSO_4$ Foliage Spray	1	12.4	15.9	28.3
	2	16.4	21.7	38.1
	3	18.3	17.0	35.3
	Total	47.1	54.6	101.7
$CuSO_4$ on Soil	1	17.7	23.2	40.9
	2	14.0	20.3	34.3
	3	19.5	23.7	43.2
	Total	51.2	67.2	118.4

APPENDIX TABLE 5

SWEET POTATO (WEIGHT YIELD -- SALABLE ROOTS)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	67.5	36.6	104.1
	2	74.9	38.2	113.1
	3	49.0	42.0	91.0
	Total	201.4	116.8	308.2
$CuSO_4$ Foliage Spray	1	82.2	30.8	113.0
	2	77.8	34.4	112.2
	3	59.9	28.2	88.1
	Total	219.9	93.4	313.3
$CuSO_4$ on Soil	1	73.2	32.5	105.7
	2	72.5	29.1	101.6
	3	70.5	42.5	113.0
	Total	216.2	104.1	320.3

APPENDIX TABLE 6

TOMATO (WEIGHT YIELD -- FRUIT)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations combined
Check	1	35.9	69.3	105.2
	2	34.5	76.3	110.8
	3	43.8	92.0	135.8
	Total	114.2	237.6	351.8
$CuSO_4$ Foliage Spray	1	35.4	79.6	115.0
	2	35.8	80.9	116.7
	3	50.7	56.5	107.2
	Total	121.9	217.0	338.9
$CuSO_4$ on Soil	1	38.5	89.9	128.4
	2	39.6	72.8	112.4
	3	31.0	60.1	91.1
	Total	109.1	222.8	331.9

APPENDIX TABLE 7

TOBACCO (FRESH WEIGHT OF STEM AND LEAVES)

FIELD EXPERIMENTS -- 1949

Treatment	Replication	Salisbury (pounds)	College Park (pounds)	Locations
Check	1	68.0	48.0	116.0
	2	51.6	62.1	113.7
	3	59.0	73.5	132.5
	Total	178.6	183.6	262.2
$CuSO_4$ Foliage Spray	1	62.2	64.4	126.6
	2	75.4	80.0	155.4
	3	59.0	42.3	101.3
	Total	196.6	186.7	383.3
$CuSO_4$ on Soil	1	67.0	63.2	130.2
	2	57.7	62.5	120.2
	3	67.7	72.0	139.7
	Total	192.4	197.7	390.1

APPENDIX TABLE 8

MEAN COPPER CONTENT OF LEAVES (PARTS PER MILLION IN DRY MATTER)

SALISBURY, MARYLAND, 1949

	Cucumber	Beet	Snap Bean	Lima Bean	Irish Potato	Sweet Potato	Tomato	Tobacco	Sweet Corn	Field Corn
Check	18.1	16.4	13.8	12.4	30.2	22.9	15.6	23.0	30.3	27.1
$CuSO_4$ on soil	19.7	17.6	12.3	18.4	33.4	22.4	14.3	21.8	33.0	27.4
$CuSO_4$ foliage spray	18.1	17.3	11.7	14.0	33.4	22.9	16.5	23.2	40.8	26.1