THE EFFECT OF METHODS AND RATES OF APPLICATION OF TWO COPPER CARRIERS ON THE YIELD AND COPPER CONTENT OF SPINACH AND SUDAN GRASS GROWN IN THE GREENHOUSE ON TWO ORGANIC SOILS

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AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
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K. Eustace E. Johnson

ABSTRACT

The Effect of Methods and Rates of Application of Two Copper Carriers on the Yield and Copper Content of Spinach and Sudan Grass Grown in the Greenhouse on Two Organic Soils.

The study was instituted to investigate sources, rates, and methods of application of copper on the yield and copper content of spinach and sudan grass grown in the greenhouse on two organic soils.

The soils were of varying acidity pH 3.7 and pH 6.0 obtained from the Anderson farm in Lapeer County and the M.S.C. Muck Experimental Farm respectively. Basic fertilizer treatments from c.p. chemicals to both soils was at the following rates: 3-9-18 at 3000 lbs. per acre, and chlordane (wire-worm control) at 10 pounds per acre. To the Anderson soil (pH 3.7) was added precipitated calcium carbonate at the rate of 10 tons per acre (which brought the pH up to 5.7) and minor elements at the following rates: zinc sulfate (monohydrate) at 25 pounds per acre, manganous sulfate (monohydrate) 100 pounds per acre, and sodium borate (decahydrate) at 100 pounds per acre.

Copper was applied to the soil at two rates, 5 and 25 pounds per acre, and also to the leaves as a dust and spray. The copper for each of these treatments was derived from two sources: copper sulfate (pentahydrate) and copper oxide (a 50 percent copper carrier sold under the trade name of Calumet Brown Copper Oxide by the Calumet and Heckla, Inc., of Calumet, Michigan.)

The first four crops were grown during the period of late fall and winter, and the second four crops from late winter to spring, with artificial lighting supplied when necessary.

Samples of the soils were taken from each of the treatments before and after cropping and analysed for copper. The crops were harvested, washed where foliar treatments were applied, dried at 60-80°C, and samples of each of the treatments were analyzed for copper.

It was observed that in dry ashing samples for copper analysis a temperature of 450°C was adequate instead of a temperature of 650°C.

The two croppings from each of the treatments did not remove enough copper to change the copper content of the soils appreciably.

On the soil from the Muck Experimental Farm there is conclusive evidence that copper is beneficial to the growth of spinach and sudan grass. On the soil from the Anderson farm only spinach showed benefits of copper applications. In most cases, the soil applications resulted in higher yields than foliar applications.

There was little or no difference in the effectiveness of copper applied as oxide or sulfate, to increase yields or influence the copper content of the plant if they are both used at the same rate of total copper.

The 25 pound per acre rate of soil application was more effective than the 5 pound per acre application in increasing the copper content of the plant tissues. Only in the second crops did the former rate give higher yields than the latter.

Dust and spray treatments used in adequate (non-toxic) concentrations are equally efficient in correcting copper deficiency and increasing yields without giving abnormal percentages of copper in the tissues. On the whole they were not quite as effective as soil applications.

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INTRODUCTION

In many organic soils it is impossible to grow certain crops without the addition of copper. Many of these soils are copper deficient to such an extent that response to copper can be observed, not only in the pasture crop, but also in the grazing herd. As early as 1817 it has been reported that (6) investigators have shown that copper is essential to plant growth, and later, have shown its necessity to higher forms of life and to man himself. Investigations concerning its addition to the soil or plant as a nutrient were initiated in 1921 (6). Several fungicides containing copper have been used — such as Bordeaux mixtures — with resulting increases in crop yields that could not be ascribed to the control of the disease.

At present the recommendations for the use of copper on organic soils are made on the basis of soil reaction (pH) and the crop itself. Several copper-bearing materials are known (17) to correct copper deficiency, but are not at present extensively recommended in Michigan.

This work was designed to investigate under greenhouse conditions the comparative effectiveness of copper sulfate ($CuSO_4 \cdot 5H_2O$), and copper oxide* in increasing crop yields. Investigations were made of two organic soils, with different rates of application and different

* Copper oxide was obtained from Calumet and Heckla, Incorporated, and contained 50 percent metallic copper. It is sold under the trade name of Calumet Brown Copper Oxide.

methods of application of copper using spinach and sudan grass as indicator crops.

REVIEW OF LITERATURE

Most of the current work on copper has been extensively reviewed by Harmer, Sommer, Lucas, Brown, Ruby and others (17,39,27,6,37) dealing with various aspects of the use of copper. In an effort to bring out the importance of copper to all forms of life, this review concerns itself with pointing out the functional role of copper in the soil, plant, animal and man himself.

Functions of copper in man and animals. Copper plays an important role in the metabolism of humans and animals. Nutritional deficiency of copper is rarely observed in man but it is frequently apparent in animals that feed on copper deficient diets, such as pastures established on copper deficient soils. It is known to be present in small quantity in animal tissue. Some invertebrates such as orthropods and mollusks, surpass plants and vertebrates in the accumulation and concentration of copper in the tissue (13). There are several processes known to require copper, yet how it functions in these processes is still not entirely clear.

Some of the functions in the animal body in which copper is thought to be essential are: (a) the process of myelinization of the central nervous system (8); (b) in erythropoesis, blood formation, chiefly red cells and haemoglobin (16); (c) for keratinization in sheep (10); (d) for hardening fresh cuticle, and toughening organs of attachment of invertebrates (13); (e) as an oxygen carrier in respiration (16); and (f) for maintenance of nutrient balance and prevention of disease (32).

Functions of copper in plants. Copper is essential for higher plants although this need is satisfied by small quantities of the element. Copper is related to metabolic activity as evidenced by its presence in enzymes or enzyme systems, and its effect on the absorption and utilization of other elements. Some workers relate its function to the prevention of certain diseases, and regulation of the phasic development of the plant.

Copper as a constituent of plants was recognized early in the nineteenth century, and the amount was determined quantitatively about fifteen years after this discovery. Increases in yields by use of copper was shown by (3,6,15,17,27,36,37) and several other workers.

Investigation as to the way in which copper is found in plants has shown that it is located chiefly in enzymes, and enzyme systems, and the greatest portion in the chloroplasts of the leaves. Some of the known copper enzymes in plants are: ascorbic acid oxidase; laccase; polyphenol oxidase or tryosinase; catechol oxidase; cytochrome oxidase; peroxidase; and an enzyme inactivating indole acetic acid, an auxin in plants (1,2,5,11,23,26,28,29,38,41). As an example of the quantity of copper, Dawson (11) points out that ascorbic acid oxidase is a specific copper protein having a molecular weight of about 150,000 and containing 6 copper atoms per molecule. The total copper in plants varies with the type of plant (27,35), its physical, topographical and chemical environment (4), as well as within tissues of the plant itself (6). It has been found by most workers to vary from 2 to 15 ppm for most plants under normal conditions (27,36), yet quantities up to 100 ppm have also been observed by others (19,36).

Arnon (3) reports Neish as having found that 74.6 percent of the total copper in clover leaves is localized in the chloroplasts, and most of it was in organic combination. The copper in organic combinations or complexes are sometimes held in what are called "chelate linkages" and these are bound so strongly that it takes most of the power of strong concentrated acids to break them down. Delf (12), reveals that copper is translocated in both phloem and xylem, but, largely in the sieve tubes of the phloem.

Several possibilities have been proposed as to the role of copper in plants yet these have not been entirely agreed upon by all workers. Copper is considered to function as an oxidizing agent and aids in respiration (3,11,21,35). Hoagland (19) suggests that it plays a role in photosynthesis. Arnon (2) believes also that it may play a part in photosynthesis due to his observation of oxidation-reduction enzymes found in the chloroplasts of leaves of plants such as spinach. Sommer (39) shows that copper is effective as a cure for "reclamation disease", premature dying of onions, and refers to evidence of its curing effects on "die-back" of citrus, rosette of pears, and other plant abnormalities. Lipman and McKinney (26) state that it is necessary for the phasic development, as evidenced by the inability of barley and flax to produce seed when copper was withheld. Other workers support this view in the case of sudan grass and oats (6,27). Winthrow (44) indicates that copper participates in certain steps of chlorophyll synthesis. Picton (36) says, "It seems to be agreed that copper must be available before iron can be used ...." which is indicative of the role in nutrient balance and governing effect on food utilization. Steinberg (40) points out that copper is essential to protein breakdown, yet the evidence was not conclusive enough to indicate with certainty that it participates in the synthesis of amino acids or proteins.

Experimental evidence is now sufficient to justify the acceptance of copper as a nutrient element and its being essential for growth of higher plants. It has been found to increase yield and quality of crops when used where deficiencies occur. There is no one concensus of opinion as to its function in the plant, but it no doubt does influence the metabolic processes, and forms part of the plant constitution.

Functional aspects of copper in soils. Copper is thought to exist in mineral soils either primarily as a cation and follow much the same pattern as calcium and potassium (42), or "strongly and irreversibly absorbed by soils especially in the organic matter" (35). In organic soils copper is believed to exist in very strongly bound complexes. According to Corwin (9) copper exhibits chelation phenomenon, which is the formation of simultaneous links to the same organic molecule, and there is so much stability of this multiple linkage, that if, for instance, a single linkage were so weak that its half-life expectancy is only about a minute at room temperature, a double link of the same strength would have a half-life expectancy of nearly a quarter of a million years, provided temperature changes or any other effects are eliminated. The protein derived components, phenolic-OH groups, R-SH, NH_4 and R-COOH groups react with copper, even within the same molecule in acid or alkaline solution to form complexes. Some of the primary copper complexes contain three atoms of copper, two molecules of cystein and one aromatic amine (9).

The availability of copper in soils has been depicted in several ways. Jamison (22) writes that much of the copper is held in three forms: non-replaceable, slowly replaceable, and slowly soluble. Truog (42) in a collective grouping uses three categories also: the readily available, moderately available, and slowly available. Adsorbed copper is believed to depend on the cupric-ion concentration, nature of the soil, exchangeable acidity and other adsorbed ions (22). It is generally agreed that copper is to be found chiefly in the upper layers of the soil (22), or more emphatically as Lucas (27) puts it, "rigidly held in the zone of placement". Holmes (20) in his analysis of United States soils reports that the copper content varied from 6 to 67 ppm with variations following no definite geographical distribution, yet he believes that parent materials and conditions of weathering influence the availability of copper.

Varmeet and Van der Bie, as quoted by Brown (6) conclude that copper released by weathering is in the ionic form, yet in contact with organic matter, it would sequentially be fixed in the organic molecule. Brown and Steinberg (7) report that ascorbic acid oxidase activity in the plant was a good index of the available copper supply whether the plant did or did not show visual copper deficiency symptoms, and that other micronutrient deficiencies can also be determined by enzymatic activities since the enzyme will be aberrant if it required the element for function.

Brown (6) reports that copper influences the uptake of iron, nitrogen, potassium, phosphorus, magnesium and silica. Picton (36) and Jacks and Sherbatoff (21) believe that copper is necessary before iron can be used, and further that organic soils containing toxic quantities of ferrous iron may be corrected by copper application. Willis and Piland (43) show that excess absorption of iron as a result of liming can be controlled by adding copper without which a lodgement of iron in the nodes and decrease in growth may occur. McMurtrey and Robinson (30) state that some of the beneficial effects derived from copper compounds may be due to the precipitation of the toxic sulfide ion found in organic soils. Lipman (25) reports antagonism of copper and zinc to sodium chloride, sodium sulfate, and sodium carbonate which was regarded as a significant observation for the use of copper in the reclamation of alkali soils.

The primary function of copper in soils is that of a nutrient element. Another function is that of an oxidizing agent. According to Lazarrev (24) organic soils which contain reduced substances such as iron and manganese do not respond to copper treatment, if the soil is treated with hydrogen peroxide. Copper and zinc are sometimes referred to as mutually coordinating catalysts for oxidation and reduction (6). Copper neutralizes harmful conditions in soils (33,42), as evidenced by the production of characteristic changes in organic acid content when there is a serious deficiency.

Much of the work with regards to copper and soil reaction has been reviewed by Ruby (37). It was shown that for the most part acid organic soils will give better response to liming if copper is also used. Harmer (17) states that in general, most crops benefit from copper if the natural pH of the organic soils is 6.0 or less, with the more responsive crops showing a need as high as pH 6.5, but, in effect, the more acid the organic soil, the greater the relative response to copper. Lucas (27) reports that the binding of copper in the soil is stronger at higher and at lower pH values, in the region of pH 3.0 and 7.2. On addition of copper acetate to saturated H-Humus, it was believed that copper was adsorbed as the divalent cation Cu^{++} and the monovalent cation complex $(CuAcO)^{+}$ which was probably precipitated when the suspension pH was increased above 4.7. Truog (42) and Hoagland (19) report contrasting results as to the availability of copper at pH 5.5-6.5. Lucas (27) embraces both points of view by saying that either the relationship of availability of copper and pH are not important if the copper content is low, or that the need for copper is more dependent on balance of nutrients within the plant.

Microbial activity is considered one of the factors associated with reduced availability of copper in soils. Mulder (33) considers that the presence of "peaty substances," H_2S bacteria, and other microbial activity assist in the formation of complex organic copper compounds. Hasler (18) shows that the affinity of humus rich soil for trivalent cations makes it possible not to influence the micro-organism content by large dosages of copper. The action of micro-organisms in organic soils is believed to be so complex that the biological test - "Aspergillus Niger" - estimated as the most reliable test for available copper in sandy soils, is regarded as unsatisfactory for organic soils (10). As previously indicated, copper is known to be essential for the normal growth and development of microorganisms (13,25), and there will be three competitors for the available copper — the plant, the microbe and the organic complex.

EXPERIMENTAL PROCEDURE

Two organic soils were obtained; one from the Muck Experimental Farm (pH 6.0) Soil 1, and the other from the Anderson Farm, Lapeer County (pH 3.7) Soil 2. The soils were partially dried and were screened through a quarter inch square mesh screen. A uniform weight of soil was placed in 108 previously weighed, four-gallon glazed jars.

An application equivalent to 3000 pounds per acre of 3-9-18 fertilizer, prepared from chemically pure grades of ammonium nitrate, potassium monophosphate, and potassium chloride, was supplied to each jar. In addition to fertilizer, to the acid Anderson muck, the equivalent of 10 tons per acre of precipitated calcium carbonate was added, which increased the pH from 3.7 to 5.7. The following minor elements were added to the limed soil at the rates listed: zinc sulfate ($ZnSO_4 \cdot H_2O$), 25 pounds per acre; manganese sulfate ($MnSO_4 \cdot H_2O$), 100 pounds per acre; and sodium borate ($Na_2B_4O_7 \cdot 10N_2O$), 100 pounds per acre. Chlordane was added at the rate of 10 pounds per acre to control wireworms.

The following nine treatments were replicated six times on each soil:

1. Control — no copper added.

2. Copper sulfate — mixed with soil — 5 pounds copper per acre.

3. Copper oxide — mixed with soil — 5 pounds copper per acre.

4. Copper sulfate — mixed with soil — 25 pounds copper per acre.

5. Copper oxide — mixed with soil — 25 pounds copper per acre.

6. Copper sulfate — Foliar application — 0.25 and 0.005 percent copper sprays.

7. Copper oxide — Foliar application — 0.25 and 0.005 percent copper sprays.

8. Copper sulfate — Foliar application — 6.25 and 2.5 percent copper dusts.

9. Copper oxide — Foliar application — 6.25 and 2.5 percent copper dusts.

The compounds were thoroughly mixed with the soil and distributed as uniformly as possible. The spray was applied by use of a hand-sprayer and the dust by use of a dusting tower. A small quantity of detergent (0.3 gm per litre of Dreft) was included with the spray as a wetting agent. During foliar applications the soil was covered with paper as close to the plant as possible to prevent any of the treatment from falling on the soil.

The moisture equivalents of 165 for soil 1, and 174 for soil 2, were used to bring the soils up to optimum moisture.

Spinach and sudan grass, the indicator crops, were planted one week after the soil was moistened and on germination were thinned to 8 and 12 plants per jar respectively. The first crops were grown during the period from November 31, 1951 to February 7, 1952 and the second crops from February 12, 1952 to July 7, 1952. Greenhouse lighting extended the light period from 5:30 P.M. to 10:30 P.M. daily. Harvesting of crops was done in the early stages of maturity, based on the emergence of seed-heads.

After harvesting fresh weights and oven-dry (60-80°C) weights were obtained. Those plants that were dusted or sprayed were washed before drying to remove particles of copper from the surface of the leaves. The method of washing was as follows:

First, the plant material was placed in about three litres of distilled water at room temperature for two or three minutes and moved upward and downward to insure the thorough wetting of the material. Next, it was similarly washed in three litres of luke warm (30-35°C) distilled water containing about 2 gms. of detergent, followed by three successive washings in the same quantity of water without detergent, and a final rinse with running distilled water. The plants were then placed on large clean porcelain dishes and dried. It was found that the wetted plant material tends to stick to paper, enamel pans, or boxes.

Determination of copper in plants. (34) The samples were ground in a Wiley Mill, using a 20 mesh screen for large samples and a 60 mesh for samples less than 2 grams. The samples were then oven-dried at 105°C for four hours to drive off moisture absorbed during grinding. The samples were removed from the oven, cooled in a desiccator, and known weights in porcelain crucibles were placed in a muffle furnace at 450°C for 10 to 12 hours for ashing. If there were visible particles of carbon left, after ashing, three or four drops of nitric acid were added to the contents of the crucibles which were then dried and re-ashed. The ashed material was treated with 3-5 ml. of concentrated hydrochloric acid and boiled for one minute. (The acid had to be added gently and under the hood; rapid addition of acid caused spattering and subsequent loss of ash). The solution was next transferred to a 200 or 250 ml. volumetric flask with boiling redistilled water, and after cooling brought up to volume with redistilled water. Aliquots of 50 ml. of less brought up to 50 ml. were placed in separatory funnels, to which 5 ml. of 15 percent citric acid were added and shaken. The solutions were made slightly alkaline (using a piece of neutral litmus paper in the funnel as an indicator) with 1:1 ammonium hydroxide. Next 10 ml. of 0.01 percent sodium-diethyl-dithiocarbamate solution were added and shaken, after which four extractions were made using about 4 ml. of carbon tetrachloride each time until no color appeared in the CCl_4 layer, and then the extract was filtered through anhydrous sodium sulphate in No. 12½ or No. 40 Watman filter paper and brought up to 25 ml. volume with carbon-tetrachloride. The quantity of copper was then evaluated photometrically using a Cenco-Sheard-Sanford photelometer equipped with a Corning lantern blue glass filter (#554) with maximum transmittancy at 450 mu.

Determination of copper in soils. (34) The above procedure was used for determining copper in the soils. The sample was ground in an agate or porcelain mortar, then oven-dried, weighed, ashed, HCl-digested, filtered, washed with hot water and diluted to a volume, aliquots of which were then analyzed for copper by the procedure described above.

EXPERIMENTAL RESULTS

In preliminary studies, the extreme differences obtained in the copper content of soil and plant samples with those previously reported (6,27,36) made it necessary to investigate the reason for this variation. The situation was further complicated by the fact that good agreement was obtained in the copper content of duplicate samples, provided the size of sample remained the same. The blank determinations were not affected by the techniques.

The temperature at which the samples were ashed appeared to be responsible for the variation. If the sample was ashed at 450°C, very good agreement between results previously reported for the sample was obtained, whereas, if ashing took place at 650°C, unsatisfactory data resulted. Values obtained were from two to five times greater when ashing occurred at the higher temperature.

In order to determine the percentage distribution after copper applications and also to find out if results from foliar applications were due to soil contamination from these treatments, samples of soil from each treatment were taken before and after cropping, analyzed and the percent copper reported in Table I.

The data in Table I show that there was essentially no change in the copper content of the soils after two croppings at the various levels of copper application.

The effect of these treatments of the two soils on the yield and copper content of the first crops of spinach is given in Table II.

TABLE I

The Effect of Cropping on the Copper Content of the Soil at the Various Treatments

Treatment			Percent copper (Soil 1)		Percent copper (Soil 2)	
Source	Pounds copper per acre	Method of appli- cation	Before cropping	After cropping	Before cropping	After cropping
	—	—	0.0011	0.0010	0.0010	0.0010
Copper Sulfate	5	soil	0.0024	0.0023	0.0024	0.0024
Copper Oxide	5	soil	0.0024	0.0024	0.0024	0.0024
Copper Sulfate	25	soil	0.0075	0.0074	0.0074	0.0074
Copper Oxide	25	soil	0.0075	0.0074	0.0074	0.0074
Copper Sulfate		spray	0.0011	0.0012	0.0010	0.0012
Copper Oxide		spray	0.0011	0.0011	0.0010	0.0011
Copper Sulfate		dust	0.0011	0.0011	0.0010	0.0010
Copper Oxide		dust	0.0011	0.0011	0.0010	0.0010

TABLE II

The Effect of Methods and Rates of Application of Two Copper Carriers on the Yield and Copper Content of the First Crops of Spinach Grown in the Greenhouse on Two Organic Soils

Treatment*			Soil 1		Soil 2	
Source	Pounds copper per acre	Method of application	Mean yield** (grams per jar)	Percent copper	Mean yield** (grams per jar)	Percent copper
	None	---	2.0	---	9.0	0.0017
Copper Sulfate	5	soil	10.0	0.0010	10.4	0.0014
Copper Oxide	5	soil	11.3	0.0011	12.1	0.0014
Copper Sulfate	25	soil	11.5	0.0016	11.4	0.0016
Copper Oxide	25	soil	11.2	0.0016	10.1	0.0016
Copper Sulfate	---	0.25% spray	2.0	0.0015	4.3	0.0015
Copper Oxide	---	0.25% spray	5.0	0.0014	6.3	0.0014
Copper Sulfate	---	6.25% dust	2.6	0.0017	6.5	0.0016
Copper Oxide	---	6.25% dust	4.5	0.0015	8.0	0.0015
L.S.D.***	1 percent		1.7	---	2.4	---
	5 percent		1.2	---	1.7	---

* A uniform application of 3000 pounds per acre of 3-9-18 fertilizer applied to all jars. Spinach harvested on December 28, 1951 and February 29, 1952 from soils 1 and 2 respectively.

** Averages of three replications dried for 72 hours at 60-80°C.

*** Difference required for significance between any two treatment means.

The data show that with soil 1, the yields obtained from all treatments except the copper sulfate dust and spray are significantly higher than the yield of the control at the 1 percent level of significance, with the soil applications giving the best results. There was no difference in yield from the 5 pound per acre applications of copper applied in the form of the oxide and the 25 pound per acre applications either as oxide or sulfate. Yields from jars treated with 5 pounds per acre of copper supplied as sulfate were significantly less at the 5 percent level than the yields obtained from jars of the three above mentioned treatments on soil 1.

With soil 2, only three treatments of the soil applications gave significantly higher yields than the control. The yields from the jars treated with 5 pounds per acre of copper as sulfate were not significantly higher than the yields from the untreated jars. Both sprays and the copper sulfate dust treatments resulted in yields significantly lower than yields of the control due to the high concentration of these treatments, as was also observed on the yields of foliar application treatments on soil 1.

The percentage of copper in the tissues was approximately the same for the 25 pounds per acre treatments and the foliar application treatments, and slightly lower in the tissues from jars receiving the lower rate of soil application. The source of copper did not influence the copper content of the plant. It is interesting to note that the copper content of the control crop was comparable to that of the 25 pounds per acre soil applications on soil 2. The copper content of the spinach control grown on soil 1 was undetermined because of insufficient plant material.

The yields of the second crop of spinach on both soils were all significantly higher than yields of the controls as shown by the data in Table III. Only one treatment, the copper oxide spray, resulted in yields significantly greater at the 5 percent level. All other yield responses were significantly greater at the 1 percent level than yields of control jars.

High rates of soil applications in the second crop of spinach resulted in highest yields with no differences due to the source of the copper. (Figs. 1 and 2)

On soil 1, the yields from jars of the oxide foliar applications were significantly higher than the yields from jars of comparable sulfate treatments.

The yields and copper content of the second crops were comparatively lower than those of the first crops of spinach on both soils.

The data in Table IV show that there was no response in yield to any of the copper treatments by the first crop of sudan grass on soil 2, while only one treatment, the sulfate spray, failed to give response significant at the 1 percent level on soil 1. (Figs. 3 and 4)

The dust applications resulted in higher yields than the 25 pound per acre treatments and spray applications, with the oxide dust showing the most outstanding performance.

There was no significant difference in yields between rate of application of copper carriers in the soil application treatments in

TABLE III

The Effect of Methods and Rates of Application of Two Copper Carriers on the Yield and Copper Content of the Second Crops of Spinach Grown in the Greenhouse on Two Organic Soils

Treatment			Soil 1		Soil 2	
Source	Pounds copper per acre	Method of application	Mean yield** (grams per jar)	Percent copper	Mean yield** (grams per jar)	Percent copper
	None	---	1.2	---	1.8	0.0010
Copper Sulfate	5	soil	6.8	0.0009	6.3	0.0010
Copper Oxide	5	soil	7.1	0.0010	7.0	0.0009
Copper Sulfate	25	soil	9.2	0.0014	7.7	0.0016
Copper Oxide	25	soil	9.7	0.0014	6.0	0.0015
Copper Sulfate	---	0.005% spray	4.7	0.0010	4.7	0.0007
Copper Oxide	---	0.005% spray	8.2	0.0011	4.0	0.0008
Copper Sulfate	---	2.5% dust	5.6	0.0009	8.0	0.0009
Copper Oxide	---	2.5% dust	9.0	0.0012	6.3	0.0004
L.S.D.***	1 percent		1.7	---	2.5	---
	5 percent		1.2	---	1.8	---

* A uniform application of 1500 pounds per acre of 3-9-18 fertilizer applied to all jars of soil 1. Spinach harvested on March 25, 1952 and April 30, 1952 from soils 1 and 2 respectively.

** Averages of three replications dried for 72 hours at 60-80° C.

*** Difference required for significance between any two treatment means.

Fig. 1. The effect of rate of application of two copper carriers on the growth of spinach on Soil 1. (0 = No copper, 1 = 5 pounds copper as oxide, 2 = 5 pounds copper as sulfate, 3 = 25 pounds copper as oxide, 4 = 25 pounds copper as sulfate.)

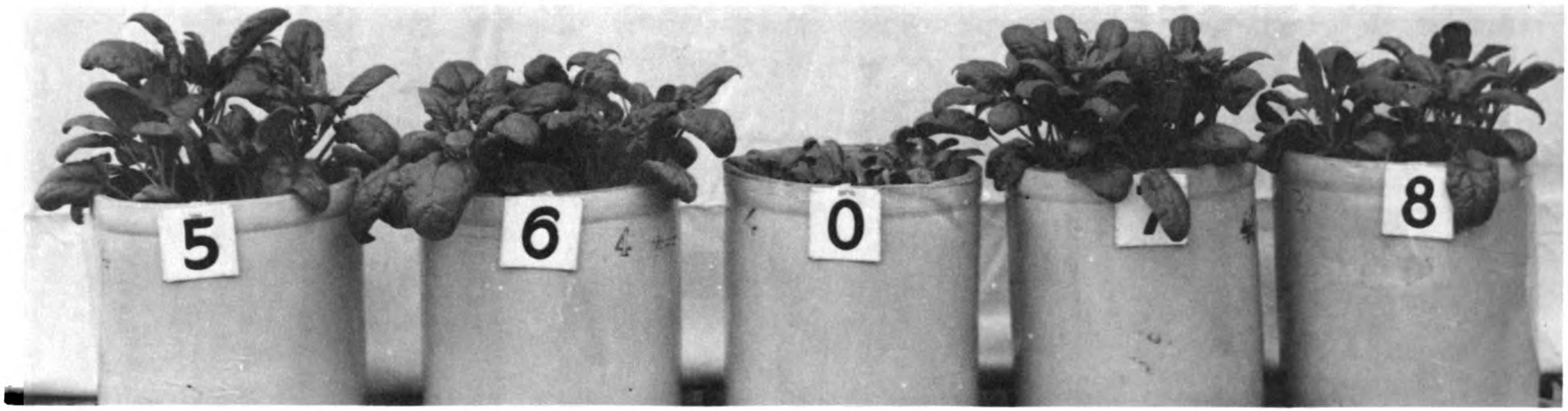

Fig. 2. The effect of foliar applications of two copper carriers on the growth of spinach on Soil 1. (0 = No copper, 5 = copper oxide dust, 6 = copper sulfate dust, 7 = copper oxide spray, 8 = copper sulfate spray.)

TABLE IV

The Effect of Methods and Rates of Application of Two Copper Carriers on the Yield and Copper Content of the First Crops of Sudan Grass Grown in the Greenhouse on Two Organic Soils

Treatment*			Soil 1		Soil 2	
Source	Pounds copper per acre	Method of application	Mean yield** (grams per jar)	Percent copper	Mean yield** (grams per jar)	Percent copper
	None	—	0.5	—	59.0	0.0018
Copper Sulfate	5	soil	61.7	0.0021	58.0	0.0015
Copper Oxide	5	soil	66.3	0.0018	62.7	0.0016
Copper Sulfate	25	soil	48.7	0.0024	60.3	0.0018
Copper Oxide	25	soil	45.0	0.0024	58.0	0.0018
Copper Sulfate	—	0.25% spray	17.0	0.0024	60.0	0.0015
Copper Oxide	—	0.25% spray	50.0	0.0024	61.0	0.0019
Copper Sulfate	—	6.25% dust	72.0	0.0023	61.5	0.0016
Copper Oxide	—	6.25% dust	102.0	0.0022	62.0	0.0027
L.S.D.***	1 percent		31.7	—	8.1	—
	5 percent		23.0	—	5.9	—

* A uniform application of 3000 pounds per acre of 3-9-18 fertilizer applied to all jars. Sudan grass harvested on February 7, 1952 and April 1, 1952 from soils 1 and 2 respectively.

** Averages of three replications dried for 72 hours at 60-80°C.

*** Difference required for significance between any two treatment means.

Fig. 3. The effect of rate of application of two copper carriers on the growth of sudan grass on Soil 1. (0 = No copper, 1 = 5 pounds copper as oxide, 2 = 5 pounds copper as sulfate, 3 = 25 pounds copper as oxide, 4 = 25 pounds copper as sulfate.)

Fig. 4. The effect of foliar application of two copper carriers on the growth of sudan grass on Soil 1. (0 = No copper, 5 =copper oxide dust, 6 = copper sulfate dust, 7 = copper oxide spray, 8 = copper sulfate spray.)

the first crop of sudan grass on soil 1.

The copper content of the first crop of sudan grass on soil 2 was not affected by the various treatments. On soil 1, the percentages differed proportionately with the rates of soil application, and the foliar treatments gave as high a percent copper in the crop as the 25 pounds per acre soil applications.

The data in Table V show that the yields of the second crops of sudan grass on both soils were lower than the first crops. The copper content was also lower, and was proportionate to rate of application with no difference due to the copper carrier. All treatments on soil 1 were significant at the 1 percent level, whereas there was no significant response in yield to copper treatments on soil 2.

TABLE V

The Effect of Methods and Rates of Application of Two Copper Carriers on the Yield and Copper Content of the Second Crops of Sudan Grass Grown in the Greenhouse on Two Organic Soils

Treatment*			Soil 1		Soil 2	
Source	Pounds copper per acre	Method of application	Mean yield** (grams per jar)	Percent copper	Mean yield** (grams per jar)	Percent copper
	None	---	0.9	---	9.1	0.0008
Copper Sulfate	5	soil	32.3	0.0008	15.1	0.0006
Copper Oxide	5	soil	34.3	0.0008	17.5	0.0007
Copper Sulfate	25	soil	35.3	0.0009	16.8	0.0011
Copper Oxide	25	soil	39.1	0.0009	17.0	0.0010
Copper Sulfate	—	0.005% spray	16.5	0.0009	12.7	0.0012
Copper Oxide	—	0.005% spray	9.3	0.0011	15.5	0.0012
Copper Sulfate	—	2.5% dust	16.3	0.0007	10.4	0.0012
Copper Oxide	—	2.5% dust	13.6	0.0010	11.1	0.0013
L.S.D.***	1 percent		7.1	---	11.5	---
	5 percent		5.2	---	8.4	---

* A uniform application of 1500 pounds per acre of 3-9-18 fertilizer applied to all jars of soil 1. Sudan grass harvested on April 30, 1952 and July 27, 1952 from soils 1 and 2 respectively.

** Averages of three replications dried for 72 hours at 60-80°C.

*** Difference required for significance between any two treatment means.

DISCUSSION

The resultant increase in values obtained by ashing the sample at 650°C could not be accounted for by techniques or reagents and the direct cause was not determined.

There was no appreciable change in the copper content of the soils after two croppings which shows the relatively small amounts of copper necessary for plant growth.

In most cases with soil 1, which responded to copper treatment, yields from the dust and spray treatments were significantly higher at the 1 percent level than the control. These yields were not as high as those obtained from soil applications. In evaluating this difference in yields between foliar and soil applications, some consideration must be given to the fact that on all first crops the concentration of copper in all foliar treatments was high enough to cause leaf damage, and in the case of the sulfate sprays some spinach plants were killed. For this reason on the second crops the concentration of foliar treatment was reduced to a point where no visible deleterious effects of the treatments were observed.

Observations on the effect of spray and dust treatments showed that if the plants were sprayed when they were too young they were more suscepitble to injury than if treated after three or four leaves have grown out. If spraying is done too late the benefit from the treatment will not be very great, and the growing period would be lengthened provided this effect is not masked by photoperiodism.

Twisted leaves compared to other leaves, were wider and smoother in the case of spinach and caused the stems and sheaths of sudan grass to increase in width.

Sudan grass was more tolerant than spinach to the higher concentrations of foliar treatments. With the 6.25 percent copper dust treatments spinach showed visible symptoms of damage from the oxide source of copper, whereas the sudan grass did not. Both crops were affected by this same concentration of copper from the sulfate source.

Soil applications proved to be most effective in the majority of cases observed. Although both soils contained equal percentages of copper, on soil 2, sudan grass did not respond significantly to any treatments yet the second crop showed a trend towards a benefit from a soil application.

The copper content of the plants was not influenced by the carrier used, provided that the same amount of copper was applied. In all cases of soil applications, the copper content of the plants varied directly with the quantity present in the soil. Where no copper was applied and plants suffered from copper deficiency, the percentage of copper was as high as other plants that showed no copper deficiency symptoms. In general the copper content of the first crops were higher than the second crops which is believed to be due to rapid tie up of copper in organic soil, or seasonal difference in crop response since the percentage of copper in the soils for both crops was essentially the same.

Copper deficiency of spinach plants resulted in stunted growth, inability to produce seeds, and intermittent marginal necrosis, as illustrated in Figure 5. In sudan grass there was stunted growth, inability to produce heads, shriveling and subsequent death of tips of leaves, especially young leaves, and in many cases chlorosis and death of the entire plant. Figure 6 shows one of the largest copper deficient plants beside a medium sized non-deficient leaf. The curling of the tips, and apparent inability to open can be observed. This curled portion of the end of the leaf was never opened, and as the leaf continued to grow this portion starting at the tip lost color gradually and dried up rapidly. It was not uncommon to observe a leaf that had one third of it completely dry towards the tip, and the adjoining two thirds pale green.

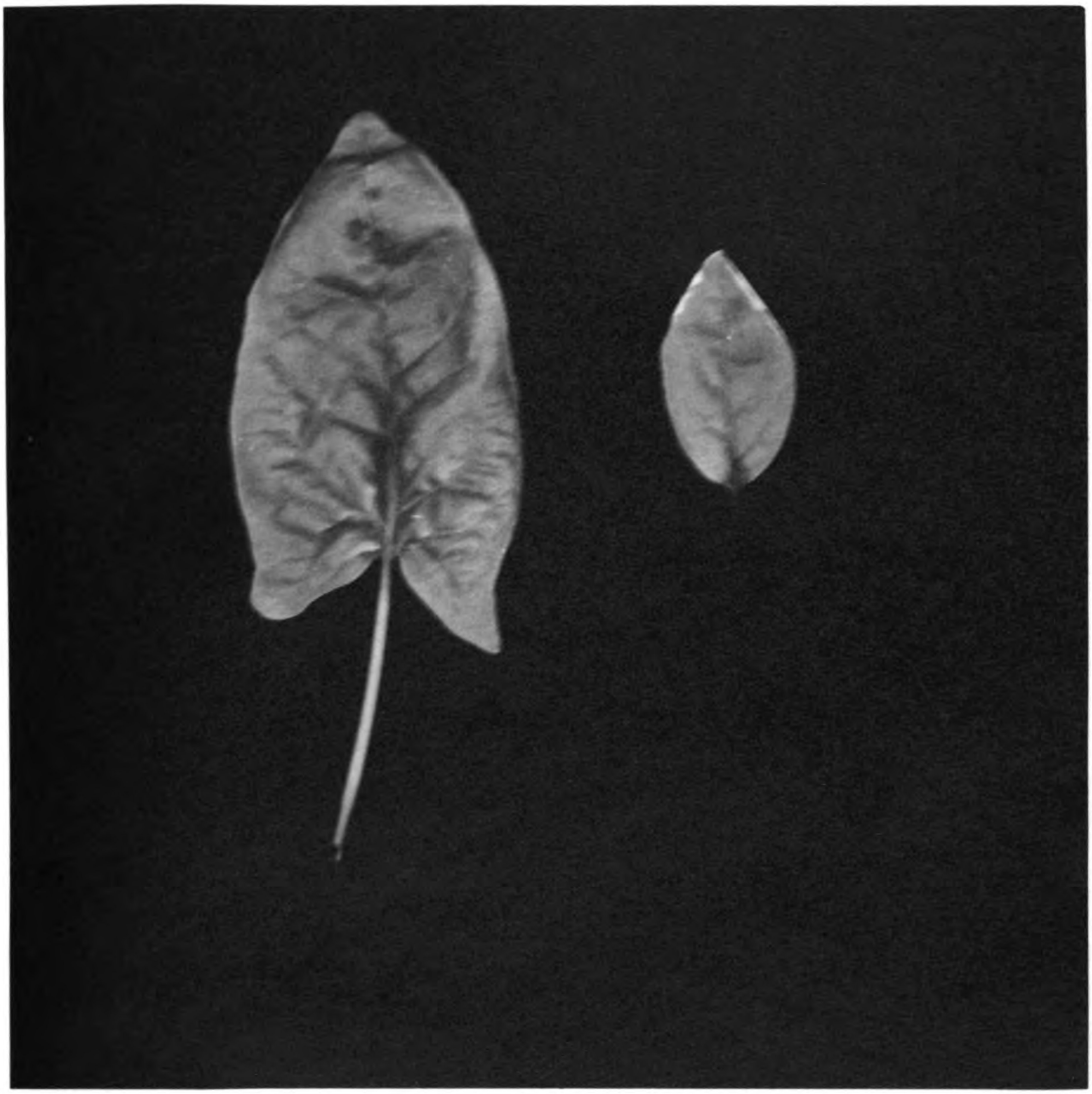

Fig. 5. Normal (left) and copper deficient (right) spinach leaves. (Note reduced size and marginal necrosis of deficient leaf.)

Fig. 6. Normal leaf (left), copper deficient plant (right). The deficient plant was one of the largest obtained in the jars on Soil 1 with no copper added.

SUMMARY

This study was set up to investigate sources, rates, and methods of application of copper on the yield and copper content of spinach and sudan grass grown in the greenhouse on two organic soils.

One of the soils with a pH of 6.0 was obtained from the M.S.C. Muck Experimental Farm and the other with a pH of 3.7 was obtained from the Anderson Farm in Lapeer County. The soils were dried to an apparent optimum moisture content and each soil was sieved through a 1/4 inch screen after which a constant weight of each was placed in 108 previously weighed, four-gallon, glazed jars.

A basic treatment of 3-9-18 fertilizer at the rate of 3000 pounds per acre and the equivalent of 10 pounds per acre of chlordane (wire-worm control) was added to all treatments on both soils. In addition to the above, on the acid Anderson soil, 10 tons per acre of precipitated calcium carbonate was added which brought the pH up from 3.7 to 5.7. In conjunction with lime certain minor elements were added; zinc sulfate ($ZnSO_4 \cdot H_2O$) at 25 pounds per acre, manganese sulfate ($MnSO_4 \cdot H_2O$) at 100 pounds per acre, and sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) at 100 pounds per acre.

Copper was applied to the soil at two rates, 5 and 25 pounds per acre, and also to the leaves as a dust and as a spray. The copper for each of these treatments was derived from two sources: copper sulfate, and copper oxide.

The first four crops were grown during the period from late fall

through winter, and the second four crops from late winter through spring, with artificial lighting supplied when necessary.

Samples of the soils were taken from each of the treatments before and after cropping and analyzed for copper. The crops were harvested, fresh weights recorded, washed where foliar treatments were applied, and dried at 60-80°C after which the dry weights were recorded. Plant samples of each of the treatments and croppings were also analyzed for copper.

The following observations were made:

1. It is necessary to ash samples for copper analysis at 450°C to obtain reproducible results agreeing with those reported previously by other workers.

2. The copper content of the soils is not changed appreciably after two croppings at the various levels of copper application.

3. On soil 1, there is conclusive evidence that copper is beneficial to the growth of spinach and sudan grass. On soil 2, only spinach showed benefits of copper applications.

4. Soil applications in most cases gave better results than foliar applications.

5. There was little or no difference in the effectiveness of either copper oxide or copper sulfate to increase yields or influence the copper content of the plant if they are both used at the same rate of total copper.

6. The 25 pound per acre rate of soil application was more effective than the 5 pounds per acre application in increasing the

copper content of the plant tissue. Only in the second crops did the former rate give higher yields than the latter.

7. Dusts and spray treatments used in adequate (non-toxic) concentrations are equally efficient in correcting copper deficiency and increasing yields but not as effective as soil applications.

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