THE BENEFIT OF FOLIAR APPLIED COPPER FERTILIZER
ON ROMAINE LETTUCE GROWN IN LOW COPPER SOILS
OF THE COASTAL SANTA MARIA,
CALIFORNIA

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Abstract

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The importance of trace elements nutrients to vegetable crops has received more attention in recent years. Accordingly this experiment investigated the benefit of foliar applied copper fertilizer on romaine lettuce (*Lactuca Sativa Longifolia*) grown in low soil Copper (Cu) concentration in the coastal Santa Maria, California. The various rates of Cu fertilizer applied include untreated (control), 100, 200, 400 and 800 grams per acre of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, using a one time and a split application methods. Three consecutive individual experimental fields were established starting in late spring through the fall of 2008. The soil texture was a loam in all three of the experimental fields. Positive responses in weight increase were found due to these treatments, however was not statistically significant. The maximum responds in weight increase was identified with the one-time application of 200 and 400 grams/acre of CuSO_4 but weight decline was observed at the higher rates of 800 grams/acre. The optimum weight increases were between 4.4 and 6 percent higher than the untreated plots. The treatments significantly increased the uptake of Cu by plants (pvalue <0.001). When analyzed collectively, significant difference was found in plant weights between the three experimental fields. Crops harvested one week earlier than scheduled produced 21% lower mean weight than those harvested on time. No apparent physical damage was identified in plants having leaf cu concentrations exceeding the suggested toxic level of 150 ppm but weight decline was observed thereafter. In 2009 an additional experimental field was established in a sandy loam, 8% clay and was completed in March. Rate of applications was modified to include untreated control, 200, 400 and 500 grams/acre, at one time application only. Again Positive responses in weight increase were found due to these treatments, however was not statistically significant. The optimum result in weight increase was found with the application of 200 grams/acre of CuSO_4 . The overall mean weights of the plants were comparatively higher than that of the three previous experimental fields. The weight increase was 7.5% higher than the untreated plots.

Key Words: Foliar; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Cu Sulfate Pentahydrate); Trace elements nutrients.

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INTRODUCTION

Purpose for research

The problem of Copper deficient soils

Recent research work on both commercial head lettuce and leaf lettuce including romaine (*Lactuca Sativa Longifolia*) in the area of coastal Santa Maria and San Luis Obispo counties of California identified copper to be the most limiting nutrient. More than 40% of the low yield fields were below the copper sufficiency range at both growth stages, mid growth and harvest. It has been suggested that the mean extractable soil copper is below what was reported by Brown and deBoer in 1985 to be representative at that time, for copper in this area (Hartz et al., 2007). Also, a significant relationship was found between soil extractable copper and pre harvest leaf copper concentration. The finding was insufficient to set a definite soil copper limit and therefore a further study was warranted.

Previous Research and Background:

Agriculture is the major producing industry in Santa Barbara county, California which includes the area of Santa Maria, California . In 2007 it had reached a gross production valued at over one billion dollars, an increase of \$86.6 million (8.5%) from the previous year. It was the second consecutive year that agriculture has surpassed the one billion dollar benchmark.

The production of vegetable crops was the second largest contributor to this revenue, next to fruit and Nut crops. In 2007, the combined acreage harvested for vegetable crops in Santa Barbara county was 71,434 acres, of which 17,087 acres was of head and leaf lettuce. Acreage harvested for leaf lettuce in 2007 was 4,252 with a total value of \$ 25,705,118 (The Agriculture Commissioner's Office, 2008).

Agricultural scientists and growers are continuing to find new ways to improve and enhance crop growth and crop quality. As the soil/plant laboratory manager of large agriculture producing firm in Santa Barbara and San Luis Obispo counties of California, this writer has, over the years, strived to keep abreast of current research, apply recently found alternate methods and test and analyze new concepts in the field.

The subject of this report is the importance of micronutrients in the soils of the above mentioned region of California, however, focusing on the paucity of the micronutrient copper in the soils and the results produced by remediation.

Research Objective

The objective the this study is to investigate the benefit of foliar applied copper sulfate on romaine lettuce grown in soils having low copper concentration and also, to determine if a one time application differs from a split application.

The role of pH and micronutrients

The established recommended range of soil pH for most vegetable crops, including lettuce is between 6.5 and 7.2. Between these pH values soil nutrients are in the forms available for plants' uptake. Micronutrients, such as iron, manganese, copper, zinc, boron and molybdenum, are needed by plants in much smaller amounts than the macronutrients (nitrate, phosphorus, potassium and sulfur) but are equally as important.

Factors influencing the availability of micronutrients in the soil

Temperature and moisture are essential factors affecting the availability of micronutrients in the soil. Most soil micronutrients tend to decrease at low temperature because of reduced root activity and low rates of dissolution and diffusion of nutrients (Cooper, 1973; Fageria et al., 2002). The availability of these micronutrients is also influenced by the pH. The micronutrients, boron, copper, iron, manganese and zinc, favor slightly acidic conditions, and therefore are available for plants at pH's between 6 – 6.5. Zinc for example, becomes less soluble as the pH approaches 7; it forms precipitates, binds with phosphorus, adsorbed on clay surfaces or on carbonates and becomes, therefore, unavailable for plants. For that reason, at slightly acidic pH values (between 6.5 - 6.8) result in favorable conditions for plant growth. One of the major contributors to high soil pH is the imbalance between the ratio of the bases, or the major cation ions [potassium (K^{1+}), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^{1+})] on the cation exchange sites. The desired ratio between calcium and magnesium on the exchange sites is about 65% calcium to 15 % magnesium for a sum of 80%. The remaining 20% is occupied by

potassium, sodium and other bases. Any of these four major elements in excess can drive the pH up and a deficiency of any of them can lower the pH, creating acidic conditions (Kinsey et al., 2006).

The Role of Copper (Cu)

Copper is essential for both plants and animals. Plant Cu concentration is influenced by plant species, stage of growth, and various soil properties and by the soil amendments applied. Soil Cu concentration is markedly affected by the parent material from which the soil formed. Alluvium is the dominant, unconsolidated surficial deposit of which soils formed in the interior valleys of California and Arizona. The mean soil Cu concentration in this area is 15 mg kg^{-1} whereas the coastal area of California has a mean Cu 54 mg kg^{-1} and a range between $8\text{-}112 \text{ mg kg}^{-1}$. No consistent difference was found between soils that formed of alluvium of mixed rocks or that of granitic alluvium (Bradford et al., 1967). Soils with the least amount of Cu, (mean concentration of 5 ppm) are found in the unconsolidated lower Atlantic Coastal Plain deposits of Florida, North and South Carolina (Kubota, 1983). A Map showing the geographical distribution pattern of Cu in legumes was prepared by Kubota, 1983 (Fig. 1), based on samples of various legume forages grown in a wide range of USA soils. The map shows coastal California where 40-70% of legumes contain $6\text{-}10 \text{ mg kg}^{-1}$ of copper.

Fig: 1. Map of the generalized distribution of Cu concentration in legumes of the USA (Kubota, 1983).

Areas with 50% or more legume have 10-12+ mg kg⁻¹ of Cu

Areas where 40-70% of legumes have 6-10 mg kg⁻¹ of Cu

Areas where 35% or more of legumes have 6 mg kg⁻¹ or less of Cu

The functions of copper in the plants

The functions of Copper in the plants are: to metabolize nitrogen and carbohydrate and to synthesize lignin which is essential for the forming of cell wall strength. It also affects flavor and coloring of the vegetables and their storage ability, which aids to the prevention of diseases.

When soil Cu is adequate roots accumulate Cu in high concentration, mostly in the apoplast (epidermal cell layer). In Cu deficient soil, root Cu concentrations are low and evidence indicates that such roots are vulnerable to fungal and bacterial attack (Mortvedt et al., 1991).

Nutrient imbalances in plants, especially latent micronutrients deficiencies, is a problem in intensive agriculture (Franck and Finck, 1980) in Marschner, 1986). The consequences are not

only for plant yield but also resistance to and tolerance of diseases and pests as well as for animal (Kubota et al., 1987) and human nutrition.

The soils of the coastal Santa Barbara and San Luis Obispo Counties

Along the coastal area of San Luis Obispo and Santa Barbara counties, there is an abundance of serpentine rocks [$\text{Mg}_6\text{Si}_4\text{O}_{10}(\text{OH})_8$] which contain high amount of magnesium. The weathering of these rocks contributes to an excess amount of magnesium in the soil, an imbalance which drives the pH up. A Survey of soil test results from coastal Santa Maria, Guadalupe and Oceano areas, showed the excessive amount of exchangeable magnesium, which occupied more than 20% on the cat-ion exchange sites. The average pH value in the surveyed area was 7.35 (Author, 2006).

Apart from the predominantly basic pH in the area, concern for a fungal disease known as *plasmodiophora brassicae Woronin* (clubroot) has prompted growers in the area to practice liming with the intention of keeping the pH high. *P. brassicae*, which infects the roots of cole crops such as broccoli, cauliflower, favors acidic conditions and well compacted soil (Campbell et al., 1985). One of the conditions caused by excess magnesium is soil hardening and tightening (compaction) (Kinsey et al., 2006). Wet field plowing as a result of intensive production in the area also contribute to soil compaction (Reade, 2006).

Various studies have been conducted to improve the quality of lettuce in general. Interest in the study of romaine lettuce also includes assessing soil and plant nutrient status to increase the weight and the plant shelf life. Plants having taken up adequate amounts of the needed nutrients are expected to be less susceptible to diseases or to secondary infections such as botrytis, which greatly reduce shelf life.

Effects of copper deficiency on plants

Various plant diseases have been found to be related directly or indirectly to copper deficiency. Wheat plants with insufficient Cu have been found to be also susceptible to stem melanosis, which is caused by bacteria. The presence of stem melanosis was noted by Kruger et al. (1985) to identify fields with possible Cu deficiency. Increased susceptibility of cereals to diseases when Cu is deficient was reviewed by (Graham (1983), and included powdery mildew (*Erysiphe graminis* f. sp. *avenae*), tan spot, ergot [*Calviceps purpurea* (Fr.) Tul.], take-all (*Gaeumannomyces graminis*), and leaf rust. All of these diseases, except ergot, are diseases of leaf or stem tissue. In addition to the effects on leaf disease susceptibility, evidence exists that pollen sterility and male sterility are enhanced when Cu is deficient in cereal crops (Graham 1975) Cu deficient plants produced smaller anthers, and the pollen had a high incidence of sterility. This might explain why a head disease of cereals, such as ergot, might have a higher incidence with Cu deficiency. If the flower is not pollinated, it has a tendency to remain open for a long period and hence become susceptible to infection by spores of the ergot fungus.

Copper deficiency may also play a role in increased infection because of its importance for cell wall lignification. The most typical anatomical change induced by copper deficiency in higher plants is impaired lignification of the cell walls. When lignification is disrupted, cell walls are more susceptible to penetration by fungi (Graham, 1983). This condition gives rise to wilting (impaired water transport) characteristics of distortion of young leaves, bending and twisting of stems and twigs in cereals; and reduced disease resistance (Graham, 1983).

Excessive soil Copper Concentration

Excessive soil copper levels have not been a problem in crop production. Toxicity however, may exist when copper is applied annually for some vegetables, either as a soil amendment or component of fungicides (Vitosh et al., 1994). When the concentration exceeds 150 ppm in pre harvest leaf tissue, toxicity may occur (Vitosh, 1994). Toxicity often results in plants stunting, bluish tint to leaf color and leaf cupping followed by necrosis. The generally recommended soil DTPA extractable Cu concentration for most vegetable crops are between 1 - 2 ppm. The rates of soil applied Cu commonly used in highly responsive crops such as lettuce and leaf lettuce are about 3 – 6 lbs per acre (Vitosh, 1994). A study to evaluate the effect of Cu carrier, Cu rate and their placement was conducted on hard red spring wheat. They found broadcast application of CuSO_4 worked extremely well, but banded application (shanked into the seed line) did not correct the deficiency. The yield obtained with broadcasted application of 23.9 lbs per acre was more than twice as much compared to banded application of the same rate (Varvel, 1983). Applications of 12 lb Cu and 4 lb Cu per acre for the first two crops, respectively, on virgin land

are usually sufficient for subsequent crops (Forsee, 1940; Kretchmer and Forsee, 1964 in Sanchez, 1990). Sanchez, 1990 stated that no further Cu fertilization is required.

Foliar Applied Fertilizer

Foliar application of copper in the form of inorganic salts, oxides, or chelates are required as a means of rapidly correcting copper deficiency in soil-grown plants but soil applied copper fertilizers are more appropriate for long-term effects (Marschner, 1986).

Researchers have found that foliar feeding maintains a better overall nutrient balance within the plant, which may not be achieved through soil uptake alone. Root distribution, soil temperature, available soil moisture, soil-nutrient imbalance and other factors can limit nutrient absorption through the roots. Copper is the most immobile micronutrient, so anything that inhibits new root growth will inhibit copper uptake. Evidence indicated that when Cu was sprayed on certain limbs of the Cu-deficient almond tree, the leaves and kernels on unsprayed limbs recovered from the Cu deficiency and their Cu levels increased significantly, suggesting phloem mobility of Cu (Kester et al., 1961 in Mortvedt et al., 1991).

Foliar application of fertilizer is not intended to replace soil fertility management and therefore should be thought of as the last resort to increase the yield (Simonne, 2007). The optimum ranges for leaf copper level for lettuce are between 5.6 and 8.2 ppm at mid growth and at pre-harvest between 5 and 8.6 ppm (Hartz et al., 2007).

Soil Applied Fertilizer

Various products, liquid or dry fertilizer, to improve the yield of romaine lettuce have been introduced and are available in the market. CATS or calcium thiosulfate (CaS_2O_3) is a soil applied liquid fertilizer recommended to improve the yield of lettuce. CATS is expected to improve plants' uptake of micronutrients by providing slight acidity to the root zone which promotes micronutrients availability. The mechanism is such that sulfate will combine with magnesium or whichever is in excess and will drive the salt below the rootzone, hence reducing the ECe (salinity) of the soil (Ruehr, 2006). Lettuce favors low salinity (ECe) therefore CATS provides an ideal condition.

A recent study to evaluate the performance of CATS and foliar fertilizers for increasing the weight and quality of romaine lettuce has produced a positive response (Author, 2008). Both fertilizer methods were applied at rates recommended by each of the two suppliers, Tessenderlo Kerley and Nutrient Technologies respectively. The amounts of nutrients applied were not comparable between the two methods, but each was promoted to improve the yield of head and leaf lettuce which includes romaine lettuce. Foliar applied fertilizer produced considerably more plant weight than CATS but both foliar and CATS performed better than the control (untreated). The mean ECe concentration (electrical conductivity in mmhos cm^{-1}), was found to be the lowest in the CATS applied soil, although not significantly so. The result of this study helped in providing support to the foliar copper study.

The Experiment Carried Out

In this experiment we applied various rates of foliar copper in the form $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (copper sulfate-pentahydrate ~25% Cu) on romaine lettuce planted in low copper soils (less than 1 ppm) by extraction with DTPA (Diethylene Triamine Pentaacetic Acid). Four rates of copper sulfate were applied in two levels, one time application (level #1) and split application (level #2) of these different rates. The location for the experiment was Betteravia Farms, a vegetable farm in coastal Santa Maria, California. The sites selected were fields previously tested to have low soil copper concentration of below 1 ppm, by Diethylene Triamine Pentaacetic Acid (DTPA) extraction. No micronutrients had been added to the soils during the recent years and no pesticide containing copper had been applied.

Our study with foliar copper began with a field selection process at the beginning of 2008. The establishment and completion of three consecutive experimental fields (experimental field #1, #2 and #3) began in May and completed in October of 2008.

Experimental field #4 was established in January, and completed in March, 2009. The data from the first three experimental fields was evaluated collectively at the completion of the experimental field #3. The data from field #4 was evaluated independently and summarized in March, 2009.

Materials and Methods

The study of foliar copper application commenced early in 2008 with composite soil samples from the top 30 cm depth being collected from the various fields scheduled to be planted with romaine lettuce. A complete soil analysis was conducted to determine the nutrient status prior to planting. The soil samples were air dried and pulverized to pass through a 10 mesh sieve (<2.0 mm). This sampling procedure was used for all of the following soil analyses.

Soil Analyses

Soil pH

The pH of each sample was measured using the saturated paste method. 200 grams of the prepared soil sample was placed into a container, deionized water was gradually added and mixed with the soil to obtain a saturated paste. The mixture was capped and allowed to sit for four hours prior to measuring the pH using a pH meter.

Electrical Conductivity (EC)

A saturated soil paste extract previously prepared for pH reading was used to obtain the soil electrical conductivity (ECe).. The saturated paste was scooped into a Buchner funnel above an Erlehnmeier flask, connected to a vacuum pump for extraction. The extract collected was

used to determine soil electrical conductivity ($\mu\text{mhos/cm}$) using a using the conductivity meter.

Particle Size Distribution of Soil

Particle size distribution was quantified by hydrometer using the modified Bouyoucos hydrometer method (Butte County Soil Survey Area 612). 50 grams of soil was weighed and transferred into 250 mL Erlenmeyer flask. 5 grams of sodium hexametaphosphate and 150 mL of deionized water was added to the flask and swirled. The flask was capped with a rubber stopper then shaken for one minute. The flask was allowed to sit for five days, shaking it vigorously two or three times a day. The sample then was transferred into a one liter graduated cylinder and the volume brought up to one liter with deionized water, the temperature at 40 seconds of the suspension was recorded. Using a brass plunger the solution was plunged up and down twenty times. After 20 seconds, the hydrometer was lowered into the suspension. After another 20 seconds, the forty seconds reading of the time was recorded. The solution was allowed to sit for six hours, after which the hydrometer was again lowered into the solution. When the hydrometer stabilized, a second time reading was recorded followed by temperature reading. The data was calculated to determine the percent sand, silt and clay and the soil texture of the sample.

Plant Available Phosphorus

Plant available phosphorus was assessed by the sodium bicarbonate extraction method (Olsen and Sommers, 1982). Two grams of soil sample was placed in a 125 mL plastic extraction Erlenmeyer. 40 mL of 0.5 N NaHCO_3 extraction solution was added to it to include a method

blank and standard quality control sample. The extraction vessels were then placed on an oscillating shaker for 30 minutes and the suspension filtered immediately. 3 mL of aliquot were measured into test bottles, 9 mL of deionized water was added to each, followed by 3 mL of ascorbic/molybdate reagent. After ten minutes, the sample was read for plant available phosphorus, using spectrophotometer at absorbance of 882 nm.

Exchangeable Basic Cations

An ammonium acetate extraction was made to test for the soil exchangeable K^+ , Ca^{2+} , Mg^{2+} and Na^+ ions. 2.5 grams of soil sample was weighed and placed into an extraction vessel. 25 mL of ammonium acetate 1N NH_4OAc extraction solution was added, and placed into a shaker for 30 minutes. These extracts were measured using the ICP-Oes to determine the concentrations of K, Mg, Ca, and Na. The exchange capacity of the soil was determined from the ratio of the above elements. (Thomas, 1982).

Cu^{2+} was analyzed using the Diethylene Triamine Pentaacetic Acid (DTPA) chelate extraction method on the atomic absorption spectrophotometer (Lindsay and Norvell, 1978). Fields having low copper concentration of less than the minimum recommended 1 ppm Cu were selected as candidates for the experimental fields.

Sample Crop Selected for Foliar Copper Application Investigation

The romaine variety of lettuce (“Hearts Delight”) was selected for the foliar copper fertilizer experiment. “Hearts Delight” is a light colored variety commonly sold as salad pack to complement the darker variety and it is most often sold by weight. The minimum desired weight is 32 lbs per carton, at 24 heads per carton which translates to 1.33 lbs per head.

Field Design and Treatment

During the early summer of 2008, the first experimental field (Exp. #1) was selected for the copper application study on romaine lettuce. The DTPA extracted soil copper concentration of the soil was 0.6 ppm. The soil texture was a loam (24% clay, 42% sand and 34% silt), soil pH was 7.78. This was established in a completely randomized design. Five treatments, at two levels of copper applications were applied, and four replicates were established in the trial for a total of 40 experimental units. The experimental field was established within an existing field of romaine lettuce. The experimental units used the variety “Hearts delight” direct seeded on May 3, 2008. The usual grower practice was that of soil testing prior to planting. Due to sufficient nutrient carryover from the previous crop, no starter fertilizer (or pre plant fertilizer) were necessary here before planting. The experimental units measured 20 feet long by 38 inches wide. The total experimental plot including borders was one tenth of an acre.

The treatment rates were, starting with control (untreated soil), then 100, 200, 400 and 800 grams of elemental copper equivalent per acre applied as a basic copper sulfate (20 % copper) as a product of Nutrient Technologies. The two levels represented differing application times. Level #1 constituted a one time application during the growing season, at mid-growth stage (six to eight weeks after planting). Level #2 (split application) constituted two applications during the growing season where half of the rate was applied at mid-growth stage and the other half was applied the following week. The identifications codes for the treatments are listed on table 1. Treatments #1 and #2 represent control; treatments #3, #5, #7, and #9 represent level #1 or single application and treatments #4, #6, #8 and #10 represent level #2 (split application). Soil temperature data were collected twice daily to determine the average daily temperature. PSNT (pre-side-dress Nitrate testing) was conducted prior to scheduled irrigation to determine nitrogen status and the need to apply additional nitrogen fertilizer (Hartz, 2000). The field, including the trial plot, received no additional nitrogen, throughout the growing season. The total amount fertilizer added to the field was recorded.

Table 1: Foliar Cu applied to romaine lettuce in grams per acre for experimental fields #1, #2 and #3 In 2008

Application level	Treatment Identification code for (CuSO ₄) applied - (grams/acre)									
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Level #1 (one application)	0		100		200		400		800	
Level #2 (split application)		0		2 X 50		2 X 100		2 X 200		2 X 400

The foliar copper fertilizer was applied at the scheduled time and rate by using a hand sprayer and dissolving the copper sulfate with water to deliver an application rate of 50 gallons per acre. For example, treatment #3 for level #1 received 100 grams of copper sulfate per acre.

Treatment #4 for level #2 received 50 grams of copper sulfate per acre initially. The following week, treatment #4, #6, #8, #10 for level #2 received the remaining: 50, 100, 200, or 400 grams per acre, respectively.

Plant Tissue Sampling and Preparation

Fifteen whole leaf plant samples were collected from each of the forty experimental units, within one week of harvest. The whole leaf samples were washed with 1% solution of liquinox (lab ware detergent), each leaf was halved lengthwise. One half of the leaves were discarded and the other halves were cut and placed on wire trays. All the trays containing the samples were placed in a convection oven at temperature 40° F for 24 hours. The dried samples were ground to a fine particle size of <1.0 mm and placed in storage bottles for further analysis.

Nitrate Nitrogen (NO₃-N) in Leaf Tissue

The concentration of leaf nitrate in parts per million was determined by extraction with an aluminum sulfate solution for subsequent determination by ion-selective electrode (ISE). The ISE determines NO₃-N by measuring an electrical potential developed across a thin layer of water, immiscible liquid, or gel ion exchanger that is selective for NO₃ ions. This layer of ion exchanger is held in place by a porous membrane. The method has been used primarily to determine NO₃-N for assessing plant nitrogen fertility (Chapman and Pratt, 1961). The extraction that follows is the standard practice of Betteravia Farms in-house laboratory.

0.25 grams of ground oven dried plant samples were weighed and placed in blender jars, 50 mL of Aluminum Sulfate extracting solution (0.03 M) were added to the samples and blended for

one minute. The samples were measured directly using Ion Selective Electrode (Hach Sense-Ion 4). The results were compared with the grower's record of nitrate-N sufficiency guideline for lettuce. Vital data relative to nitrogen $\text{NO}_3\text{-N}$ and phosphate phosphorus ($\text{PO}_4\text{-P}$) were gained from the laboratory testing. Although their importance does not bear directly on the subject of this study, it is anticipated that future research into soil nutrients will deal with their significance.

Phosphate Phosphorus (PO_4P) in Leaf Tissue

The concentration of leaf phosphate phosphorus in parts per million was determined by extraction with deionized water and subsequent determination using the spectrophotometer. The following was the standard practice of Betteravia Farms' in-house laboratory in Santa Maria, California to assess plant's phosphate sufficiency levels. 0.50 grams of oven dried ground samples were weighed and placed in blender jars. 100 mL of deionized water and $\frac{1}{4}$ teaspoon of charcoal was added to the solution and blended for one minute. The samples were filtered through Whatman #2 filter paper and the filtrates analyzed using Hach DR2000 spectrophotometer at 890 NM.

Metal Ion Concentrations in Leaf Tissue

Dry ash method quantitatively determines the concentrations of potassium (K^+) and copper (Cu^{2+}) using a high temperature dry oxidation of the organic matter and dissolution of the ash with hydrochloric acid. One gram of the plant samples were weighed and placed in porcelain crucibles. Each crucible was placed in a muffled furnace with ramp temperature to 500°C over two hours, then ashed for four hours at an additional 500°C . The samples were then

allowed to cool at room temperature in the oven with the door slightly open. After cooling, each sample was dissolved with 10 ml of 1.0 N Hydrochloric acid. The contents were transferred into 50 ml volumetric flask and diluted to volume with deionized water. The flasks were capped and inverted three times and filtered through Whatman #2 filter paper into sample beakers. Each sample was analyzed for the percent potassium (K^+) and parts per million of Cu^{++} using atomic absorption spectrometry (AAS). The level of leaf copper in each treatment was compared to the established recommended DRIS pre-harvest optimum level for lettuce (Hartz et al., 2007).

Field Harvest Procedures

On the scheduled harvest day, a commercial harvester was used to cut all the marketable heads of romaine in each experimental unit. A total of 480 heads of romaine were weighed and measured in the field. Evaluation was done on weight per plant basis as opposed to cartons per acre, considered as the best method to evaluate the treatments. One box of romaine lettuce heads from each treatment was randomly selected and weighed in the field and then placed into cold storage (using a commercial cooler). These boxes were retrieved after one week for further evaluation by Betteravia Farms' quality assurance person for of the shelf life and quality of the plants.

Experimental Field #2

The experiment was repeated (replicated) in a nearby field where the soil texture was loam (24% clay, 46 % sand, 30 % silt), soil pH was 7.32. The initial DTPA extractable soil copper level was 0.53 ppm . The same light variety of romaine lettuce ("Hearts delight") was planted in June 17th

and harvested in August 21st, 2008. The field received no fertilizer prior to planting. Based on the PSNT (pre side dress nitrate testing) conducted between irrigation schedules the field received no additional fertilizer through the life cycle of the crop. As in the previous experimental field #1, no copper fertilizer or copper containing fungicide had been applied to the field in recent years.

Experimental Field #3

The experimental field #3 was established in a field of romaine lettuce where the soil texture was loam (18% Clay, 48 % sand, 34 % silt), the soil pH was 7.70. The initial DTPA extractable soil copper level was 0.65 parts per million. Light colored variety of romaine (“Heavy Heart”) was planted in August 5th and harvested in October 13th, 2008. “Heavy heart” is the same light colored variety as “Hearts delight”. This change in romaine variety was due to its only availability of light variety in the fall planting season.

Similarly, the first foliar copper sulfate application was made between six to seven weeks after planting, and the second foliar copper sulfate application was made one week later. The treatments applied for level #2 were the same as those used for level #1 on the same plots (as listed in table 1). As in the first experimental field, the second and the third experimental fields were harvested by using a commercial harvester. The harvest and evaluation procedure was identical to the one done in the first field assurance official.

Experimental Field #4

In 2009, we placed an additional experimental field, transplanted in a field of romaine lettuce where the soil texture was sandy loam (8% Clay, 70 % sand, 22 % silt). The initial DTPA extractable soil copper level was 0.8 parts per million and the pH was 7.36. The same light colored variety of romaine Hearts Delight was planted in January 7 and harvested in March 20, 2009.

The treatment rates were modified, as listed on table 2. Starting with control (untreated soil), then 200, 400 and 500 grams of elemental copper equivalent per acre applied as a basic copper sulfate (20 % copper) as a product of Nutrient Technologies. All the three rates of Cu fertilizer were applied at once, and replicated five times for a total of 20 experimental units.

The foliar copper sulfate application was made between six to seven weeks after planting and plant tissue sampling was done one week before harvest. As in the previous experimental fields, harvesting was done using a commercial harvester and the evaluation procedure was identical to the previous fields. Five representative commercial boxes of romaine lettuce were placed into the cool storage for five days prior to further evaluation by a quality assurance official.

Table 2: Foliar Cu applied to romaine lettuce in grams per acre
Experimental Field #4 Jan - March 2009

Treatments	CuSO ₄ (grams/acre)			
	#1	#2	#3	#4
Rate	0	200	400	500

Table 3: Summary of the time frame, planting method and pertinent features of the soils in the four experimental fields

Experimental Fields	Plant date	Harvest date	Method	# of days	Soil Texture	Soil Cu (ppm)	Soil pH
#1	1-May-08	14-Jul-08	Direct seeded	71	loam	0.6	7.78
#2	17-Jun-08	21-Aug-08	Direct seeded	65	Loam	0.53	7.32
#3	5-Aug-08	13-Oct-08	Direct seeded	69	loam	0.65	7.7
#4	7-Jan-09	20-Mar-9	transplant	72	sandy loam	0.8	7.36

Statistical analysis of the final plant data

The final plant data relative to Cu application were evaluated using statistical analysis to determine whether any of the treatments had a statistically significant effect on the weight and quality of the romaine lettuce. The statistical analysis also permitted the differentiation between the single (one time application) and the split application of foliar copper sulfate. The data was analyzed using SAS program version 4.1 and Minitab.

The Experimental field #4 was analyzed independently using the same statistical program and analysis.

Results

For the experimental Fields #1, #2 and #3

The concentration of leaf NO_3 -Nitrogen, PO_4 -Phosphorus and K_2O -potassium were well within the sufficiency level at harvest in all plant samples. The data was analyzed collectively as randomized complete block design, blocked by the experimental fields. The analysis of variance, general linear model, was used to evaluate the effect of the experiment on the weight of the romaine (lbs/plant). A significant difference was identified between the mean weights of the romaine (lbs/plant) of the three experimental fields with p value < 0.0001 (table 4). The experimental field #3 was harvested one week earlier than scheduled which became apparent by the low overall mean weight per plant in all of the subsequent treatments (table 6). As shown on the analysis of variance on table 4, no significant difference was found in the weight increase (lbs/plant) due to neither the level of application (p value 0.5137), nor due to the treatments with Cu sulfate, p value 0.5972. The interaction between the levels of application and treatments was not statistically significant, p value 0.2796. However, in the three experimental fields, the plants responded with the increase of mean weight due to the treatments (table 6). In the experimental fields #1 and #3 the highest mean weight increase was obtained by a one time application of 200 grams/acre of CuSO_4 (Treatment #5). In experimental field #2 a one time application of 400 grams per acre of CuSO_4 (Treatment #7) produced the highest mean weight (table 6). The optimum weight increase in experimental fields #1, #2 and #3 were 4.4, 6 and 4.5 percent respectively (table 7).

The effect of the treatments (foliar Cu sulfate) on the uptake of Cu by plants was most significant at p value <0.0001 as shown on statistical analysis table 5. Cu uptake by plants due to the level of applications was also significant at p value 0.0187. Level #2 (split application) produced a higher leaf Cu uptake than level #1 (one time) application, as illustrated by the graph on fig. 2. No significant interaction was identified between the level of application and the treatments with CuSO₄.

Table 4: Analysis of variance table for the effects of the experimental fields, the levels, the treatments and the interaction between the levels and the treatments on the weight of the plants.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1.51904710	0.13809519	35.59	<.0001
Error	108	0.41910026	0.00388056		
Corrected Total	119	1.93814736			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Expt	2	1.48661613	0.74330806	191.55	<.0001
level	1	0.00166582	0.00166582	0.43	0.5137
TRT	4	0.01078459	0.00269615	0.69	0.5972
level*TRT	4	0.01998057	0.00499514	1.29	0.2796

Table 5. Analysis of variance table for the effect of the experimental fields, level of application and the treatments, on leaf Cu concentration (ppm).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	785101.8181	71372.8926	46.57	<.0001
Error	108	165531.8131	1532.7020		
Corrected Total	119	950633.6312			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Expt	2	74656.5776	37328.2888	24.35	<.0001
level	1	8741.5470	8741.5470	5.70	0.0187
TRT	4	698211.5538	174552.8884	113.89	<.0001
level*TRT	4	3492.1398	873.0349	0.57	0.6852

Table 6: The mean weight of romaine lettuce (lbs/ plant) due to the treatments.

Treatment #	Exp #1	Exp #2	Exp #3
1	1.283	1.327	1.137
2	1.252	1.325	1.135
3	1.306	1.342	1.106
4	1.297	1.396	1.098
5	1.341	1.392	1.188
6	1.313	1.376	1.051
7	1.290	1.408	1.092
8	1.322	1.360	1.043
9	1.313	1.390	1.053
10	1.298	1.387	1.158

Table 7. Summary of the plants' weight increase due to the treatments in the experimental Fields: #1, #2 and #3

Exp Fields	Cu SO ₄	Level	Untreated wt	final wt	% increase
	(grams/ac)				
#1	200	one time	1.283	1.341	4.4
#2	400	one time	1.327	1.408	6
#3	200	one time	1.136	1.188	4.5

The established optimum concentration of leaf Cu at harvest is between 5 and 8.6 ppm (Hartz et al., 2007). The untreated control (treatments #1 and #2) in all of the three experimental fields had sufficient amounts of copper in the leaf tissue at harvest. The highest mean weights were achieved by plants having mean leaf copper concentrations of: 38.050, 110.628 and 87.885 ppm in the experimental fields #1, #2 and #3 respectively (table 8).

Table 8: Summary of the weight of the romaine lettuce relative to the leaf cu concentration at harvest

Treatment #	Exp. Field #1		Exp. Field #2		Exp. Field #3	
	Weight lbs/plant	Leaf Cu ppm	Weight Lbs/plant	Leaf Cu ppm	Weight lbs/plant	Leaf Cu ppm
1	1.283	10.188	1.327	6.031	1.137	16.368
2	1.252	11.538	1.325	7.00	1.135	22.65
3	1.306	12.688	1.342	35.53	1.106	53.875
4	1.297	37.710	1.396	27.838	1.098	58.738
5	1.341	38.050	1.392	42.36	1.188	87.885
6	1.313	58.250	1.376	53.734	1.051	123.075
7	1.290	73.737	1.408	110.628	1.092	120.188
8	1.322	85.597	1.360	85.942	1.043	228.018
9	1.313	174.637	1.390	201.17	1.053	276.015
10	1.298	241.660	1.387	747.79	1.158	289.748

Fig. 2: The effects of the treatments, the experimental fields and the application level on the uptake of leaf Cu

Fig. 3: The correlation between leaf cu concentration (ppm) and the weight of the romaine (lbs)

Results

For the Experimental field #4

As in the previous experimental fields, the concentration of leaf NO_3 -Nitrogen, PO_4 -Phosphorus and K_2O -Potassium were well within the sufficiency level at harvest in all plant samples.

The statistical analysis of the data from the experimental field #4 indicated no significant difference p value 0.190, between the mean weights of the romaine due to the treatments, as shown on the analysis of variance table (table 9). However, as in the previous three experimental fields, the plants responded with an increase in weight as recorded on table 10.

The overall mean weights in all of the treated and untreated plants in this field were higher than the treated plants in the three previous experiments. As illustrated in Fig.5, the treatment with 200 grams per acre of Cu sulfate, produced the highest mean weight. Treatment #4 (500 grams/acre) produced better result than #3 (400 grams/acre), table 10. The optimum weight increase in the experimental field #4 was 7.58%, obtained by treatment #2 (200 grams/acre), shown on table 11.

Table 9: Analysis of variance table for the effect of treatments on the weight of the romaine in experimental field #4.

One-way ANOVA: weight versus treatment					
Source	DF	SS	MS	F	P
treatment	3	0.02953	0.00984	1.79	0.190
Error	16	0.08811	0.00551		
Total	19	0.11763			
S = 0.07421 R-Sq = 25.10% R-Sq (adj) = 11.06%					

Table 10: The effect of treatments on the weight and leaf cu concentration at harvest on experimental field #4

Treatment #	grams/ac	Weight (lbs/plant)	Leaf Cu (ppm)
1	0	1.522	7.035
2	200	1.638	26.764
3	400	1.549	41.766
4	500	1.608	38.516

Table 11: Summary of the plants' weight increase due to the treatments in the experimental field #4

Treatment	Grams/acre	Control (0)	Treated weight	% wt increase
#2	200	1.5224	1.6378	7.58
#3	400	1.5224	1.549	1.75
#4	500	1.5224	1.6085	5.65

Fig.4: The effect of the treatments on the mean weight of the plants in Experimental field #4

Summary of experimental fields #1, #2 and #3

Vitosh et al. (1994) suggested that toxicity may occur when leaf copper concentration exceeds 150 ppm in pre harvest leaf tissue. In all the experimental fields, herein the highest weight per plant had less than 150 ppm leaf copper at harvest. Weight declines were observed at harvest leaf copper concentrations of 58.25, 85.9 and 123 ppm (table 8). No evidence of toxicity such as bluish tint or cupping on the leaf tissue was observed in the plants having copper concentration as high as 290 ppm but excessive copper may have contributed to the weight decline.

Harvesting the plants one week too early as in the case of field #3 produced significantly lower plants' mean weight than those harvested on time. The mean weight of experimental field #3 was 21 percent lower than the previous two experimental fields (table 6). The variety used in experimental #3 was although the similar light colored variety, was not the same therefore, may have had some influence on the outcome.

The effect of leaf Cu on the mean weight of romaine was evaluated to include only experimental field #1 and field #2. As shown on fig. 3, an upward trend of weight per plant was apparent, which peaked around 100 ppm leaf Cu and decreases thereafter. However, the correlation between the weight and leaf Cu concentration was not statistically significant (R^2 8.5%). Post harvest evaluation was conducted by the growers' quality assurance official. Representative samples of each treatment are illustrated in fig. 5, 6, and 7. All experimental plants were found to be of acceptable quality for sale and sale after one week in cold storage.

Fig: 5. Photograph illustration of samples from untreated and treated experimental units after one week in cold storage

Fig.6. Photograph illustration of untreated control and treatment #7 (400 g/acre) after one week in cold Storage.

Fig. 7: Photograph illustration of samples from untreated control and treatment #9 (800 grams/acre) after one week in cold storage

The expected minimum weight for commercial romaine lettuce is 32 lbs per carton of 24 plants (growers' communication), and equivalent to 1.33 lbs per plant. A carton of romaine lettuce weighing 35 lbs (1.46 lbs/plant) falls into the category of premium grade, the weight should not exceed 40 lbs. The optimum weight increase due to the treatments for each of the individual experimental fields was previously discussed and summarized on table 7.

As shown in Fig. 8 the application level 1 (one time application) produced significantly better results than level #2 (split application). The combined experimental fields indicated that, treatments #5 (a one time application) of 200 grams per acre of Cu sulfate produced the best result in increasing the weight of the romaine. Treatment #10 (split application of 800 grams/acre) also responded, in weight increase, although the cost of double application may outweigh the benefit. One time application of 400 grams/acre of CuSO_4 may prove to be more economically beneficial, as previously summarized on table 7 (page 26).

Fig.8: The effect of the application level and treatments on the mean weight of the plants in Experimental fields #1, #2 and #3

Summary of experimental field #4- confirming previous observations

As shown on table 10, the highest increase in weight in the experimental Field #4 was obtained by the application of 200 grams per acre of Cu sulfate. The mean weights of the untreated plants in the experimental field #4 were higher than the maximum weights per plants observed in the three previous experiments. Soil texture was sandy loam 8% clay for the experimental field #4, whereas in the three previous experimental fields the soil texture were loam 18-24% clay, a factor which must be noted. The mean soil Cu concentration on field #4 at 0.8 ppm was slightly higher than the three experimental fields at 0.5-0.6 ppm. The two factors combined may have influenced the outcome.

It was apparent that the overall mean weights of the plants in field #4 were higher than the previous three fields. The untreated field has a mean weight equivalent to 36.5 lbs/carton which would be considered premium. As in the previous three experimental fields, the mean leaf Cu concentration was sufficient at 7.035 ppm, with a mean soil Cu concentration of 0.8 ppm.

Fig.9 illustrates the treated and untreated samples from field #4, at harvest.

From photograph illustration of the untreated and treated cross-section samples of the plants in the experimental field #4 (Fig. 10), we observed larger core area and leaf density on the treated plants as compared to the untreated.

Fig. 9: photograph illustration of samples from untreated control and treated samples from experimental field #4 at harvest, March 20, 2009

Fig. 10: photograph illustration of the untreated and treated cross-section samples of the plants from the experimental field #4. March 20, 2009

Conclusions:

The application of foliar fertilizer does not replace good soil nutrient management. However, the result of these experiments indicated that romaine lettuce grown in low copper soils (<1 ppm DTPA extractable copper), responded to and benefited from the application of foliar copper sulfate.

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