Copper

IN TOBACCO PRODUCTION

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Copper is an element essential to plant growth, although the requirement is extremely low, being less, for instance, than that of boron. Only a trace seems to satisfy the need for the catalytic function it performs in the plant.

It has been suggested[2] that copper functions indirectly in chlorophyll formation, in such a way that copper compounds unite with the chlorophyll. The proteids of the chlorophyll complex, hence the coloring matter, become more stable and are less influenced by fluctuations in light. Moreover, reference has been made to the possible role of copper in the enzyme system, again functioning as a stabilizer.

The amount of copper needed depends on the fixing power of the soil. The fixing or buffering properties may be organic matter, lime, or high content of other bases.

According to McMurtrey,[3] copper deficiency in tobacco is shown by severe and permanent wilting of the upper leaves, and by a reduction in growth, somewhat in proportion to the extent of copper shortage.

Although the wilting symptoms never have been observed on field grown tobacco, reduced crop growth is often puzzling to the investigator. In some cases, the reduction cannot be laid to known disease troubles or nutrient deficiencies.

In the Everglades of Florida, relatively large quantities of copper sulfate are applied to vegetable crops. There the material is not applied primarily as a plant nutrient but as a conditioner for high-organic soils, in order to get a proper utilization of the large amounts of fertilizer that they require.

The function of copper sulfate as a soil amendment for tobacco has been demonstrated in Virginia, the Carolinas and Georgia, according to reports from the Crop Protection Institute, Washington, D. C.[4] In a summary of 1935 data, they state that a fertilizer containing 50 pounds of copper sulfate per ton, applied at the rate of 1,000 pounds per acre, produced an average increase in value of tobacco of 54.29 per cent. The average yield was increased 43.9 per cent and the quality, 10.39 per cent. The test included 14 trials. In 1936, in 11 trials, the increased value of tobacco amounted to 33.85 per cent, of which yield accounted for 18.4 per cent and quality, 15.45 per cent.

The tobacco soils in Connecticut undoubtedly contain sufficient copper for normal plant growth. However, due to the relatively high fertilizer require-

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[2] Sommer, Anna L. Copper in plant growth. Soil Sci. 60: 71-79. 1945.

[3] McMurtrey, J. E. Plant nutrient deficiency in tobacco. Hunger Signs in Crops: 33-35. 1941.

[4] Churchman, W. L., M. M. Manns and T. F. Manns. Copper sulfate as a plant nutrient and soil amendment. Bul. Ser. 63: 4-14. 1936.

ments for cigar tobacco, it may be that we are not receiving full benefit from the annual applications of fertilizer. Unknown disease or other malady factors may prevent growers from obtaining still better yields and gradings. Would applications of copper sulfate benefit Connecticut tobacco as it has crops in other districts? In order to find the answer to this question, a series of experiments was undertaken here. The following is a report on three years of research.

PLAN OF EXPERIMENT

As a preliminary trial in 1947, three plots (19 x 40 ft.) were fertilized at the regular rate of 3,400 pounds 6-3-6 to the acre. Copper sulfate, mixed with the fertilizer, was added at the rate of 18, 36 and 72 pounds per acre. The adjacent part of the field, receiving the equivalent amount of fertilizer but no copper, served as a check. The entire field was planted with Havana seed tobacco.

In 1948 a more extensive experiment was laid out. Results from the previous year had indicated the necessity of a closer observation on amount of copper sulfate application between the 18 and 36 pound rates. Thus, it seemed logical to include a rate of 27 pounds. The 72-pound rate was omitted. Therefore, four rates of 18, 27, 36 and 54 pounds of copper sulfate per acre were employed. Each rate was applied to quadruplicate plots, with no copper sulfate added to four check plots. All the plots were arranged at random within the four blocks. The same acre-rate and type of fertilizer were used as in 1947, and Havana seed tobacco was planted.

Based on 1948 results, only one rate, 20 pounds copper sulfate to the acre, was tried in 1949. Quadruplicate plots with this treatment were compared with the same number of check plots, all randomized in one block. The plot size was increased to 1/40 acre. Otherwise, the fertilization and plantings were the same as in previous years.

Soil samples were taken and analyses made during the 1948 season for the purpose of estimating the effect of copper on nitrification and the amount of active copper in the soil.

RESULTS OF 1947 FIELD EXPERIMENTS

In 1947 the tobacco on the copper sulfate plots grew considerably larger than tobacco in the surrounding areas. In fact, the tobacco on the three plots in question appeared to be the largest on the experimental farm.

There was little difference among plots receiving various amounts of copper sulfate. Early in the season, however, it was noticed that the plants on the plot with the highest rate of copper sulfate were darker green and progressed slower than the rest of the tobacco. It is possible that the higher rate had a depressive effect, although the final results (Table 1) indicated that possible injury from surplus $CuSO_4$ was of a passing nature.

TABLE 1. YIELD AND GRADING RECORDS OF COPPER SULFATE TEST, 1947

Pounds copper sulfate per acre	Yield pounds per acre	Grade index [1]	Crop index [2]	Relative crop value
None	2100	.468	982.8	100.0
18	2449	.514	1258.8	128.0
36	2362	.546	1289.7	131.2
72	2423	.546	1323.0	134.6

[1] Grade index is a figure which represents the *relative* value of a lot of tobacco computed on the percentage weight of each grade of leaves in the lot and the relative values of these grades. Assuming that the light wrapper is the perfect leaf of Havana Seed tobacco, we assign to it a value of 1. The other grades are assigned values of the same proportion to 1 as their market value was to the price of the light wrapper when this system was established. Thus, medium wrappers have a value of .60; long seconds (19 inches or more) are .60; short seconds (15 inches and 17 inches) are .30; long darks are .30; dark stemming (short darks of 15 inches and 17 inches) are .20; fillers and brokes are .10. It is true that the values of these grades have fluctuated considerably during the 15 years that we have used this system of comparing lots of tobacco, but in order to be able to average results over a period of years it seems advisable to retain the same system for the present. To obtain the grade index figure, the percentage of each grade in a lot of tobacco is multiplied by the relative values noted above and then the products are added.

[2] Crop index is the product of grade index and yield.

From these results it is seen that both yield and grading (quality) of tobacco improved 28, 31.2 and 34.6 per cent, respectively, from applying 18, 36 and 72 pounds copper sulfate per acre to tobacco land. The improvement was balanced about equally between yield and grading.

The maximum increase in yield was reached with the lowest application of the copper salt, while higher grade index values were obtained with the higher rates. The optimum rate, therefore, seemed to fall between 18 and 36 pounds per acre.

In addition, burn tests,[1] made on cured (not fermented) leaf samples showed the following results:

Pounds copper sulfate per acre	*Av. duration of burn in seconds*
18	41.3
36	45.3
72	48.3

Thus, it seems that copper sulfate has no adverse effect on burn. No reduction in duration occurred; rather, duration of burn increased as copper sulfate applications were increased.

The results from this pilot experiment were sufficiently promising to warrant more extensive testing the following year.

RESULTS OF 1948 FIELD EXPERIMENTS

In 1948 the tobacco grew vigorously on the test plots and was noticeably better than that on surrounding fields. In places, however, independent of treatments, areas of inferior growth were found. Examination of the roots of plants showed the presence of many nematodes which may have accounted for the poor spots in the field. An adjacent field showed a near crop failure,

[1] The capacity of tobacco to hold fire is often tested by holding the leaf taut between the hands and touching it with a lighted cigar. In the laboratory, a hot electric filament is used and the time (in number of seconds) the leaf will remain glowing is recorded.

definitely caused by nematodes. In compiling the yield and grading data, therefore, it was necessary to correct statistically four of the individual plot results (Table 2).

TABLE 2. YIELD AND GRADING RECORDS OF COPPER SULFATE PLOTS, 1948

Treatment	Plot	Yield lbs. per A. Plot	Yield lbs. per A. Av.	Grade Index Plot	Grade Index Av.	Crop index	Relative crop values	Original (not corrected) relative crop values
Check (No Cu)	A	1889[1]		.438[1]				
	B	1875	1988	.487	.456	906.5	100.0	100.0
	C	1980		.440				
	D	2209		.457				
18 lbs. $CuSO_4$ Per A.	A	1983[1]		.490[1]				
	B	1969	2102	.495	.490	1030.0	113.6[2]	116.3
	C	2204		.500				
	D	2250		.508				
27 lbs. $CuSO_4$ Per A.	A	1969		.498				
	B	2160		.448				
	C	1940[1]	2027	.435[1]	.475	962.8	106.2	106.6
	D	2037		.479				
36 lbs. $CuSO_4$ Per A.	A	2016		.476				
	B	2070		.490				
	C	2250	2091	.495	.475	993.2	109.6	116.3
	D	2029		.440				
54 lbs. $CuSO_4$ Per A.	A	1861		.438				
	B	2063		.465				
	C	1956[1]	1968	.446[1]	.455	895.4	98.8	105.5
	D	1990		.469				

[1] Corrected values.
[2] Statistically significant difference from check, at odds 19 to 1.

The highest yield and grading were obtained at the acre-rate of 18 pounds copper sulfate. The 13.6 per cent increase in relative crop value over the control was significant at odds 19 to 1. Contrary to expectation, the 27-pound rate did not result in maximum or optimum values. The 36-pound rate showed results superior to the latter, even with corrected data. It is possible that nematodes played a part here, which, however, could not be adjusted by simple probability technique. In the last column of Table 2 the relative crop values without adjustment are given.

The land used in 1948 was on a different location than the 1947 plots and might have required less copper for proper growth and development of tobacco, in view of the fact that the 54-pound rate seemed to have a slight depressive effect.

RESULTS OF 1949 FIELD EXPERIMENTS

The early part of the growing season in 1949 was extremely dry. It was, therefore, necessary to irrigate the field five times at about a week to 10-day intervals. With ample soil moisture there was vigorous growth and slightly better appearance of the tobacco receiving "copper".

The improvement, however, was chiefly in grading, as revealed when the crop was sorted and weighed. Data collected at that time are shown in

Table 3. It was found that both yield and grading of tobacco grown with copper sulfate were far superior to the check. The improvement in crop value (yield and grading), amounting to 26 per cent, is highly significant. Of this, grading accounted for about 15 units and yield, for the balance.

TABLE 3. YIELD AND GRADING RECORDS OF COPPER EXPERIMENTS, 1949

Treatment	Plot No.	Yield lbs. per A. Plot	Yield lbs. per A. Av.	Percentage of grades [1] L	M	LS	SS	LD	DS	F	B	Grade index Plot	Grade index Av.	Relative crop value
Regular	1	1902		2	3	42	5	28	8	8	4	.417		
fertilizer	2	1892	1855	6	5	29	9	31	10	6	4	.414	.403	100.0
without	3	1835		4	6	32	8	31	10	8	1	.414		
$CuSO_4$	4	1791		3	4	23	13	33	14	8	2	.368		
Regular	1	2015		8	8	36	6	29	6	5	3	.459		
fertilizer	2	2121	2028	9	7	35	6	29	6	6	2	.467	.463	126.0[2]
with 20 lbs.	3	1995		10	8	31	8	28	7	6	2	.464		
$CuSO_4$ per A.	4	1980		9	8	32	8	28	9	5	1	.462		

[1] See footnote under Table 1.

[2] Highly significant; odds 99 to 1.

In general, the riper (but not the ripest) leaves, situated two-thirds up the stalk of the plant, are the most valuable. The copper sulfate treatment reduced the amount of less desirable leaves ("darks") at the upper part of the plant by about 6 per cent (by weight). (See percentage of grades, Table 3.) This suggests that the copper sulfate treatment speeded up the ripening processes to some extent.

Burn tests on leaves from the 1949 crop gave these results, in duration of burn[1]:

Check	"seconds"	14 seconds	
	"darks"	7 "	Av. 10.5 seconds
Cu treatment	"seconds"	23 "	
	"darks"	8 "	Av. 15.5 "

Each final average figure for the check and the treatment is based on 160 individual tests. The burn may be significantly better for the copper treatment, but it is sufficient to conclude that copper sulfate will in no way impair the burn of tobacco.

THE EFFECT OF COPPER SULFATE ON NITRIFICATION

As early as 1916[2] the effect of copper sulfate on nitrification was observed in Colorado. The rate of 100 pounds per acre on fallow soil reduced the amount of nitrates by about 60 per cent, as compared with the check plot.

It is conceivable that such a relatively high rate of application might kill some of the nitrifying organisms. The tendency seems to be the reverse with small amounts of copper sulfate, as suggested from the results of nitrate

[1] The magnitude of burn depends on the potash level in the soil; thus, a high duration is concomitant with a high level of K_2O and, reversely, a low level means a low average content of K_2O in the soil.

[2] Jensen, C. A. Nitrification and total nitrogen as affected by crops, fertilizers and copper sulfate. Jour. Amer. Soc. Agr. 8: 10-22. 1916.

determinations in 1948 (Table 4). The lowest rate of copper sulfate application (18 pounds per acre) consistently caused a higher rate of nitrate production than the check plots. Some stimulation occurred also at the rate of 27 pounds per acre. Thus, it is safe to conclude that a rate of 20 pounds copper sulfate per acre will have no harmful effect on nitrification, but might instead be beneficial in that respect.

TABLE 4. NITRATE NITROGEN IN SOILS OF COPPER SULFATE PLOTS, 1948

Copper sulfate per A.	NO_3-N [1] in parts per million			Relative NO_3-N producing capacity
	June 15	July 15	August 15	
None	8.7 [2]	61.8	3.7	100
18	10.1	83.5	6.6	135
27	9.1	75.9	6.1	123
36	8.5	59.3	3.4	96
54	8.2	59.7	3.7	96

[1] The field before application of fertilizer contained only .44 p.p.m. NO_3-N.
[2] Each figure represents the average of four replicates.

COPPER IN THE SOIL

Analyses of both total and available copper in test plots were made in 1948. The methods of determining copper in soils are not satisfactory and cannot be expected to give accurate results. The use of a spectrograph is probably the best means so far devised.

Total copper was determined spectrographically [1] on soil samples from all plots in the 1948 tests. The results, each an average of four plots, are given below:

Pounds $CuSO_4$ per acre	*Total copper (p.p.m.)*	*Expected content of Cu [2] (p.p.m.)*
None	5	5
18	6.5	7
27	7.5	8
36	6.5	9
54	10.0	11

With one exception, the recovered copper was only one-half to one part per million less than the expected amount. The results suggest that practically all the copper added remains in the soil. The following tests show that most of it stays in a fixed form.

Active or readily available copper in the soil was determined according to a method suggested by Hurwitz,[3] which involves the use of oat-straw or alfalfa meal. It was found that soybean (straw) meal gave better results than the two materials mentioned. Thus, with this modification of the method, the soils were extracted and Cu determined colorimetrically. It was difficult to obtain stable colors, but fairly reliable results were obtained from three treatments in quadruplicate plots. The averages are given below.

[1] The spectrographic analyses were made by the Department of Analytical Chemistry at the Station.
[2] Assuming that the determination for the check plot is correct, 18 pounds $CuSO_4$ per acre would be expected to furnish an additional 2 p.p.m. Cu.
[3] Hurwitz, Charles. Extraction of copper from soil as affected by soluble components of oat-straw and alfalfa meal. Soil Sci. 65 (3): 275-300. 1948.

Check	0.375 p.p.m. active Cu
36 lbs. $CuSO_4$ per A.	0.715 " " "
54 " " " "	0.900 " " "

For more reliable results, soil extracts from all the treatments in quadruplicate were subjected to spectrographic analysis. The results are shown below. Each figure represents an average of four replicates.

Check	0.475 p.p.m. active Cu
18 lbs. $CuSO_4$ per A.	0.200 " " "
27 " " " "	0.710 " " "
36 " " " "	1.340 " " "
54 " " " "	1.190 " " "

The content of active Cu, calculated on air-dried soil, seems rather irregular, but the magnitude is about the same as obtained colorimetrically. The results suggest that the amount of active Cu in the soil is not very high and is only slightly influenced by additions of copper sulfate to the land. There is little likelihood that all the so-called active Cu would be readily absorbable, as judged by the amounts found in the leaf tissue.

COPPER DEPOSITED IN THE LEAF

Analyses of the amounts of copper found in the leaf were made in all three years the experiments were conducted. After grading the crop, representative samples of the various grades were oven-dried and portions were ground to a fine powder in a porcelain mortar. Weighed portions were ashed and dissolved in solutions of strong acids and subjected to spectrographic analysis for Cu. The results are listed below.

Average Cu content (p.p.m.) of quadruplicate tests, air-dried basis:

Pounds $CuSO_4$ per acre	*1947*	*1948*
None	58	47.5
18	56	44.5
27	..	55.1
36	37	37.0
54	..	37.0
72	23	...

The 1949 results, based on only one application rate (20 pounds of copper sulfate per acre), showed that an average of 72 p.p.m. of copper was found in leaves from the copper treated plants, compared with 88 p.p.m. from the untreated plots.

With one exception, there is less copper deposited in leaves with copper sulfate supplied in the soil than without it, as indicated by the results of three years. There is an apparent trend for less deposit of copper in the leaf, the more copper sulfate is supplied to the crop. While the average of the check for the first two years amounts to 53 p.p.m. Cu, the average of all the copper treatments was only 41 p.p.m. To be sure, what is determined as active copper in the soil cannot be synonymous to absorbable copper. If it were, at least the highest amounts found should have a toxic effect on plant growth. It is commonly known that a fraction of one part per million of Cu in solution cultures is injurious to plant growth.

OTHER CHEMICAL CONSTITUENTS IN THE LEAF

Besides copper, some of the other mineral constituents in the leaf were determined. As shown by the results in Table 5, the content of chemical constituents in the leaf is not altered by adding copper sulfate to the fertilizer on which the crop is grown. Therefore, copper is not likely to tie up essential plant nutrients in the soil.

TABLE 5. AVERAGE CONTENT OF SOME MINERAL CONSTITUENTS IN TOBACCO LEAF FROM THREE CROPS OF THE $CuSO_4$ EXPERIMENTS, IN 1947, 1948 AND 1949

Treatment	Percentage on air-dried basis [1]									p.p.m.
	K	Ca	Mg	P	Mn	Fe	Al	Zn	Na	B
Check	3.64	4.24	1.10	.30	.051	.078	.073	.029	.07	53
Copper	3.45	4.35	1.03	.30	.055	.078	.068	.030	.09	50

[1] Each figure represents 12 observations and 24 determinations, by spectrographic methods.

DISCUSSION AND CONCLUSIONS

The dividing line between materials used as soil amendments and those used as plant nutrients cannot be sharply drawn, because most of the materials used for improving the land contain some plant food. With respect to copper sulfate, the material serves both as an amendment and a nutrient, at least on organogenic soils.[1]

Mineral soils, on which most of Connecticut tobacco is grown, cannot fix large quantities of copper sulfate. The copper in the small amounts added in the experiments was apparently not utilized as a nutrient. Therefore, it must be concluded that copper sulfate applied to Connecticut tobacco land serves as a soil amendment. Within the range of 18 to 27 pounds per acre, the results suggested that nitrification is stimulated by copper sulfate. However, it is not easily understood what course the material takes in its amelioration of soil conditions. This problem must be studied further.

At present, this course may be postulated on the idea that copper sulfate "neutralizes" some of the compounds in the soil that actually injure or retard plant growth. Sulfides are known to be very injurious to plant growth, even at extremely low concentrations. They may result from action of desulfofying bacteria or certain fungi, or be brought about by chemical changes in the organic compounds in the soil. Another feature is the great competition for oxygen in nitrate production and the evolution of CO_2. Nitrides, too, might be formed under conditions analogous to those of sulfides and would also be injurious to plant growth.

Moreover, there is the known affinity of copper for those two reduced anions. Copper sulfate applied to the soil would form CuS and Cu_3N which are insoluble compounds. The lessened Cu absorption by tobacco when copper sulfate is applied lends support to this theory. The possibility, however, is not excluded that copper sulfate might have a fungicidal value. This would probably function although the copper reverted into insoluble compounds.

[1] Felix, E. L. Correction of unproductive muck by the addition of copper. Phytopath. 17 (1): 49-50. 1927.

In view of the remarkable improvement in crop values obtained through the use of copper sulfate, with no harmful effect on the crop or to the land, it is concluded that small amounts of the material should be added to the tobacco fertilizer. *It is suggested that copper sulfate be used at the rate of 20 pounds to the acre.* Occasional checking of the status of active copper in the soil is advisable. This is particularly important if tobacco is to be grown on old potato land where copper fungicides have been used. A couple of sprayings with Bordeaux mixture would deliver more copper per acre than the recommended 20-pound rate of copper sulfate.

SUMMARY

Three years of experiments with copper sulfate applied to Havana seed tobacco at an acre rate of 18 to 20 pounds gave an increase of 13 to more than 26 per cent in crop value.

Total copper added to the soil may be detected by spectrographical analyses. Active copper, also best determined spectrographically, does not increase to any great extent through copper sulfate applications to the land.

No *absorbable* copper was formed, since Cu-content in leaf tissue of tobacco grown *with* copper sulfate invariably was less than in leaves grown on land where no copper sulfate was added.

Nitrification appeared to be stimulated within the range of 18 to 27 pounds of copper sulfate per acre.

The fire-holding capacity (burn) of tobacco was not in the least retarded through the use of copper sulfate; neither were the potash and the chief mineral constituents of the tobacco leaf altered as compared with tobacco from check plots.

The addition of copper sulfate to tobacco land at the rate of 20 pounds to the acre seems advisable, but growers are cautioned to check occasionally the status of active copper in the soil.