Soil and Root Copper. Evaluation of Copper Fertilization by Analysis of Soil and Citrus Roots

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Copper sulfate and copper oxide were applied to young citrus trees grown on Leon fine sand. Comparative rates and placements of these sources were used. Feeder roots were excised and the adjacent soil was sampled. Soil copper extracted either by water, 1*N* ammonium acetate at pH 4.8, or 1*N* HCl reflected treatment differences and rates. Copper content of the roots increased proportionately to the amount of copper applied. Linear regression relationships were established between root and soil copper by the three extractants. Foliar copper deficiency symptoms were noted where root copper was less than 3 p.p.m. of the fresh weight, and copper extracted with 1*N* HCl was less than 2 p.p.m. Root analysis provided a basis for the calibration of soil test values for copper.

Copper fertilization commonly is employed on citrus grown on naturally poorly drained soils in Florida. Approximately 25 million young nonbearing trees are involved. However, copper accumulation, primarily from copper sprays used for many years is are cognized problem on the older groves on well drained soils. Correction of copper-induced iron deficiency (*5*) and improved root growth from this correction (*3*, *8*) reduce the severity of the copper injury in these groves. In groves where the soil contains more than 50 p.p.m. of copper, the pH should be maintained between 6.5 and 7.0 (*6*). Soluble copper in excess of 0.1 p.p.m. is toxic to citrus roots (*1*, *7*).

Experiments were established to determine the response of young citrus trees to various copper treatments. The present study attempted to define the copper status of the soil in terms of extractable copper and the copper content of the feeder roots.

Experimental

Materials. Double-row beds on Leon fine sand at the Villa Grove citrus development near Avon Park, Fla., were planted with Hamlin orange trees on sweet orange rootstock in February 1962. Incorporation of materials at this time was made in the treatments with a single copper fertilization either in the planting hole or broadcast around the tree. Other treatments where the copper was used in a spray or in the fertilizer were applied uniformly each year. Details of this grove fertilization and management were similar to those reported for soil amendment studies nearby in the same grove (*4*), except that copper treatments were varied and fertilization was uniformly applied. Each plot consisted of four trees and there were four replications. The whole experiment contained 33 treatments. Of these, 17 (Table I) were used for the sampling in June 1965 when information was sought concerning copper sulfate and cupric oxide applied at several rates but equivalent in copper. In March 1966, 10 of these treatments (Table I) were sampled; two copper spray treatments and one with cuprous oxide were included.

A grove on Leon fine sand near Balm, where Queen orange on rough lemon rootstock was planted, exhibited severe copper deficiency and was used for another copper study; 13 copper treatments of which seven were sprays were applied in 1965. In March 1966, samples from 10 of these treatments (Table II) from four replications were taken.

Methods. The top inch of soil was scraped aside to remove recent copper fertilization or spray residues. Feeder roots were removed from the next 6-inch depth of soil and placed in a polyethylene bag along with the damp soil adjacent to the roots; each sample was obtained from two trees per plot. Where copper was placed in the planting hole, the samples were taken from this area. The samples were kept refrigerated enroute to the laboratory.

The roots were separated manually from the soil and those larger than 2 mm. in diameter were removed. The root mass was washed until free of sand and debris. The roots were damp dried in paper towels and weighed. Five to ten grams were extracted with 1*N* HCl according to the method of Fiskell and Brams (*2*).

The soil was screened through a 20-mesh aluminum sieve. Three methods were used to extract copper. A 10-gram sample of air-dried soil was extracted with 50 ml. of 1*N* HCl for a period of 2 hours and centrifuged. A 10-ml. aliquot of the supernatant liquid was analyzed for copper. A 25-gram sample of soil was extracted with 100 ml. of 1*N* ammonium acetate (pH 4.8) for 2 hours and filtered through 12.5 cm. No. 40 Whatman paper; 50 ml. were taken for copper analysis. Another 100 grams of soil were shaken in 200 ml. of deionized water for 2 hours and filtered; 100 ml. of the filtrate were taken for analysis. The 2,2′-biquinoline method (*1*) was used for both the root and soil copper determinations. The pH of the above soil-water suspension was measured with a glass electrode.

Samples from the 1966 sampling were treated as de-

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Table I. Effect of Copper Treatments on Copper Content of Soil Adjacent to Citrus Feeder Roots and of These Roots

Treatment				Copper, P.P.M.[a]	
No.	Material	Placement	Rate	1N HCl extraction of dry soil	Total in fresh roots
		SAMPLED IN JUNE 1965			
1.	Copper sulfate	Planting, broadcast	0.80 lb. per tree	16.8 def	24.0 cde
2.	Cupric oxide	Planting, broadcast	0.27 lb. per tree	7.3 ef	15.8 de
3.	Copper sulfate	Planting, broadcast	0.80 lb. per tree	13.5 def	18.0 cde
	Agr. lime		16.00 lb. per tree		
4.	Cupric oxide	Planting, broadcast	0.27 lb. per tree	12.3 ef	27.2 cd
	Agr. lime		16.00 lb. per tree		
5.	Copper sulfate	In fertilizer	0.25% CuO	12.4 ef	15.5 de
6.	Cupric oxide	In fertilizer	0.25% CuO	11.6 ef	20.3 cde
7.	Copper sulfate	In fertilizer	1.00% CuO	39.4 bc	53.2 ab
8.	Cupric oxide	In fertilizer	1.00% CuO	32.5 bc	35.6 bc
9.	Copper sulfate	In fertilizer	2.00% CuO	53.6 ab	57.6 a
10.	Cupric oxide	In fertilizer	2.00% CuO	34.7 bcd	50.2 ab
11.	Copper sulfate	In fertilizer	1.00% CuO	12.3 ef	15.4 de
	Dolomite		20.00 lb. per tree		
12.	Cupric oxide	In fertilizer	1.00% CuO	11.0 ef	19.4 cde
	Dolomite		20.00 lb. per tree		
13.	Copper sulfate	Planting hole	0.090 lb. per tree	22.1 def	19.9 cde
14.	Cupric oxide	Planting hole	0.030 lb. per tree	64.5 a	49.1 ab
15.	Copper sulfate	Planting hole	0.090 lb. per tree	26.7 cde	30.2 bc
	Dolomite		2.00 lb. per tree		
16.	Cupric oxide	Planting hole	0.030 lb. per tree	36.6 bcd	58.9 a
	Dolomite		2.00 lb. per tree		
17.	Check			2.9 f	8.4 e
		SAMPLED IN MARCH 1966			
1.	Copper sulfate	Planting, broadcast	0.80 lb. per tree	31.8 gh	24.8 h
2.	Cupric oxide	Planting, broadcast	0.27 lb. per tree	24.6 hi	19.4 hi
3.	Copper sulfate	Planting, broadcast	0.80 lb. per tree	19.6 hi	16.8 hij
	Agr. lime		16.00 lb. per tree		
4.	Cupric oxide	Planting, broadcast	0.27 lb. per tree	15.0 ij	17.8 hij
	Agr. lime		16.00 lb. per tree		
5.	Copper sulfate	In fertilizer	0.25% CuO	4.4 k	10.7 jk
7.	Copper sulfate	In fertilizer	1.00% CuO	10.3 ijk	14.3 ijk
9.	Copper sulfate	In fertilizer	2.00% CuO	38.2 g	32.6 g
11.	Copper sulfate	In fertilizer	1.00% CuO	8.7 ijh	9.4 jk
	Dolomite		20.00 lb. per tree		
17.	Check			2.5 k	9.9 jk
18.	Copper sulfate	In fertilizer	0.50% CuO	6.6 jk	14.5 ijk
19.	Cuprous oxide	In fertilizer	1.00% CuO	12.5 ij	22.2 hi
20.	Tribasic copper	Spray, annual	1.5 lb. in 100 gal.	4.4 k	8.2 k
21.	Tribasic copper	Spray, semi-annual	1.5 lb. in 100 gal.	5.4 k	9.6 jk

[a] Average of four replications; treatment means having the same letter are not statistically different by Duncan's multiple range test at the 0.05 probability level.

scribed above except that the roots were rinsed in a 1% Calgon solution followed by a thorough washing with water prior to the analytical steps.

Results and Discussion

The extent that treatments of the soil with copper were reflected by the total copper extracted from the soil by 1*N* HCl and the total root copper is shown in Table I. From the statistically significant grouping, copper sulfate and copper oxide used at equivalent rates and methods of application resulted in similar root and soil copper values. The exceptions were where copper was applied initially in the planting hole; such differences reflected the problem in obtaining roots precisely where the copper was placed. Where agricultural lime was broadcast with the copper (treatments 3 or 4, Table I), the soil and root copper both were not statistically different than where copper alone was broadcast. When copper was applied in the planting hole, dolomite inclusion showed an apparent significant effect. (Compare treatments 13 and 14 to treatments 15 and 16, Table I.) This was attributed to sampling. The lime treatment increased the soil pH from 5.0 to 5.5. Low rates of copper fertilization were not recognized as statistically different from the check treatment in the data.

Copper recovered from the samples by the acid acetate method also significantly reflected treatment effects; these values were approximately one tenth of the total copper (Figure 1). The high correlation between values from these two methods was important since the acetate method currently is employed for soil testing purposes. Water-extractable soil copper was 440 times less than the total copper, and this amount also significantly reflected the higher copper fertilizations.

Table II. Effect of Residual Copper from a Single Spray on the Copper Content of Soil and Roots from a Grove Exhibiting Copper Deficiency

Treatment			Copper,[a] P.P.M.	
			1*N* HCl	Total
No.	Material	Rate, lb./100 gal.	extraction of dry soil	in fresh roots
1.	Tribasic copper	1.5	1.84	4.86
2.	Tribasic copper	3.0	3.47	4.66
3.	Bordeaux mixture	1.5	2.61	5.61
4.	Bordeaux mixture	3.0	2.26	6.22
5.	Bordeaux mixture	6.0	1.61	5.62
6.	Copper chelate (Rayplex)	3[b]	2.15	7.16
7.	Check		0.81	3.12

[a] Not significant between treatments; average of four replications.
[b] Gal./100 gal.

The 1966 samples from this experiment provided information for soil and root copper similar to that presented for the earlier samples. However, these data (Table I) were not identical with the previous data for the same treatments. The soil values, as well as the root values, were different from the previous sampling. This variation involved sampling error and the extent that the roots differed in copper accumulation between dates of sampling. Treatment differences were recognized statistically for the higher rates of copper fertilization. Copper sprays over the 4-year period appeared to have little effect on the soil and root copper values obtained. Cuprous oxide was nearly equivalent to copper sulfate in its effect on the root copper content.

Where copper sprays were applied to young trees which exhibited copper deficiency, the trend for increased soil and root copper values was not significant (Table II). This meant that, under these conditions, foliar response to copper sprays was independent of the soil copper factors. In these plots, the most severe deficiency was found where soil copper was 1 to 2 p.p.m. and root copper was less than 3 p.p.m.; moderate deficiency was observed where soil copper was between 2 and 3 p.p.m. and root copper between 3 and 5 p.p.m. This relationship between root and soil copper was highly significant (Table III). Soil reaction range was pH 4.7 to 5.4.

The relationships between soil and root copper are described by linear regression equations (Table III). When 1*N* HCl was employed, the slope of these equations ranged from 0.47 to 0.89 for the Villa Grove samples. The low copper values from the Balm Grove resulted in a regression equation with a slightly steeper slope. In a similar study with roots from mature citrus trees on well drained soils, Fiskell and Brams (*2*) re-

Table III. Linear Regression Equations and Correlation Coefficients Describing the Relationships Found between Root Copper Content (X) and Soil Copper (y)

Plots	Soil Extractant	Regression Equation	Linearity F Test[a]	Correlation Coefficient[a]
		1965 SAMPLING		
All (Table I)	1*N* HCl	$X = 13.5 + 0.71y$	48.0	0.646
All	1*N* NH_4OAc[b]	$X = 16.8 + 7.1y$	35.4	0.807
All	H_2O	$X = 19.8 + 196y$	53.1	0.668
Copper sulfate	1*N* HCl	$X = 6.6 + 0.89y$	77.6	0.849
Copper sulfate	1*N* NH_4OAc[b]	$X = 12.8 + 8.7y$	11.9	0.847
Copper sulfate	H_2O	$X = 11.7 + 373y$	3.8	0.724
Cupric oxide	1*N* HCl	$X = 17.5 + 0.65y$	17.3	0.720
Cupric oxide	1*N* NH_4OAc[b]	$X = 12.8 + 8.7y$	23.1	0.699
Cupric oxide	H_2O	$X = 21.9 + 149y$	24.9	0.674
		1966 SAMPLING		
All (Table II)	1*N* HCl	$X = 9.8 + 0.47y$	73.4	0.771
All (Table III)[c]	1*N* HCl	$X = 2.3 + 1.03y$	11.4	0.662

[a] Significant at the 0.01 probability level.
[b] Ammonium acetate acidified to pH 4.8.
[c] Copper deficiency prevalent.

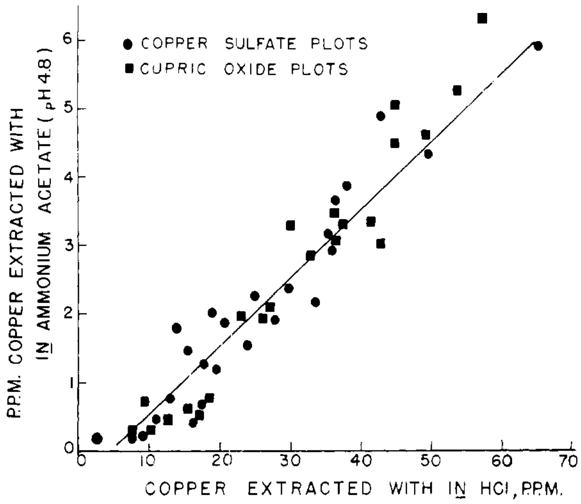

Figure 1. Relationship between acetate-extractable (X) and acid-extractable (y) copper from Leon fine sand

Linear regression equation, $X = 0.98y - 0.45$, is highly significant ($t = 21.7$); correlation ($r = 0.936$) is also significant at the 0.01 level

ported a 0.66 slope where the soil copper was expressed as parts per million. Such equations are useful in predicting root copper content.

Since soil testing laboratories, particularly in Florida, use 1N ammonium acetate at pH 4.8 as the extractant, the prediction equations for root copper from these soil copper values (Table III) and the linear relationship with total soil copper (Figure 1) are valuable for interpretation of the soil test values. The acetate method for soil copper was better suited for soil testing than the water extraction because a smaller sample size is used and less hazard of contamination at the very low copper levels found by water extractions is involved. The interpretation of these soil values was aided greatly by the significant relationship to the root data.

The copper fertilization of citrus both at relatively low and high rates resulted in proportionately similar increases in root copper. This effect was identified fully by correlation and regression analysis. These relationships included sampling and treatment variations. Copper treatment differences were differentiated, however, only at the higher rates. The technique of utilizing root copper to interpret soil copper values appears to be successful as a diagnostic measure of copper fertilization. Similar procedure for evaluating relationships between copper fertilization and root copper may be useful for other soils or other crops.

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Received for review September 6, 1966. Acceptea December 8, 1966. Division of Fertilizer and Soil Chemistry, 152nd Meeting, ACS, New York, N. Y., September 1966. Florida Agricultural Experiment Station Journal Series Number 2492.