Infected trees of Persian lime on sour orange rootstocks observed under field conditions may not have been quite as vigorous as the surrounding trees in some instances. At the same time these infected trees were not in a serious state of decline. The exact effects of tristeza virus on the acid Persian lime and of other possible virus combinations that may be encountered in any topworking operations have yet to be determined.

It is not expected that this warning as to the virus hazards will stop the practice of topworking citrus, but when the grower does decide to topwork he should take every possible precaution to insure that his source of budwood is healthy and the trees he topworks are as healthy as possible.

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RELATION OF pH AND SOIL TYPE TO TOXICITY OF COPPER TO CITRUS SEEDLINGS

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In a previous study (5) it was shown that the topsoils in most bearing citrus groves in the sandy soils of central Florida have accumulated between 100 and 500 pounds of total copper (calculated as CuO) per acre-six-inches. Natively such soils usually contain between 5 and 20 pounds of CuO per acre in the topsoil. This accumulation results largely from the fixation of copper added in mixed fertilizers and to a lesser extent from that in residues of fungicidal or nutritional sprays containing copper. For the past decade or more it has been common practice to apply 10 to 25 pounds of CuO (from copper sulfate) per acre in the regular fertilizer program each year and in some cases an additional 5 to 25 pounds per acre per year from foliage sprays. In other studies (6) it was shown that under acid soil conditions, copper concentrations well within the ranges commonly found in mature grove soils caused stunted, abnormal root development, and under certain conditions, so-called iron chlorosis of the foliage of young citrus seedlings in pots.

This paper summarizes the results obtained from additional pot studies designed to clarify further the relation of type, cultural history, acidity and zinc and manganese contents of soils to toxicity of copper to citrus seedlings.

Materials and Methods

Soil number 1 described in table 1 was obtained in Lake County from land newly cleared in anticipation of planting to citrus.

Table 1. Description and composition before treatment of soils used in pot studies.

Soil No.	Type	Depth (inches)	Cultural history	Exch.cap. (me./100gms.)	Total CuO (lbs./A)	Total P_2O_5 (lbs./A)
1	Lakeland sand	0 - 4	Virgin	1.51	5.7	690
2	Lakeland sand	6 - 12	Virgin	1.15	1.8	680
3	Orlando fine sand	0 - 6	Old grove	7.22	262.0	7,690
4	Orlando fine sand	0 - 6	Virgin	6.15	3.5	2,640

Similar soil is common in the rolling hills of central Florida, and is extensively used for citrus culture. Soil number 1 is typical of the lighter, sandier phase of the Lakeland series known as "blackjack" land. Soil number 2 is subsoil of a type similar to soil 1, but obtained from a different location.

Soil number 3 described in table 1 was obtained in a 30-year-old Valencia orange grove affected periodically in the past four to five years with mild iron chlorosis. Soil number 4 was obtained in a newly planted grove near the source of soil number 3. The area had been recently cleared of native growth. Soil approximating virgin status was collected from the unfertilized middles. Soils 3 and 4 were obtained in locations having similar profiles, and were judged to have been fairly comparable in texture and composition in their native states.

The exchange capacity data in table 1 indicate that the two Orlando soils have between 4 and 5 times as much organic matter and clay as the Lakeland sand. The survey made by Peech and Young (2) indicates that the topsoils of most well-drained soils used for citrus culture in the central part of the state fall between the two extremes in exchange capacity represented by the two soil types used in these pot experiments.

Large lots of soil from the locations described were screened and thoroughly mixed. Soil acidity was adjusted by adding sulfuric acid or lime to 40 lb. portions of air-dry soil. Similarly, the various copper levels were obtained by addition of powdered copper sulfate to the soil. After the necessary chemicals were added, each soil portion was thoroughly mixed, wetted somewhat, and mixed again. In this manner a homogenous mixture of the added materials and soil was achieved. After treatment, the soil was distributed equally among three 8-inch clay pots. Each pot was planted with one large sweet orange seedling and two small Cleopatra mandarin seedlings in March, 1952.

During the first month, some adjustment of pH was necessary. This was accomplished by removing the seedlings, remixing the soil

Table 2. Effect of copper level and acidity of virgin Lakeland topsoil on growth of citrus seedlings in pots.

Treatments and statistical indices	Total fresh weight (gms./pot)	Dry weight of rootlets (gms./pot)	Iron-deficiency index	Nitrogen-deficiency index
6 lbs. CuO/A	154	6.1	0.0	0.0
125 lbs. CuO/A	93	3.9	0.6	0.8
250 lbs. CuO/A	67	2.6	0.3	1.3
375 lbs. CuO/A	47	1.9	0.3	2.6
500 lbs. CuO/A	39	1.5	0.2	4.1
1000 lbs. CuO/A	13*	0.6*	0.4*	5.0*
L.S.D. at .05	16	1.0	N.S.	0.8
pH 4.0-4.5	51	2.3	0.3	3.5
pH 5.0-5.5	55	2.5	0.6	4.2
pH 5.5-6.5	65	3.1	0.3	3.8
pH 7.0-7.5	104	4.9	0.7	2.3
L.S.D. at .05	13	0.9	N.S.	0.7

*Some plants died; data incomplete.

NOTES

Treatments: Each of the copper level means presented represents the over-all effect when the four pH treatments are combined, and each of the pH means the over-all effect when the six copper levels are combined.

L.S.D. at .05 = least difference required for significance between two means by odds of 19:1. N.S. indicates that effect of treatment was not significant.

Copper is expressed as lbs. of CuO per acre-six-inches of *air-dry* soil. To convert to parts per million of Cu, multiply by 0.399.

Total fresh weight is the mean fresh weight of 3 seedlings per pot, roots and tops combined.

Dry weight of rootlets is the mean over-dry weight of fibrous roots from 1 orange seedling per pot.

Iron-deficiency index was obtained by visually rating a vein chlorosis of leaves suggesting iron deficiency as follows: None in plants of a pot = 0, very slight = 1, slight = 2, moderate = 3, severe = 4, and very severe = 5.

Nitrogen-deficiency index was obtained by visually rating an over-all yellowing of leaves suggesting nitrogen deficiency, using the same numerical categories as above.

with an appropriate amount of sulfuric acid or lime, and then replacing the soil in the pots and replanting the seedlings. It was not possible to control pH rigidly, but with minor exceptions the limits indicated in tables 2 and 3 define the range for each treatment.

The plants were fertilized with a liquid fertilizer made up of equal parts by weight of KNO_3, KH_2PO_4, $Ca(NO_3)_2$ and NH_4NO_3. Enough fertilizer to provide about 300 mgms. of N per pot was applied in dilute solution about every two or three months during the growing period. Moisture was maintained by additions of rain water as required. In November, 1952 the pots were transferred to a greenhouse where they remained until harvested in July, 1953.

Results

The data summarized in table 2 show that growth of citrus seedlings in pots of virgin Lakeland topsoil is sharply reduced as the level of added copper is increased. Adding even 125 lbs. of CuO per acre reduced total growth an average of about one-third when effects of all the pH treatments are pooled (table 2), but about one-half for acidity treatments below pH 7 (see figures 1, 2, and 3). The relative weights of fibrous roots of orange paralleled closely the relative fresh weights of the entire plants. Further studies would be required to establish just what the optimum levels of copper might be for growth of seedlings in pots in this soil at various acidity levels, but it is safe to conclude that all would be less than 125 lbs. of CuO per acre in the slightly acid to very acid range.

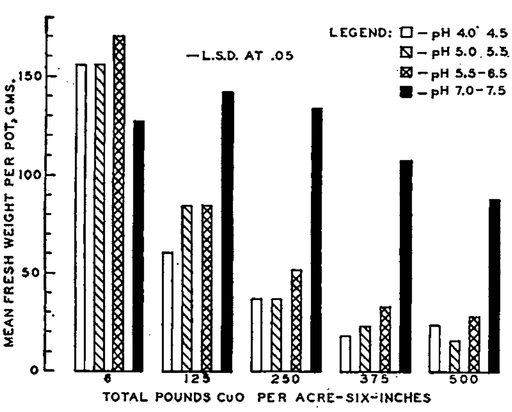

Figure 1. Interrelation of soil acidity and total copper level with growth of citrus seedlings in virgin Lakeland sand. (L.S.D. at .05=30 grams).

Figure 2. Comparative effect of increasing levels of added copper (expressed as lbs. per acre-six-inches of CuO) in virgin Lakeland topsoil in unlimed, acid soil (pH 4) and in heavily limed, neutral soil (pH 7).

The data in table 2 also show that growth of citrus seedlings in Lakeland sand throughout a wide range of copper increments is strongly influenced by pH. Total growth or fibrous root development was about one-third greater at pH 5.5 to 6.5 and about double at pH 7.0 to 7.5 as compared to pH 4.0 to 4.5. Thus these data clearly show that heavy liming resulting in a pH of 7.0 to 7.5 is by far the best method of treating this soil to reduce the toxic effect of added copper on the growth of citrus seedlings (see figure 2). In this very sandy Lakeland soil, this was achieved in pots by adding lime at a rate equivalent to about two tons of agricultural-grade, finely-ground, high-calcium limestone per acre.

There were very few symptoms of iron chlorosis on leaves of citrus seedlings growing in the virgin Lakeland soil regardless of the amount of copper added or the pH level (table 2). On the other hand, stunting of the tops and the fibrous roots by copper additions was fairly regularly correlated with an increasing degree of over-all yellowing of the leaves sug-

Figure 3. Comparative effect of pH on growth of citrus seedlings in virgin Lakeland topsoil with no copper added (upper pots) and with 250 lbs. of added copper (calculated as CuO) per acre-six-inches.

gesting nitrogen starvation. Thus in this soil citrus seedlings grown in pots exhibit a predominance of leaf symptoms suggesting nitrogen starvation rather than iron deficiency in response to the addition of toxic amounts of copper.

The data summarized in figure 1 show the interrelation of copper level and soil acidity to the growth of seedlings in the Lakeland soil. These indicate that acid soil stunted the seedlings only when copper was added (see also figure 3). Heavy liming (pH 7.0 to 7.5) of this virgin soil without adding copper tended to stunt growth slightly and induce symptoms suggesting copper deficiency. However, seedlings in the heavily limed pots were markedly more vigorous than those from more acid soil that had received added copper (figures 1 and 2). The growth of seedlings in the heavily limed soil with 500 lbs. of added CuO per acre equaled or exceeded that of those in the more acid soil with only 125 lbs. of added CuO (figures 1 and 2). The vigorous growth of seedlings in the heavily limed series in relation to the growth of seedlings in the more acid series clearly put the pH 7.0-7.5 treatment in a class by itself (figures 1 and 2).

In a supplemental experiment with Lakeland sand topsoil, three treatments were compared at each of three acid pH ranges. These treatments were as follows: 1. Added copper at the rate of 375 lbs. per acre of CuO. 2. The same as treatment 1 plus 250 lbs. of ZnO from zinc sulfate and 500 lbs. of MnO from manganous sulfate. 3. The same as treatment 1 plus 1,880 lbs. per acre of P_2O_5 from $Ca(H_2PO_4)_2$. The mean fresh weight of plants from these treatments (grown for about the same period as plants in table 2) were respectively 25, 25 and 37 grams per pot when pH effects were pooled. The difference between the growth of seedlings in treatment 3 and the other two was not quite large enough to attain significance at the 5% level of confidence. However, plants in pots treated with added P_2O_5 tended to show a moderate amount of iron chlorosis, but this result was obtained only at pH 5.5 to 6.5, the highest range of soil pH tested. These were the only seedlings in the Lakeland soil series of tests that showed more than a trace of iron chlorosis symptoms on a few leaves. These limited supplementary experiments suggest that zinc and manganese in concentrations normally found in soils in mature groves in Florida do not appreciably supplement or otherwise influence the toxic action of copper, but more extensive studies are needed over a wider range of conditions. Nevertheless, these results appear to be in harmony with the results obtained in solution cultures by Smith and Specht (7) which indicated that copper is several-fold more toxic to citrus seedlings than either zinc or manganese. The phosphate results suggest that the relation of phosphate level in the soil to copper toxicity and iron chlorosis also should be studied in more detail.

In still another series of tests, subsoil of Lakeland sand (soil 2, table 1) was treated as follows: (1) No CuO added, pH 4.5. (2) 37.5 lbs. CuO per acre, pH 4.5. (3) 75 lbs. CuO per acre, pH 4.5. (4) 150 lbs. CuO per acre, pH 4.5. The mean fresh weights of plants grown in the treated subsoil were respectively 95, 102, 68 and 28 grams per pot. Thus these data indicate that under the highly acid conditions common in Lakeland subsoil, 75 lbs. of added CuO was definitely toxic to citrus seedlings, but 37.5 lbs. was not.

Some results obtained with soils 3 and 4 described in table 1 were reported in an earlier paper (6). Copper levels and acidities of portions of the two soils saved from these initial studies were adjusted to give the combinations of treatments indicated in figure 4. The data presented in table 3 indicate some of the

Table 3. Effect of copper level, acidity and cultural history of Orlando soils on growth of citrus seedlings in pots.

Treatments and statistical indices	Total fresh weight (gms./pot)	Dry weight of rootlets (gms./pot)	Iron-deficiency index	Nitrogen-deficiency index
250 lbs. CuC/A	151	7.7	0.3	1.7
500 lbs. CuO/A	102	6.6	1.2	1.7
750 lbs. 8uO/A	52	2.5	1.8	4.5
L.S.D. at .05	23	1.4	0.5	0.6
pH 4.0 - 4.6	53	2.5	1.9	3.9
pH 5.5 - 6.1	151	8.7	0.3	1.3
L.S.D. at .05	19	1.2	0.4	0.5
Old grove soil	141	7.6	2.0	1.7
Virgin soil	63	3.6	0.3	3.6
L.S.D. at .05	19	1.2	0.4	0.5

Notes: Each of the copper level means presented represents the over-all effect with both types of soil when the two pH treatments are combined. Likewise, the pH and soil type means represent the over-all effect of these treatment factors when the effects of the others are pooled.

See also footnotes of table 2.

major over-all effects of these treatments. Copper levels of 500 lbs. CuO per acre or above sharply reduced growth of seedlings as compared with 250 lbs. of CuO in the range of soil acidity from about pH 4 to 6. Growth was reduced more in very acid soil than in moderately acid soil. The average size of plants in the old grove soil was appreciably larger than in virgin soil throughout a wide range of copper and pH treatments.

In contrast with the results on Lakeland soil, a moderate amount of iron chlorosis symptoms

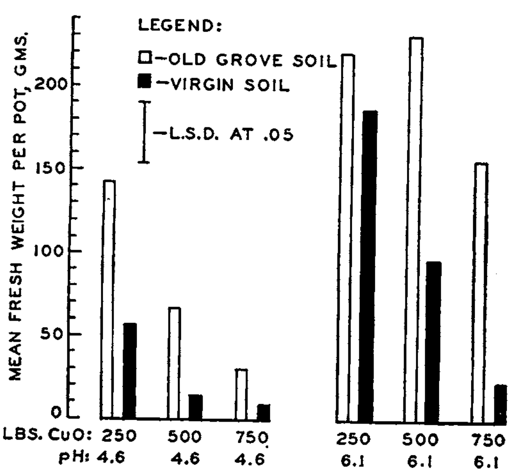

Figure 4. Interrelation of soil acidity, total copper level and cultural history of Orlando soil with growth of citrus seedlings.

showed up in the leaves at the two higher copper levels in the old grove soil of the Orlando series. This was most pronounced at the lower pH range. Markedly depressed growth was also associated with an over-all yellowing of the foliage suggesting nitrogen deficiency in much the same manner as with the Lakeland series.

The interrelation of copper level, pH and cultural history of the soil is indicated from the Orlando soil series of treatments by the data summarized in figure 4. Inspection of these means indicate, and statistical analysis confirm, that the higher copper increments are more toxic in the virgin than in the old grove soil (see also figure 5). In addition, the highly acid soil treatment intensified the stunting effect of the higher copper treatments more in the virgin than in old grove soil. Parallel interactions are clearly *indicated* by the data obtained on fibrous root development.

Discussion

It cannot be emphasized too strongly that the results obtained in these pot experiments do not always apply directly to groves. In mature Florida citrus groves having a history of heavy copper applications over a period of years, toxic or potentially toxic concentrations usually occur only in the top soil (5). The major portions of the fibrous roots of mature citrus trees in such groves are usually located in the subsoil which is virtually devoid of copper. In these pot experiments, the entire root system of the seedling was confined to soil having a uniform copper content throughout.

Undoubtedly the toxic action of copper on the plant as a whole is far greater when the entire root system is confined in a high-copper soil than when only a portion is subjected to this unfavorable condition. Moreover, the possibility that the plant as a whole may react somewhat differently to high-copper in these two situations must be considered. For example, previous studies (6) showed that chlorosis of seedlings was produced when the entire root system was confined in old grove soil, but no chlorosis developed when only a portion of the root system was grown in high-copper soil. Thus pot studies such as these appear useful for evaluating the favorableness of the soil for development of healthy, vigorous roots much phosphate as similar soil did in its virgin state. It seems likely that the accumulated phosphate plays a major role in the reduction of toxicity (growth stunting) of added copper (and possibly also in increasing iron chlorosis, paradoxical as this may seem) in the old grove soil as compared with the virgin soil (table 3 and figure 5). Of course, other tenable explanations are possible.

Some additional evidence favoring the viewpoint that heavy phosphate concentrations reduce copper toxicity is the fact that heavy phosphate fertilization has been shown to depress the concentration of copper in citrus leaves (3). Also, there was a suggestion in the present pot studies that the addition of a massive single dose of soluble phosphate salt to virgin Lakeland soil may have lessened copper toxicity. It appears that this safening factor present in old grove soil is not as effective in reducing copper toxicity as liming (figure 5).

Very slight iron chlorosis symptoms developed on the foliage of plants in the virgin Lakeland soil (table 2). Also, little chlorosis developed on plants on the virgin Orlando soil (table 3). On the other hand, iron chlorosis of the foliage was prevalent at the higher copper levels on the somewhat more vigorous plants growing in the Orlando soil with the high phosphate content obtained in an old grove. These observations suggest that toxic concentrations of copper cause a general derangement of normal growth and metabolic processes in roots which results in appreciable iron chlorosis of the foliage only under special conditions yet to be clearly defined, but possibly having some relation to the amount of phosphate in the soil.

The very marked reduction in toxicity of copper obtained by liming the Lakeland soil to pH 7.0 to 7.5 suggests that this sort of treatment should be tried in low-vigor groves having poor fibrous root development in the topsoil associated with high copper content. It is suggested that small experimental plots of a few trees be established in such groves and that liming sufficient to bring the top six inches above pH 7 be tried. Usually this will require 2 to 5 tons of high-calcium limestone per acre, depending on the exchange capacity of the soil and the initial pH. Some of the heavier soils which have become very acid may require even more than this. Such treatment may disturb the availability of other nutrients such as zinc and manganese, but these deficiencies can be dealt with by means of foliage sprays if necessary.

Summary and Conclusions

A series of pot experiments with citrus seedlings are described. These were designed to study the relation of type, cultural history, and acidity of certain soils to toxicity of copper in soil. The data obtained indicate the following results and conclusions.

1. A very sandy Lakeland-type soil became toxic to citrus seedlings at a much lower level of total copper than did a better Orlando type soil of higher exchange capacity and organic matter content. These results suggest that acid, sandy soils of the Lakeland type having 1.5 to 3 milli-equivalents of exchange capacity per 100 grams are definitely unfavorable for normal growth of citrus roots when they contain 120 to 240 lbs. or more of CuO per acre-six-inches. Acid soils of the Orlando type having 4 to 6 milli-equivalents of exchange capacity would have an equivalent degree of toxicity at 320 to 480 lbs. of CuO per acre. Slight to no toxicity to roots might be expected at copper levels about one-half of these concentrations. Zinc and manganese in these concentrations do not appear to be toxic.

2. Heavy liming of Lakeland sand to produce a neutral to alkaline reaction (pH 7 or above) greatly reduced the toxicity of added copper as compared with treatments in the slightly acid (pH 6) to very acid (pH 4) range. This suggests that heavy liming treatments should be tried on a small experimental scale in low-vigor groves having poor root development in topsoil associated with high copper.

3. An old grove soil, when compared with an analogous virgin soil, had some factor or factors in it which reduced toxicity of added copper to citrus seedlings, yet increased the prevalence of iron chlorosis symptoms on their leaves. It is postulated that the high phosphate content of the old grove soil is a possible causal factor.

4. Citrus seedlings growing in the virgin Lakeland and Orlando topsoils did not develop appreciable iron chlorosis of the foliage throughout a wide range of copper and pH treatment combinations. An over-all yellowing of the foliage suggesting nitrogen starvation, however, was associated with the stunting of top growth and fibrous root develop-

ment caused by additions of toxic amounts of copper to the soil. This indicates that prominent iron chlorosis symptoms are not always associated with copper toxicity.

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MINERAL CONTENT OF ORANGES IN RELATION TO FRUIT AGE AND SOME FERTILIZATION PRACTICES

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There is little published information on the mineral content of Florida oranges. Certain minerals in oranges (unpublished) and in grapefruit (3) were determined by Fudge several years ago. In 1940 Bryan (1) published a rather complete mineral analysis of citrus fruit including both orange and grapefruit values from all available sources. Fertilization practices have changed considerably since that time and most groves today receive more nitrogen and less phosphorous and potassium, in relation to yield, than was customary a few years ago. The present report is concerned with the mineral content of whole fruit and of juice samples from two orange groves used for fertilizer studies the past few years. In one case, fruit samples at different stages of development were taken in order to obtain information as to when the minerals entered the fruit. The samples taken in relation to fertilization, however, were of mature fruit.

Experimental Procedure

For each sample 20 to 24 random-sized fruit were taken from several trees. None of the orchards received nutritional sprays during the particular year that samples were taken. Dust was removed by detergent washing, but this was not of any apparent benefit since the main contaminating chemical element removed is iron (10) and recontamination by this element resulted from the grinding process. The fruit was cut into several pieces, dried at 65° C. and ground through a large Wiley mill. After mixing, a portion of the ground material was saved for chemical analysis.

[1]/The authors wish to gratefully acknowledge the assistance of both A. W. Specht and G. Hrnciar in performing the laboratory work involved in this report.

Some analyses for potassium in expressed juice were made by allowing fermentation to remove the sugar, acidifying with hydrochloric acid, boiling and filtering. Determinations were then made on the filtrate. The various elements were determined by the methods used for leaf analysis (7, 9). The micro-elements were determined partly by spectrographic methods and partly by standard chemical methods.

Results and Discussion

Mineral content in relation to age of fruit. In June 1948, 5 samples of 24 Valencia oranges were collected from plots of 12 trees each. These fruits were about 1 inch in diameter and less than 3 months of age. Five additional samplings from the same plots were made on the dates indicated in table 1. The trees were about 5 years of age and on Rough lemon stock. The soil was about average Lakeland fine sand. Conventional complete starter fertilizer mixtures had been used along with supplementary applications of nitrate of soda. The trees were vigorous and showed no signs of malnutrition. Detailed leaf analyses from these trees for this same period have been published (7, 9).

Table 1 shows the mean weights of the fruit and the amounts of the various elements in each fruit. The fruit of the last two collections were about commercial size 200 (2.75 to 3.00 inches in diameter). The fact that the crop was picked early in February indicates that the fruit was nearly mature at the last date of sampling. There were more potassium, nitrogen and calcium and less copper and