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SOIL PROPERTIES CONTRIBUTING TO CITRUS CHLOROSIS AS REVEALED BY SEEDLING TESTS

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SOIL PROPERTIES CONTRIBUTING TO CITRUS CHLOROSIS AS REVEALED BY SEEDLING TESTS

BY W. T. McGEORGE

INTRODUCTION

Much has been written on the subject of micro-nutrient elements and the part they play in the economy of plant life and the physiological behavior of crops. Since these elements rarely occur in large amounts, either in soils or in plants, most of the research on the subject has dealt with deficiencies. It has become a common practice to identify various deficiencies by characteristic patterns resulting from the partial destruction of chlorophyll in leaves. Also, it is the practice to assume that these patterns can be traced to actual deficiencies of micro-nutrient elements in the soil or to factors tending to reduce availability or uptake. In acid soils the deficiency is usually actual and rarely a matter of availability. In alkaline and calcareous soils the deficiency may be actual but is usually attributed to the effect of alkalinity on the solubility, availability, and /or plant uptake. The relation between the various leaf patterns and micro-nutrient element deficiency is based almost entirely on qualitative physiological symptoms. There is no information in the literature on plant nutrition as to the optimum or actual amount needed in plants for normal plant growth. In other words, research on chlorosis has been more concerned with the symptoms than with underlying causes.

To cite a few examples, it is of interest that Chapman (5) produced a manganese deficiency pattern on citrus leaves by growing citrus trees in phosphorus deficient cultures, a zinc deficiency pattern where an excess of phosphorus was present, and an iron deficiency pattern where an excess of zinc was present in the nutrient culture. Willis and Piland (20) showed that an excess of copper will bring on an iron deficiency pattern. When the characteristic pattern is induced, a correction is obtained by treating the leaves with salts of the elements identified as being associated, as a deficiency, in the formation of the pattern.

It is of further interest that cases have been noted where response was obtained from micro-nutrient element fertilization on crops which did not show any chlorotic leaf pattern. It seems clearly evident that as research progresses from the qualitative to the quantitative status that the micro-nutrient element problem is far more complex than a simple deficiency relationship, and that even a loss or destruction of chlorophyll is not necessarily always a characteristic.

In Arizona, citrus is the principal crop exhibiting micro-nutrient deficiency symptoms, and all the citrus groves are located on alkaline-calcareous soils. It has been generally assumed that there is no actual deficiency of any of the elements in these soils, but that the alkalinity, especially the alkalinity of calcium carbonate, reduces the solubility and uptake to the point where the trees are inadequately supplied. During the past fifteen years many citrus leaf samples have been analyzed in this laboratory. The samples have been taken from many different groves representing leaves of all ages, both normal and chlorotic types. In the absence of experimental evidence on the quantitative requirements of citrus leaves one cannot draw any final conclusions from these analyses. However, they do indicate the presence of iron, manganese, copper, and zinc in what appear to be adequate amounts in both green leaves and in the chlorotic and mottle-leaves. In other words, there is little or no evidence in the leaf analyses that availability in the soil and uptake by the tree are seriously reduced by the alkalinity or any other inherent soil characteristic. Furthermore, the analyses do not show any consistent deficiency difference between the chemical composition of the different chlorotic patterns.

In view of the above it appeared advisable to conduct a quantitative study of micro-nutrient element availability in alkaline-calcareous soils. The physiological behavior of a crop is fundamentally influenced by soil characteristics and properties, but the influence may be direct or indirect. The investigation presented in this bulletin was designed to determine the manner in which the alkaline-calcareous characteristic contributes to micro-nutrient element deficiency symptoms in Arizona soils. A seedling technic was selected for a study of the problem. Hibbard (11) employed a seedling technic for zinc availability studies on California soils, using water and sand cultures. The procedure which he followed was to add 10 gm, of soil to the culture, or to 1 kilo of sand, in preparing a medium for growth. The results obtained were considered qualitatively indicative of the available zinc present in the soils.

THE SEEDLING TECHNIC

The seedling technic selected for this investigation is essentially that developed by Neubauer, and known as the Neubauer method for determining plant food availability in soils. It was originally designed to determine quantitatively the available phosphorus and potassium in soils. It involves the growth of 100 rye seedlings in 100 gm. of soil and sand controls for a period of fifteen to seventeen days, and then analyzing the 100 plants to determine the difference in composition between those grown in sand and those grown in 100 gm. of soil.

While the method was intended only as a routine test for available nutrients, it possesses certain merits as a research tool.

In the Chemistry and Soils Department of the Arizona Experiment Station, it has been extensively employed in soil investigations. For example, it has been used to study calcium uptake from calcareous soils, as a procedure for studying the effect of many factors on phosphate uptake, the suitability of various phosphatic fertilizers for Arizona soils, the advisability of using silty river water on the sandy lands of the Yuma mesa, and others. Since the micro-nutrient elements are most abundant in actively growing plant tissues and less so in the fibrous and woody tissues, the seedling test, as a means of studying micronutrient element problems suggested itself.

METHODS

As mentioned under seedling technic, the seedlings were grown for fifteen to seventeen days after planting. In harvesting the plants, the roots were washed thoroughly with running water under the tap to remove soil and sand. Washing was conducted over a 20-mesh sieve so as not to lose any ungerminated seed or broken plant parts. In most cases, on the basis of an experimental study, the roots were given an additional rinse in 0.1 N HCl. The plants were then dried and ashed. In the first experiment the plants were ashed as a whole, but in all subsequent experiments the roots and tops were ashed and analyzed separately.

For ashing, the air dry plant material was placed in a 150 ml. pyrex beaker and heated in an electric muffle furnace at very low redness until free or practically free of carbon. After cooling, 5 ml. of con. HC1 and 20 ml. of distilled water were added and boiled for a few minutes on the hot plate. It was then filtered into a 200 ml. volumetric flask, and the residue on the filter paper was washed several times with hot water. The residue and filter paper were then transferred to a platinum dish and heated to a thoroughly white ash in an electric muffle at red heat. Five ml. of con. HC1 was then added, the dish covered with a watch glass, and then digested on a steam bath to a volume of 1 ml. Ten ml. of distilled water was then added and digestion continued for one-half hour. This was then filtered into the original 200 ml. flask and the filtrate made to volume. Aliquots of this solution were used for the determination of iron, manganese, zinc, and copper.

Iron.—If the acid extract represents the whole plant, a 5 ml. aliquot is used for the iron determination. If the roots and tops are analyzed separately, a 5 ml. aliquot is used for the solution prepared from the roots and a 20 ml. aliquot for the tops. This aliquot was placed in a 50 ml. graduate, 3 drops of con. HNO_3 added, diluted to a volume of 40 ml., and 10 ml. of 20 per cent KSCN solution added and shaken. The color developed was compared with a standard prepared in the same way with a standard iron solution containing 0.0025 mg. iron per ml.

Manganese.—A 150 ml. aliquot was used for the manganese determination regardless of whether the solution represented the whole plant or roots and tops separately. This aliquot was placed in a 250 ml. pyrex beaker, 5 ml. of con. H_2SO_4 added, and the whole evaporated on a hot plate to dense white fumes in order to remove all HC1. The beakers were cooled, diluted with 50 ml. of distilled water, and filtered to remove any residue of $CaSO_4$ that might have been deposited during the evaporation. The volume of the solution at this point should be about 75 ml. Add 2 gm. of sodium sulphite, 10 ml. of 0.3 per cent $AgNO_3$ solution, and heat to boiling. Ammonium persulphate was then added until the maximum permanganate color was produced. The beakers were cooled in a cold water bath. The depth of color was then measured by comparison with $KMnO_4$ of known strength. The standard used in this determination contained 0.00387 mg. Mn per ml.

Zinc and copper.—These were determined by the dithizone method, using a 20 ml. aliquot. This aliquot was pipetted into a 100 ml. pyrex beaker, 2 ml. of 0.5 molar ammonium citrate solution added, 1 drop of metacresol purple added, and 4 N NH_4OH solution added, stirring constantly until the solution became purple. The solution was then transferred to a 125 ml. separatory funnel in which it was shaken successively with dithizone and chloroform until the chloroform layer was green. The chloroform extracts were combined and shaken twice with 0.02 N NH_4OH until free from excess dithizone. Zinc and copper were then separated by shaking with 20 ml. of 0.01 N HC1. The acid fraction was made alkaline with NH_4OH using metacresol purple as an indicator, and then extracted with dithizone and chloroform as described under the original extraction. The chloroform extract from which the zinc had been separated was then extracted with 0.02 N NH_4OH to remove excess dithizone, and this residual red color was determined as copper. A Cenco photolometer was used for the zinc and copper determinations.

All the reagents used were made as nearly free of zinc and copper as possible. NH_4OH, HC1, and the distilled water were redistilled as recommended by Hibbard. Ammonium citrate was washed free of zinc and copper by shaking the 0.5 molar solution with chloroform and dithizone until the chloroform layer was green. In preparing the acid extract of the plant ash, the same volume of HC1 was always used and accurately measured. An equal volume of acid was made to 200 ml. and aliquots analyzed. The plant analyses were corrected for any values thus obtained for the reagents.

EXPERIMENT 1.—A PRELIMINARY EXPERIMENT

The first experiment was more or less of an exploratory nature and designed to determine how many cultures of 100 plants each would be required to supply sufficient plant material for the determination of iron, manganese, copper, and zinc in aliquots of a single acid extract of plant ash. Nine different soils were selected for the test. Both rosen rye and Sacramento barley were grown in duplicate cultures: the two rye cultures combined for one analysis, and the two barley cultures combined for another. The plants were dried, ashed at very low redness in a muffle furnace, the ash boiled in 10 ml. of con. HC1 plus 50 ml. of distilled water, then filtered into a 200 ml. flask, and washed on the filter to volume.

All the distilled water used throughout this work was redistilled in pyrex to avoid traces of the micro-nutrient elements, especially zinc and copper.

For the iron determination, an aliquot of 5 ml. was found to be satisfactory, for the zinc and copper, 20 ml., and for the manganese, 150 ml. was used.

For these nine soils the zinc varied from .380 to .490 mg. per two cultures; for copper .120 to .340 mg.; for iron 1.1 to 6.4 mg.; and for manganese 0.49 to 1.41 mg.

The experiment shows that the seedling method does have possibilities. The data were significant in that the highest copper value was obtained from a soil from the Safford valley in the upper basin of the Gila River. This river carries much water that originates or passes through one of the large copper mining areas of the state.

EXPERIMENT 2.—ACCURACY OF SEEDLING METHOD

Since the fact was established that the seedlings contained sufficient micro-nutrient elements for quantitative determination and study, the next experiment was designed to determine just how closely the uptake of elements could be duplicated in separate cultures grown in individual soils. For this experiment two soils were selected: one, a Palo Verdes soil, noncalcareous, and the other, a Gila soil which is highly calcareous. Cultures of rye and barley were grown in quadruplicate in each soil, and two of each combined, giving duplicate plant ashes for analyses. The procedure followed in Experiment 1 was changed in that the tops and roots were ashed and analyzed separately. The depth of color obtained in the colorometric methods employed on the whole plant in Experiment 1 indicated that two cultures of 100 plants each would supply sufficient material for analyzing the roots and tops separately.

It seemed advisable to analyze the roots and tops separately for several reasons. First, if the seedling method is to be useful, it should, in as many ways as possible, differentiate the micro-nutrient element relationships between roots and tops of plants, for manifestation of a deficiency usually appears in the latter. Distribution of micro-nutrient elements between roots and tops, movement of these elements to the tops after they have been

TABLE 1.—ANALYSIS OF ROOTS AND TOPS (EXPERIMENT 2)

	Iron (Fe)*		Manganese (Mn)*		Zinc (Zn)*		Copper (Cu)*	
Soil and seedling	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
Palo Verdes, rye	1.7	.24	.37	.18	.200	.110	.050	.022
Palo Verdes, rye	2.1	.24	.31	.21	.200	.110	.050	.022
Palo Verdes, barley	3.6	.37	.51	.23	.236	.170	.050	.050
Palo Verdes, barley	3.8	.40	.49	.29	.236	.170	.050	.050
Gila, rye	1.9	.35	.43	.12	.200	.130	.090	.030
Gila, rye	1.8	.36	.43	.11	.200	.130	.090	.030
Gila, barley	3.2	.68	.55	.15	.220	.110	.115	.040
Gila, barley	3.3	.48	.59	.19	.220	.118	.115	.040

* Mg. per two cultures.

absorbed by the roots, whether there is a difference in fixation in the roots of plants growing in alkaline-calcareous and acid soils, are important. Second, under some soil conditions, especially if roots are slightly flaccid, the soil colloids are not easily washed off the roots. This will obviously introduce an error of considerable magnitude which can be partly avoided by analyzing the tops separately.

This experiment shows that duplicate cultures can be closely checked for uptake of micro-nutrient elements, both in the roots and in the tops, and that the value of the method is greatly enhanced by analyzing the roots and tops separately.

EXPERIMENT 3.—VALUES FOR SAND CONTROLS

In using a seedling technic, such as the Neubauer method, it is important to determine a value for the sand control; that is, a value which represents the nutrient element content of the same number of seedlings grown in the absence of any soil, but in the same weight of sand as is mixed in the soil cultures. The difference between this value and the value obtained from seedlings grown in 100 gm. of soil indicates the amount of available elements in the soil. In the case of micro-nutrient element determinations it is necessary to use the same volume of reagents for extracting the ash and for making the extract to volume; it is also necessary to duplicate carefully any other analytical procedure.

A large number of sand controls for both rye and barley were run in order to establish a reasonable control value. The values obtained from these are given in Table 2. The analyses are

TABLE 2.—MICRO-NUTRIENT ELEMENTS IN SAND CONTROL*

	Iron (Fe)*		Manganese (Mn)*		Zinc (Zn)*		Copper (Cu)*	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
	Rye Seedlings							
	.63	.15	.16	.09	.239	.148	.122	.078
Root:top ratio	4.2		1.8		1.6		1.6	
	Barley Seedlings							
	.54	.16	.19	.10	.237	.144	.115	.073
Root:top ratio	3.4		1.9		1.6		1.6	

* Analyses are expressed as milligrams per two cultures. Values represent average of ten duplicate cultures.

given as milligrams per two plant cultures of 100 seedlings each.

For the analytical data presented in the several tables in this bulletin, sand controls have not been subtracted from the seedling soil cultures. They have merely been used to help interpret the data.

EXPERIMENT 4.—RINSING ROOTS IN ACID

The next experiment was designed to study the high nutrient element content of the seedling roots. If the seedlings are making vigorous growth at the time of harvest or if they have a healthy and active root system, the roots can be washed under the tap to a clean white color. On the other hand, if root growth is inhibited by any factor resident in the soil, the soil colloids are difficult to remove by simply washing with water. In several cases seedlings were discarded because of extremely high iron values obtained from the analysis of the roots. The color of the ash in some cases also indicated that the soil had not been completely washed from the roots. We must not overlook the possibility that iron was fixed on the surface of the roots rather than being present as soil colloids absorbed on the surface. At all events an experiment was conducted in which replicated cultures were washed with running tap water only, and with running tap water followed by a double rinse in 0.1 N HCl. The data obtained from this experiment are given in Table 3.

TABLE 3.—SHOWING THE EFFECT OF AN ACID RINSE ON ANALYSIS OF ROOTS

Soil No.	Iron (Fe) Water	Iron (Fe) Acid	Manganese (Mn) Water	Manganese (Mn) Acid	Zinc (Zn) Water	Zinc (Zn) Acid
1 Barley	3.0	3.0	.49	.39	.302	.302
2 Barley	3.9	3.2	.30	.20	.296	.258
3 Rye	1.4	1.0	.55	.14	.204	.196
3 Barley	1.4	1.2	.46	.13	.186	.164
Sand	.32	.24	.14	.13	.270	.248

This experiment indicates that it is advisable to rinse the seedling roots in dilute HC1 after washing as thoroughly as possible with running water under the tap. Soil No. 3 is an acid soil from the northern part of the state. It is evident that the greater amount of micro-nutrient elements in the roots and the reduction obtained by washing the roots with dilute HC1 is true for the seedlings grown in acid soils as well as those grown in alkaline soils. The fact that seedling roots from plants grown in sand also gave values lower when rinsed in acid indicates that there is a precipitation on or near the root surface which cannot be attributed to soil colloids attached to the root surface.

EXPERIMENT 5.—AVAILABILITY OF MICRO-NUTRIENT ELEMENTS

For the next experiment a group of seven soils, representing a fair cross section of the state, was selected for the seedling test. The data obtained from the analyses of these seedlings, both barley and rye, are given in Table 4 as milligrams per two cultures.

A comparison of the uptake of micro-nutrient elements by rye and barley seedlings from these seven soils shows a good agreement. The barley seedlings, on the whole, show a slightly higher uptake for this particular group of soils which may be due to the fact that barley makes a more vigorous growth and produces a greater weight of plant material than rye over a seventeen-day period of growth. This is especially true for the comparative root growth produced by these two grains in alkaline-calcareous soils. On a percentage dry matter basis the rye values would be definitely higher than the barley. On an average, two rye cultures will produce 2.2 gm. roots and 1.5 gm. tops (air-dry basis) while two barley cultures will produce 3.4 gm. roots and 1,8 gm. tops.

Zinc.—In three of the rye soil cultures and four of the barley soil cultures the zinc content of the seedling roots is less than for the sand controls. In the tops, in every case, it is greater for the seedlings grown in soil. This indicates that the seedlings are amply supplied with zinc and that the zinc content of the grain is sufficient for growth during the seedling stage. The ratio of zinc in roots and tops is very significant in that it is lower in all the soil-grown seedlings. This shows a greater proportionate transfer of zinc from roots to tops and a greater need for zinc in the tops of these soil-grown seedlings than for those grown in sand. It is important to note that calcareous soils do not interfere with the movement of zinc from roots to tops as shown by the favorable root: top ratio.

Copper.—The copper determinations are not as consistent as the zinc analyses but on the whole the distribution and behavior correlates with that noted for zinc. In several cases the values are again lower for the soil-grown seedlings than for those grown in sand. Except in two cases, the root: top ratio is lower for the soil-grown seedlings which again shows that the calcareous soils probably do not seriously interfere with the movement of copper from roots to tops. Attention is called to the high copper values for both rye and barley seedlings grown in the soil from Safford valley through which the Gila River flows.

Iron.—The greatest difference between roots and tops is shown in the iron determinations, and they also show the highest root: top ratio. It is significant that the lowest ratio was found for the rye seedlings grown in soil No. 1 which is a noncalcareous Palo Verdes soil. There is a strong indication in these

TABLE 4.—AVAILABILITY OF IRON, MANGANESE, ZINC, AND COPPER IN ARIZONA SOILS

	Iron (Fe)*			Manganese (Mn)*			Zinc (Zn)*			Copper (Cu)*			Fe:Mn (ratio)	
Soil from	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops
					Rye Seedlings									
Tucson area	1.72	.37	4.6	.66	.41	1.6	.216	.166	1.3	.120	.076	1.6	2.60	0.9
Safford area	2.04	.33	6.2	.78	.22	3.5	.204	.180	1.1	.186	.128	1.5	2.67	1.5
Mesa area	2.60	.25	10.4	.27	.12	2.2	.180	.160	1.1	.140	.100	1.4	9.6	2.1
Tucson area	3.20	.29	11.0	.43	.21	2.0	.255	.236	1.1		.100	.	7.4	1.4
Yuma mesa	2.32	.20	11.6	.37	.14	2.6	.275	.185	1.5	.100	.104	1.0	6.3	1.4
Casa Grande area	1.68	.24	7.0	.76	.22	3.5	.247	.190	1.3	.114	.086	1.3	2.2	1.1
Marana area	1.88	.21	13.7	.45	.12	3.8	.280	.170	1.6	.124	.060	2.1	4.2	1.7
Sand control	0.63	.15	4.2	.16	.09	1.8	.239	.148	1.6	.122	.078	1.6	3.9	1.7
Av. soils	2.35	.27	9.2	.55	.22	2.7	.237	.184	1.3	.131	.093	1.4	4.3	1.3
					Barley Seedlings									
Tucson area	2.08	.25	8.3	.90	.53	1.7	.228	.198	1.1	.110	.136	0.8	2.3	0.5
Safford area	2.06	.40	5.1	.93	.31	3.0	.240	.224	1.1	.180	.124	1.4	2.2	1.3
Mesa area	2.72	.29	9.4	.36	.18	2.0	.237	.193	1.2	.104	.100	1.0	7.6	1.6
Tucson area	1.80	.29	6.2	.78	.25	3.0	.202	.190	1.1	.100	.102	1.0	2.3	1.2
Yuma mesa	2.64	.20	13.2	.36	.17	2.1	.224	.168	1.3	.101	.195	0.5	7.3	1.2
Casa Grande area	1.76	.31	5.7	.65	.26	2.5	.242	.180	1.3	.155	.070	2.2	2.7	1.2
Marana area	3.60	.28	12.8	.45	.18	2.5	.234	.176	1.3	.104	.080	1.3	8.0	1.6
Sand control	0.54	.16	3.4	.19	.10	1.9	.237	.144	1.6	.115	.073	1.6	2.8	1.6
Av. soils	2.38	.29	8.7	.63	.27	2.4	.230	.190	1.2	.122	.116	1.2	3.8	1.1

* Mg. per two cultures.

iron data that this element is largely fixed in the roots or on the surface of the roots. All the soil cultures showed an appreciably higher iron content for both roots and tops than the sand cultures, and this can be interpreted as showing a good availability and uptake of iron in all the seven soils.

Manganese.—All the soil cultures show an appreciable manganese availability with the root content being notably higher than the top. However, the manganese is much lower in roots than iron and the ratio indicates a more active movement of manganese than iron to the tops. The root: top ratio for the soil cultures as compared with the sand cultures is quite significant, being higher in every case except soil No. 1 which is the non-calcareous soil. These data, together with the iron determinations on the rye seedlings grown in soil No. 1, indicate that the calcareous soils exercise some retarding influence on the movement of iron and manganese from the roots to the tops. It is significant that this retarding influence applies largely to the iron and manganese and little or none to zinc and copper. The data for manganese show that all these soils are well supplied with available manganese which is readily absorbed by the seedling roots.

As a second part of this experiment four acid soils were selected and rye seedling tests conducted with them. Soils No. 4 and 5, extremely high in organic matter, are from forest areas in the northern part of the state. Number 6 is a laterite type of soil also high in organic matter. Number 7 is an acid soil from Iowa. The analyses of the seedlings grown in these soils are given in Table 5, roots and tops separately, as mg. per two cultures.

Apparently the seedlings grown in acid soils behave in much the same way as those grown in alkaline-calcareous soils with respect to uptake of micro-nutrient elements and distribution between roots and tops. However, there are some important differences.

Zinc and copper.—Again, there is less zinc and copper in the roots of the soil-grown seedlings, and the root: top ratio is similar to the values for seedlings grown in alkaline-calcareous soils. The significant difference between the seedlings grown in the two types of soil is in the lower content of seedling tops from the acid soils. The roots also contain less zinc and copper. This indicates that seedlings grown in acid soils have a lower zinc and copper requirement, and that zinc and copper in seedlings grown in the acid soil environment are more active and more efficiently utilized within the plant. Both zinc and copper values are very low for the seedlings grown in the Iowa soil. Since these values are so much lower than the sand controls, it shows a high absorption capacity of this soil for these elements. It is assumed that the zinc and copper set free during the decomposition of the grain was absorbed by the soil.

Iron.—As in the alkaline-calcareous soils, the iron uptake

TABLE 5.—ANALYSES OF RYE SEEDLINGS GROWN ON ACID SOILS, MG. PER TWO CULTURES

Soil No.	Iron (Fe) Roots	Iron (Fe) Tops	Iron (Fe) Ratio	Manganese (Mn) Roots	Manganese (Mn) Tops	Manganese (Mn) Ratio	Zinc (Zn) Roots	Zinc (Zn) Tops	Zinc (Zn) Ratio	Copper (Cu) Roots	Copper (Cu) Tops	Copper (Cu) Ratio	Fe:Mn (ratio) Roots	Fe:Mn (ratio) Tops
4	2.8	.17	16.4	1.0	.31	3.2	.196	.139	1.4	.160	.058	2.8	2.8	0.55
5	2.2	.15	14.7	0.4	.28	1.4	.204	.100	2.0	.124	.036	3.4	5.5	0.54
6	3.8	.13	29.1	0.8	.46	1.7	.170	.120	1.4	.100	.058	1.7	4.7	0.28
7	1.0	.16	6.2	1.0	.24	4.2	.074	.060	1.2	.020	.020	1.0	1.0	0.67
Sand	0.6	.15	4.2	0.16	.09	1.8	.239	.148	1.6	.139	.086	1.6	3.7	1.67

and the root: top ratio are the greatest of the four micro-nutrient elements. In agreement with the zinc and copper, the iron content of the tops is significantly lower for the seedlings grown in acid soils. This again shows a lower iron requirement and better activity of iron within the plant for seedlings grown in acid soils as compared with those grown in alkaline-calcareous soils.

Manganese.—Of the four micro-nutrient elements, the manganese values for acid and alkaline-calcareous soils are in closest agreement. The experiment indicates that manganese uptake and distribution between roots and tops are similar for both types of soil. The seedlings grown in acid soil contain slightly more manganese.

Iron:manganese ratios.—The most significant difference between seedlings grown in acid soils and alkaline-calcareous soils is in the Fe:Mn ratio. This ratio is much lower in the seedling tops grown in acid soils, being approximately one-half the ratio for the alkaline-calcareous soils. The difference in ratio is largely due to the lower iron values for the acid soils. This indicates a greater iron activity in these seedlings. Comparing the analyses of seedlings grown in the two types of soil, there is a tendency toward higher iron content and lower manganese content in the seedling tops for the acid soils. This may be of interest in explaining why manganese toxicity sometimes occurs on plants growing in acid soils and why manganese deficiency often follows the overliming of acid soils.

This experiment indicates that there is no material basis for the theory that the reaction (high pH and potential alkalinity) of alkaline-calcareous soils *seriously* reduces micro-nutrient element solubility, availability, or uptake and transfer from roots to tops. The difficulty is entirely one of micro-nutrient element activity within the plant and the failure of the plant to utilize these elements when the roots are growing in an alkaline-calcareous substrate. As will be shown later, highly calcareous soils may reduce uptake, but there is no evidence that this reduction in uptake reaches a point which represents a deficiency.

EXPERIMENT 6.—SEEDLING TESTS ON INCUBATED SOILS

Among the factors contributing to chlorosis on calcareous soils, overirrigation or a continuously high moisture content in the subsoil has been frequently observed and has been recognized as important in Arizona (3). Obviously such soil environment will seriously interfere with normal microbiological activities in the soil which function best in well-aerated soils. Where an excess of calcium carbonate is present in the soil, a high pH might arise from poor aeration, for under such conditions calcium hydroxide may accumulate from hydrolysis of calcium carbonate.

An experiment was conducted in which the 100-gm. portions of soil were incubated just previous to their being planted with seedlings. They were incubated for thirty days at 30 degrees C. under well-aerated conditions and optimum moisture content. At the same time these cultures were grown, others were grown in the same soil unincubated.

Five alkaline-calcareous soils, low in organic matter, as they usually are in the irrigated valleys of the state, were used. The data from this experiment are given in Table 6. This experiment shows that keeping the soils moist and properly aerated for thirty days has no apparent effect on the uptake of micro-nutrient elements. The experiment indicates that a continuously wet soil contributes to chlorosis because of a more or less anaerobic condition which adversely affects respiration and ion uptake.

TABLE 6.—EFFECT OF SOIL INCUBATION ON AVAILABILITY OF MICRO-NUTRIENT ELEMENTS

	Mg. per two cultures								
	Iron (Fe)			Manganese (Mn)			Zinc (Zn)		
Soil No.	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops	Ratio
				Dry Soil					
8	16.5	.86	19.2	.78	.25	3.1	.230	.165	1.4
9	6.7	.47	14,2	.36	.24	1.5	.180	.175	1.0
10	15.3	.73	20.9	.50	.14	3.6	.232	.184	1.2
11	7.1	.54	13.1	.32	.22	1.4	.196	.154	1.1
12	7.1	.93	7.6	.32	.44	0.8	.211	.195	1.1
Av.	10.5	.71	15.0	.46	.26	2.1	.210	.175	1.2
				Incubated Soil					
8	18.0	.50	36.0	.74	.18	4.1	.213	.150	1,3
9	6.8	.42	16.3	.43	.18	2.6	.200	.168	1.2
10	14.7	.62	23.7	.54	.24	2.3	.207	.192	1.1
11	9.0	.67	13.4	.42	.33	1.3	.217	.162	1.2
12	7,3	1.20	6.1	.71	.67	1.1	.237	.170	1.3
Av.	11.1	.68	19.1	.57	,32	2.3	.215	.170	1.2

EXPERIMENT 7.—SEEDLING TESTS ON SOILS FROM CITRUS GROVES

For the next experiment, soil samples (1-foot depth) were taken from twelve different citrus groves in the Salt River Valley, six of which were severely chlorotic, two of which were only slightly chlorotic, and four of which were nonchlorotic. No differentiation was made between mottling and chlorosis, as in every case, both chlorotic and mottle-leaves were present on the same trees. It was assumed, so far as the soil itself is concerned, that the presence of any chlorotic leaf pattern was indicative of the physiological disturbances that are manifested as micro-nutrient element deficiencies, arising from the alkaline-calcareous soil character. Both rye and barley seedling tests were conducted on these soils. Roots and tops were analyzed separately, the data being given in Tables 7 and 8. The data

TABLE 7.—ANALYSES OF RYE SEEDLINGS GROWN IN SOILS FROM CITRUS GROVES, MG. PER TWO CULTURES

Grove No.	Iron (Fe) Eoots	Iron (Fe) Tops	Iron (Fe) Ratio	Manganese (Mn) Roots	Manganese (Mn) Tops	Manganese (Mn) Ratio	Zinc (Zn) Roots	Zinc (Zn) Tops	Zinc (Zn) Ratio	Fe:Mn (ratio) Roots	Fe:Mn (ratio) Tops
Severely Chlorotic Groves											
A	2.66	.14		.32	.07		.213	.200	1.1	8.3	2.0
B	7.28	.17		.55	.07		.255	.220	1.2	13.2	2.4
C	1.52	.19		.17	.10		.228	.184	1.2	9.0	1.9
D	2.32	.12		.26	.09		.190	.151	1.3	8.9	1.3
E	2.30	.18		.21	.10		.310	.185	1.7	11.0	1.8
P	1.78	.17		.26	.10		.193	.230	.8	6.8	1.7
Av.	2.98	.16	18.6	.29	.09	3.2	.231	.195	1.2	10.3	1.8
Slightly Chlorotic Groves											
G	2.56	.18		.28	.11		.210	.175	1.2	9.1	1.6
H	1.32	.20		.20	.10		.188	.145	1.3	6.6	2.0
Av.	1.94	.19	10.4	.24	.11	2.2	.199	.160	1.2	7.9	1.7
Nonchlorotic Groves											
I	1.52	.16		.18	.09		.185	.150	1.2	8.4	1.8
J	2.12			.25	.11		.200	.170	1.2	8.6	
K	1.52	.20		.19	.11		.213	.170	1.2	8.0	1.8
L	2.74	.24		.36	.13		.222	.170	1.3	7.6	1.8
Av.	1.97	.20	9.8	.24	.11	2.2	.205	.165	1.2	8.2	1.8
Sand	0.63	.15	4.2	.16	.09	1.8	.239	.148	1.6	4.0	1.7

TABLE 8.—ANALYSES OF BARLEY SEEDLINGS GROWN ON SOILS FROM CITRUS GROVES, MG. PER TWO CULTURES

Grove No.	Iron (Fe) Roots	Iron (Fe) Tops	Iron (Fe) Ratio	Manganese (Mn) Roots	Manganese (Mn) Tops	Manganese (Mn) Ratio	Zinc (Zn) Roots	Zinc (Zn) Tops	Zinc (Zn) Ratio	Fe:Mn (ratio) Roots	Fe:Mn (ratio) Tops
Severely Chlorotic Groves											
A	7.60	.20		.64	.17		.211	.180		11.9	1.2
B	7.44	.27		.66	.17		.200	.170		11.3	1.6
C	3.02	.28		.32	.16		.240	.216		9.4	2.0
D	2.81	.26		.31	.17		.200	.196		9.0	1.5
E	1.82	.38		.24	.16		.184	.200		8.0	2.4
F	2.64	.23		.34	.14		.216	.204		7.9	1.6
Av.	4.22	.27	15.6	.42	.16	2.6	.209	.194	1.1	10.0	1.8
Slightly Chlorotic Groves											
G	2.84	.16		.36	.14		.182	.140		8.0	1.2
H	2.14	.19		.28	.18		.180	.180		7.7	1.0
Av.	2.49	.18	13.8	.32	.16	1.4	.181	.160	1.1	7.8	1.1
Nonchlorotic Groves											
I	2.07	.20		.28	.16		.164	.140		7.4	1.2
J	2.44	.21		.32	.18		.247	.168		7.6	1.2
K	2.50	.34		.30	.16		.204	.160		8.3	2.1
L	2.82	.22		.39	.21		.190	.160		7.2	1.0
Av.	2.46	.24	10.2	.32	.18	1.7	.201	.157	1.3	7.7	1.4
Sand	0.54	.16	3.4	.19	.10	1.9	.237	.144	1.6	2.8	1.6

will be discussed on the basis of averages of each group of soils rather than as individual soils.

Zinc.—There is more zinc in both roots and tops of seedlings grown in the soils from the severely chlorotic groves. The root: top ratio is also more favorable, indicating that there is more active movement from roots to tops. Finally, zinc values for this group of soils approach most closely the values for the sand controls. The high zinc content of roots and the high total zinc of roots plus tops for the sand controls are significant. It again indicates that for the seventeen-day growing period of the seedlings, the zinc content of the seed is sufficient for growth. Regardless of this, the zinc data are useful as zinc content of tops and root: top ratio. These data show that calcareous soils do not seriously interfere with transportation of zinc within the plant, at least in seedlings. The rye and barley seedlings are in substantial agreement.

Iron.—The data on iron are of special interest. The average iron content of the roots is highest for the seedlings grown in soils from the severely chlorotic citrus groves, and the lowest for the rye tops. It is recognized that there is some overlapping of values for seedlings grown in the three groups of soil The data in Tables 7 and 8 indicate very strongly that there is nothing seriously wrong with the availability of iron in these soils, nor in the uptake of iron from the soils by the seedling roots.

There is evidence in the data that if an iron deficiency symptom is a true deficiency, it is one of low activity or of failure to utilize iron within the plant itself. The data further indicate that an inherent soil characteristic or factor, residing in certain alkaline-calcareous soils, contributes to inactivity or nonutilization of iron within the plant. Further evidence of this is noted in the data, showing the iron root: top ratios which are very much the highest for the seedlings grown in the soils from the severely chlorotic groves. It is of interest that the data for both the rye and barley seedlings are in general agreement. These data are also in agreement with Milad's (15) findings for pear trees: namely, higher iron in the roots of chlorotic trees than normal green trees. The seedling experiments show that the data they yield are indicative of conditions noted for tree crops on calcareous soils.

Manganese.—The manganese data are in substantial agreement with iron for the roots but not necessarily in the seedling tops. The average manganese content of the roots is highest for the cultures grown in the soils from the severely chlorotic groves, but there is no difference in the tops for this group of soils. The root: top ratio, like that for iron, is highest for the group of soils from the severely chlorotic groves, even though it is lower than iron, and shows that the retardation of movement from roots to tops is of less magnitude in proportion to the total taken up by the roots. The root: top ratios again give evidence of an inherent soil characteristic or factor which retards transportation or utilization within the plant itself. The data for rye and barley seedlings are in substantial agreement.

Iron:manganese ratio.—The ratios of iron: manganese show the highest values for seedling roots grown in the soils from the severely chlorotic groves. The ratio is quite constant for the soils from the slightly chlorotic and nonchlorotic groves. The

Fe:Mn ratios for the seedling tops are slightly higher for the soils from the severely chlorotic groves.

Before presenting the next experiment it seems advisable to present some chemical and nutritional data bearing on the soils from these citrus groves. The seedling tests presented in Tables 7 and 8 show a rather definite relation between micronutrient uptake and the group classification in which the soils were placed on the basis of the severity of the chlorosis of citrus trees growing on these soils. Chemical analyses and Neubauer tests were made on this group of soils in order to determine their reaction, salinity, nutritional rating, and calcium content. The data obtained are given in Table 9.

Total soluble salts given in column two show no particular relationship even though the average salt content of the soils where trees are chlorotic is slightly higher than for the other two groups of soils. The same may be said for the pH values, namely, higher average in the first group of soils but many overlapping values in the three groups. The pH values indicate that if alkalinity contributes to chlorosis it is in part the total or potential alkalinity and not entirely the OH-ion concentration. The fertility tests, that is, the solubility of potassium and phosphorus in carbonic acid and the potassium and phosphorus Neubauer values yielded no correlating values. The several calcium determinations are highly significant and indicative that calcium carbonate, probably because of its activity and potential alkalinity, is a contributing factor. Three separate determinations were made. These were active calcium, total $CaCO_3$, and the Neubauer calcium value. The active calcium was determined by shaking 10 gm. of soil with 250 ml. of 0.2 N ammonium oxalate, filtering this, and titrating the excess of oxalate in the filtrate with standard $KMnO_4$ after acidifying with sulphuric acid. The oxalate absorbed by shaking the soil with the solution is calculated as having reacted with and having been precipitated by the active calcium in the soil. Total $CaCO_3$ was determined by the A. O. A. C. method. The calcium Neubauer value represents mg. Ca per 100 rye seedlings.

With only one exception the calcium Neubauer values for the soils from the severely chlorotic groves are significantly higher than for the soils from the other groves, showing a high availability and uptake of calcium in this group. The active calcium and the total $CaCO_3$ are significantly higher in three of these soils and appreciably higher in the rest of the soils in this group. The first five soils represent two rather distinctly different soil types. Soils A and B are of stony or gravelly loam texture not considered highly calcareous, while C, D, and E are silty loam soils, highly calcareous. Gile (8) found that chlorosis will develop on less $CaCO_3$ in a sandy soil than in a clay soil and his observation may have some application here.

TABLE 9.—CHEMICAL AND NUTRITIONAL DATA ON SOILS FROM CITRUS GROVES SHOWING CHLOROSIS OF DIFFERENT SEVERITY

Grove	Total salts (p.p.m.)	pH soil paste	pH 1:10	Avail. P_2O_5 (p.p.m.)	Avail. K_2O (p.p.m.)	Active Ca (per cent)	$CaCO_3$ (per cent)	Neubauer values P_2O_5	Neubauer values K	Neubauer values Ca
				Severely Chlorotic Groves						
A	825	8.50	9.15	7	159	0.38	2.46	1.9	21.1	9.2
B	478	8.65	9.20	13	83	0.29	2.02	3.2	16.2	9.3
C	615	8.40	9.15	8	177	1.76	10.45	2.8	21.7	20.1
D	545	8.40	9.15	7	214	1.14	6.72	4.2	24.3	19.1
E	1110	8.20	9.00	9	218	0.97	4.20	3.6	20.7	9.4
F	610	8.50	9.15	11	83	0.73	2.54	3.7	26.3	2.0
Av.	697	8.44	9.12	9	156	0.88	4.77	3.2	21.7	11.5
				Slightly Chlorotic Groves						
G	540	8.50	9.25	10	114	0.14	0.48	4.2	21.3	2.3
H	520	8.20	9.05	4	187	0.22	0.60	4.5	23.7	3.9
M	945	8.45	8.90	11	214	0.51	1.92	8.0	25.2	4.2
Av.	668	8.40	9.07	8	172	0.29	1.00	5.9	23.4	3.5
				Nonchlorotic Groves						
I	405	8.20	9.05	4	113	0.22	0.65	4.5	20.6	2.8
J	452	8.30	8.90	10	160	0.26	1.18	3.4	24.6	4.5
K	590	8.30	9.05	6	144	0.48	2.40	5.3	19.7	2.9
L	408	8.20	8.45	15	187	0.33	0.70	3.7	25.0	2.6
Av.	464	8.25	8.86	9	151	0.32	1.23	4.2	22.5	3.2

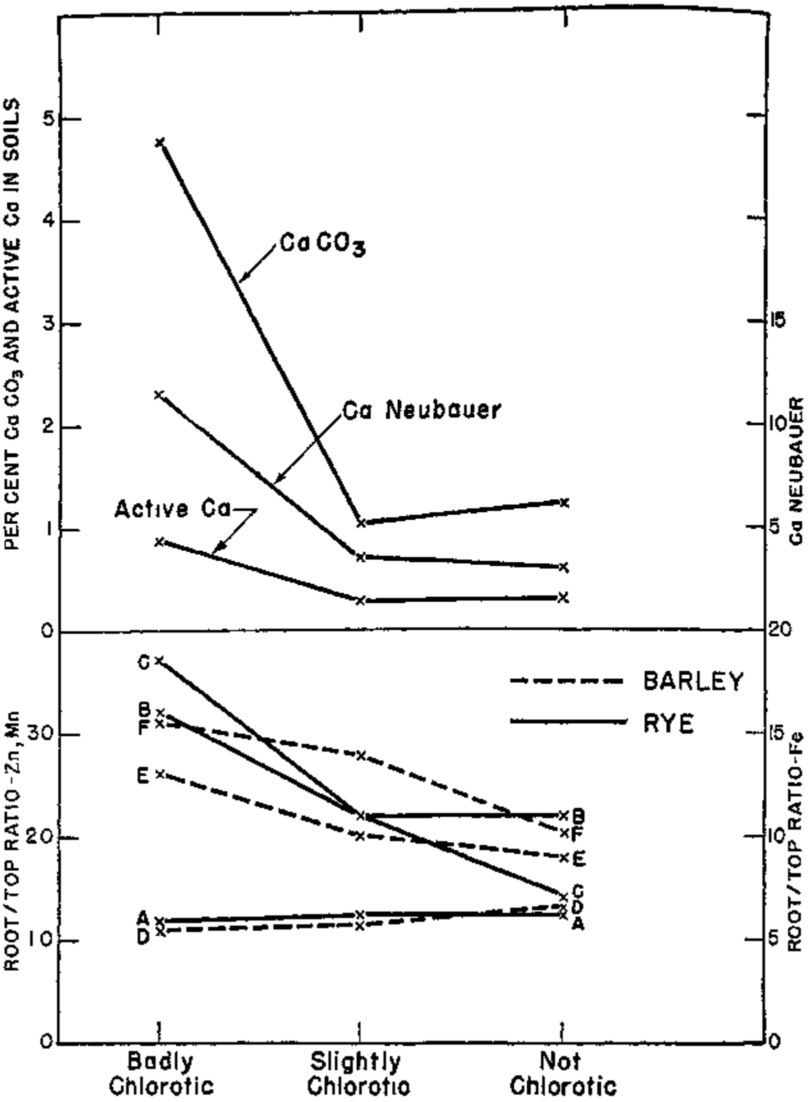

Figure 1.—Showing the relation between root:top ratios for iron, manganese, zinc, and active calcium, $CaCO_3$, and Ca Neubauer values: A and D, zinc; B and E, manganese; C and F, iron.

At all events the differences in the several calcium values presented in Table 9 and especially the uptake of calcium by the seedlings show definite characteristics in soils from severely chlorotic groves as compared with those where chlorosis is mild or entirely absent. The values obtained in the Neubauer test are for the whole seedling. It has not been feasible as yet

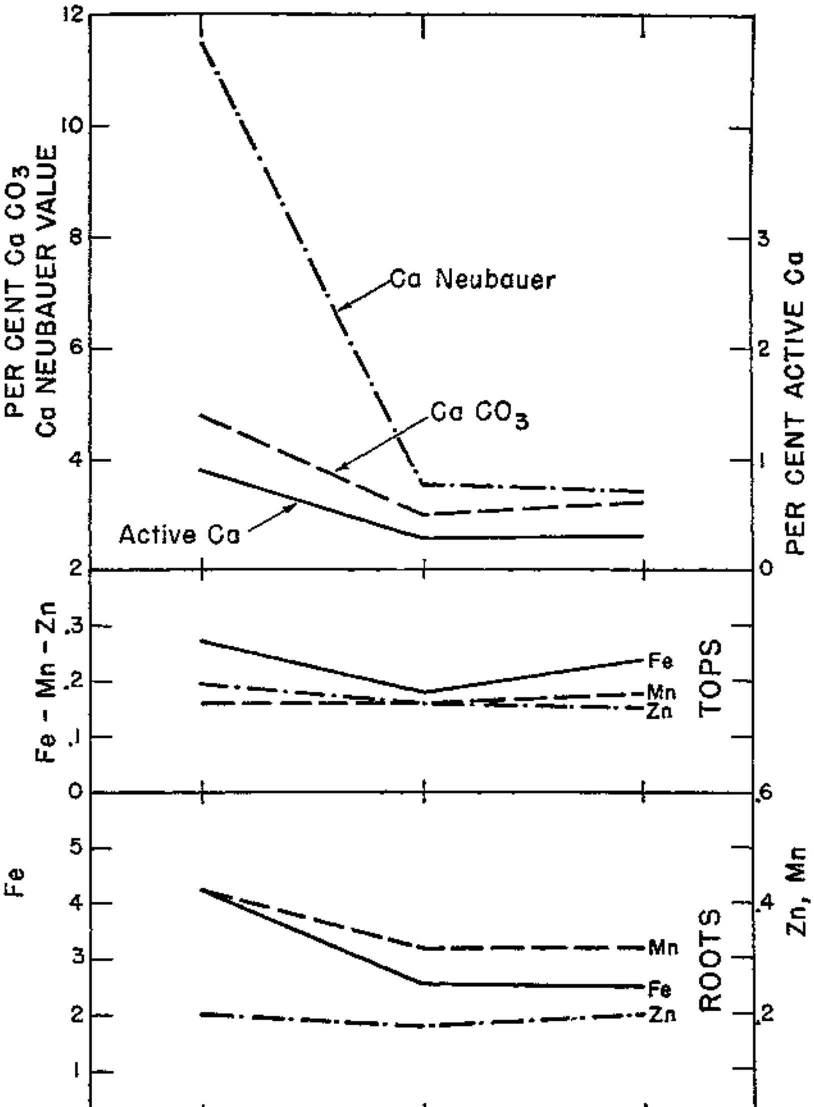

Figure 2.—Showing relation between Ca Neubauer values, per cent active Ca and $CaCO_3$, and uptake of Mn, Zn, Fe by barley seedlings from citrus soils. Mg. per two cultures, roots and tops separately.

to make analyses of citrus roots to study the calcium relations, but it is significant that chlorotic citrus leaves from the trees grown in soils represented in the severely chlorotic soil group are lower in calcium than green leaves.

The relation between calcium availability, total calcium carbonate, and calcium uptake as measured by the Neubauer method (Table 9), and the uptake of iron, manganese, and zinc by seedlings (Tables 7 and 8) for soils from the chlorotic and nonchlorotic groves is shown graphically in Figures 1 and 2, Figure 1 shows that none of the calcium values appreciably affect the root: top zinc ratios: that is, movement of zinc from roots to tops is quite similar for each group of soils. The curves for iron and manganese indicate that these two micro-nutrient elements are probably the ones which contribute most to micro-nutrient element deficiency symptoms occurring in Arizona citrus groves. Root: top ratios for both these elements are highest for the soils from the severely chlorotic groves which are likewise highest in calcium carbonate, active calcium, and calcium Neubauer values. This means that the high calcium values may be contributing to chlorosis by fixation or by precipitation of calcium, iron, and manganese in the roots and probably also to lower activity of these elements in the tops.

In Figure 2 the milligrams of iron, manganese, and zinc in roots and tops of seedlings grown in this same group of soils is compared with active calcium, calcium carbonate, and calcium Neubauer values. The greatest uptake of micro-nutrient elements correlates with the highest calcium values. This is quite definite evidence that micro-nutrient element deficiency symptoms are not actual deficiencies for plants grown in calcareous soils but are symptoms of failure to utilize.

In studies on tree crops, most of the analyses have been on leaf samples because the visible disturbances are manifested there, and root samples are difficult to obtain. For analytical data on tree roots reference should be made to Milad (15) who found higher iron content in the roots of chlorotic pear trees than in normal pear trees growing in calcareous soils. A comparison of his observations with the seedling studies here presented testify to the value of a seedling technic in studies of chlorosis.

EXPERIMENT 8.—APPLICATION OF SULPHUR TO THE SOIL

Some success in the correction of chlorosis and mottle-leaf of plants growing in calcareous soils has been obtained from the application of sulphur or sulphur-manure mixture to the soil. This response has generally been attributed to the acidity developed during the oxidation of sulphur to sulphuric acid which in turn should increase the solubility and availability of micro-nutrient elements in this type of soil and lower the pH of the soil. There is also some evidence that sulphur tends to offset the inherent soil characteristic which retards activity and utilization within the plant. The next experiment was designed to determine the effect of sulphur applications on the uptake of micro-nutrient elements by barley seedlings.

Two separate incubation experiments were conducted with sulphur and sulphur-manure mixture. For these experiments the sulphur was mixed with the soil, incubated for a definite period, and then these soils were used in seedling tests, following the Neubauer technic. In one experiment, sulphur was mixed with the soil at the rates of 250, 500, and 1,000 mg. per 100 gm. of soil. In another part of this experiment, a mixture of equal parts of sulphur and manure was mixed with the soil at the rates of 250, 500, and 1,000 mg. per 100 gm. of soil. They were incubated in covered tumblers for thirty days at 30 degrees C., and at an optimum moisture content. Other experiments conducted in this laboratory have shown that practically all the sulphur is oxidized in this period of time. These 100-gm. portions of soil were then planted with 100 barley seeds. The seedlings were harvested at the end of seventen days and analyzed, roots and tops separately, for iron, manganese, and zinc. In the second experiment, the same procedure was followed, using two different soils and 250 and 2,000 mg. of sulphur and 500 and 4,000 mg. of sulphur-manure mixture. The data obtained from both experiments are given in Table 10.

TABLE 10.—EFFECT OF SULPHUR ON UPTAKE OF MICRO-NUTRIENT ELEMENTS, MG. PER TWO CULTURES

	Iron (Fe)			Manganese (Mn)			Zinc (Zn)			Fe:Mn (ratio)	
Treatment	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops
Control	2.5	.34	7.3	.26	.25	1.0	.302	.160	1.89	9.6	1.3
250 S	3.7	.56	6.7	.26	.22	1.2	.360	.346	1.04	14.2	2.5
500 S	2.7	.52	5.2	31	.21	1.5	.376	.300	1.28	8.7	2.4
1,000 S	3.2	.70	4.5	.36	.19	1.9	.332	.216	1.53	8.9	3.7
250 S-M	3.6	.40	9.0	.28	.13	2.2	.344	.256	1,34	12.8	3.1
500 S-M	2.8	.40	7.0	.23	.16	1.4	.344	.304	1.13	12.1	2.5
1,000 S-M	2.8	.40	7.0	.23	.22	1.0	.372	.300	1.26	12.1	1.8
Control	2.4	.28	8.6	.20	.15	1.3	.250	.120	2.08	12.0	1.9
250 S	3.2	.34	9.4	.24	.15	1.6	.310	.244	1.29	13.2	2.3
500 S	3.1	.68	4.6	.26	.17	1.5	.304	.256	1.19	11.9	4.0
1,000 S	2.8	,76	3.7	.21	.16	1.3	.306	.244	1.25	13.4	4.7
250 S-M	2.9	.60	5.0	.18	.16	1.1	.282	.244	1.16	16.1	3.7
500 S-M	2.9	.90	3.2	.19	.22	0.9	.300	.284	1.06	15.2	4.1
1,000 S-M	3.0	1.00	3.0	.21	.20	1.0	.282	.244	1.17	14.3	5.0
Control	6.8	.42	16.2	.43	.18	2.4	.200	.168	1.19	15.9	2.3
250 S	5.6	.57	10.0	.36	.19	1.9	.195	.183	1.07	15.5	3.0
2,000 S	7.8	.40	19.5	1.26	.50	2.5	.270	.200	1.35	6.2	.8
500 S-M	6.3	.60	12.0	.48	.20	2.4	.207	.155	1.33	13.1	3.0
4,000 S-M	7.2	.46	13.7	1.39	.86	1.6	.252	.220	1.15	5.2	.5

* Three different soils. Control represents soil without any added sulphur. S represents sulphur, S-M represents sulphur-manure mixture.

Zinc.—The zinc uptake of both the tops and roots was greatly increased by both the sulphur and sulphur-manure mixture.

The root: top ratio decreased following fertilization with sulphur and sulphur-manure mixture. This is quite definite evidence that sulphur fertilization increases zinc uptake and also stimulates movement from roots to tops, as shown by root: top ratios.

Iron.—The iron uptake of roots and tops was also increased by sulphur fertilization. The root: top ratio of iron content was decreased, which shows an improved utilization of iron by the seedlings.

Manganese.—The data obtained in the chemical analyses of the seedlings for manganese are not as consistent as the zinc and iron analyses. However, since the heaviest applications, 2,000 mg. of sulphur and 4,000 mg. sulphur-manure mixture produced a greater increase in uptake in both roots and tops, it is evident that the trend is the same as for zinc and iron. It is of interest that where sulphur applications have been made to soils in chlorotic citrus groves the chemical analyses of citrus leaves have shown an increase in per cent manganese in the leaves.

Fe:Mn ratio.—Sulphur applications increased the Fe:Mn ratio in every case in both roots and tops, except in the two heaviest applications where the seedling tops were very high in manganese. This shows that sulphur increases iron uptake at a proportionately greater rate than manganese except when heavy applications are made to the soil. This experiment indicates that if a low Fe:Mn ratio is contributing to chlorosis, light applications of sulphur should benefit, but if a high Fe:Mn ratio is contributing, then heavy applications of sulphur should be employed.

It is extremely significant that the Fe:Mn ratios in seedling tops obtained from heavy applications of sulphur agree closely with the Fe:Mn ratio for seedling tops grown in acid soils. This is all the more significant since chlorosis does not occur in acid soils except when the soil is deficient.

This experiment shows definitely that sulphur applications to the soil will increase uptake and promote better utilization of micro-nutrient elements within the plant.

EXPERIMENT 9.—BARLEY SEEDLING TESTS ON CITRUS SOILS FROM TEXAS, CALIFORNIA, ARIZONA, AND FLORIDA

The next experiment was designed to compare the availability and uptake of micro-nutrient elements for citrus soils from Arizona, Texas, Florida, and California. The data obtained from the seedling tests with these soils are given in Table 11.

Texas soils.—The three samples of Texas soils are from three widely separated groves in the Rio Grande Valley. The Bayview samples are from the extreme eastern end of the valley, the Goodwin samples are from the Mission district in the extreme western end of the valley, while the Engleman samples are centrally located. All are calcareous soils.

California soils.—These soil samples were taken from five different groves in the Coachella Valley, two in the Imperial Valley, one each in the Brawley and El Centro districts. The soils from the Coachella Valley are sandy types, while those from the Imperial Valley are clay soils. All are calcareous.

Arizona soils.—For representative tests on soils from Arizona citrus groves an average of the data presented in Table 8, for severely chlorotic groves, slightly chlorotic groves, and groves where no chlorosis was present, is presented for comparison.

Florida soils.—The Oak Hill sample is a highly organic, acid soil. The Sugar Loaf sample represents the Norfolk fine sand extensively cropped to citrus in the central part of the state. The Smith Island sample is a highly organic soil from Lake County, and very faintly acid. The Sligh sample is a highly organic, calcareous soil, and the last sample is a virgin, highly calcareous soil.

Sand.—At the bottom of the table the Neubauer micronutrient element values for sand controls are given for the convenience of the reader.

Zinc.—The seedlings grown on the Texas soils show the lowest zinc content for roots, but the zinc in the tops is about the same as for the other three sets of soil samples. In fact, there is a close agreement in zinc content of seedling tops for all four areas. This holds true even for the two samples from Florida which have a high zinc content in the roots because they had been fertilized with zinc sulphate. It is somewhat puzzling to note that while the zinc content of the seedling tops in all soil cultures is greater than for the seedlings grown in sand, the zinc content of the roots, as well as the total zinc (roots plus tops), is less than in the sand controls.

Iron.—Again, the most significant part of the data is the large amount of iron fixed in the roots. This is true for the acid soils of Florida as well as the calcareous soils from the other three states. The root: top ratio shows a wide variation between individual samples but no consistent difference between areas as a whole.

Manganese.—The manganese availability does not vary greatly between the soils from the four states. The manganese content of the roots and tops is more or less the same except for the two Florida soils, which had been fertilized with manganese sulphate. It is interesting to note that the manganese content of the roots, except for the seedlings grown in Arizona soils, is closely the same as the sand controls.

Fe:Mn balance.—The Fe:Mn ratio is lowest for seedling tops grown in the acid Florida soils, and highest for the Texas and California soils. The seedlings grown in Arizona soils are slightly higher than the acid Florida soils but lower than Texas and California soils. The ratios for the Florida soils are of the same order as those obtained from other tests on acid soils (Table 4). It is evident that this ratio is characteristically lower for seedlings grown in acid soils than for those grown in calcareous soils.

TEXAS SOILS

These soils are all calcareous, and one would expect the seedling data to be somewhat similar to the data on Arizona and California soils but this is hardly true. The Texas soils are higher in organic matter than the California or Arizona soils. This may favorably affect utilization of micro-elements in the plant.

Zinc.—The zinc values obtained by the seedling technic are the lowest of the four groups, and considerably lower than the controls grown in sand. This is true for the zinc content of the roots and the total zinc (roots plus tops), but the zinc content of the tops is equal to that of the seedlings grown in the other three soil groups, and greater than zinc in the seedlings grown in sand controls. A difference in zinc availability and plant behavior for the seedlings grown in Texas soils is further indicated by the low root: top ratio. These data show that either there is a lower supply of available zinc in Texas soils, or that the seedlings make a more efficient utilization of zinc. Considerable amounts of sulphur are used in Texas citrus soils, and the fact that studies show a more active movement of zinc where seedlings are fertilized with sulphur may be a factor to consider in interpreting these data.

Iron.—While there is some overlapping in the iron values because of the wide variation in the iron content of roots, tops, and the root: top ratios, the average for the Texas soils is significant. The seedlings from this group of soils consistently show the highest iron content in tops and the lowest root; top ratio. This indicates a more efficient utilization of iron by the seedlings. Again, the question arises concerning the sulphur used in the fertilization of Texas soils.

Manganese.—The data for the manganese content of the seedlings grown in Texas soils are significant. In every case except one, the manganese content of tops is greater than roots. The manganese values of the roots are the lowest of the four soil groups, and the average is no greater than the sand control. Regardless of this, the manganese content of the seedling tops is on a par with the other soil groups and does not indicate a deficiency. The root: top ratio is less than unity, namely, 0.8 average.

It is interesting to note that all three of the elements, zinc, iron, and manganese, show more efficient utilization and active movement in seedlings grown in the Texas soils and indicate a favorable response to sulphur or a favorable influence of higher organic matter in these soils.

CALIFORNIA SOILS

These soils are all calcareous and the existing semiarid desert conditions are somewhat similar to Arizona citrus areas.

Zinc.—Zinc availability and uptake appear to be slightly greater in the sandy soils of the Coachella Valley than in the heavy clay soils of Imperial Valley. The root: top ratio indicates a good translocation of zinc from roots to tops.

Iron.—The iron relationships appear to be favorable for this group of soils both in roots and tops and are about on a par with the Texas soils.

Manganese.—The manganese data are similar to those for Texas and Florida soils. The root: top ratio is more favorable than for Arizona soils.

ARIZONA SOILS

The data for Arizona soils are taken from Table 8. Since the data in Table 8 are separated into groups according to the severity of the chlorosis, the average figure for each group is used for brevity and to avoid repetition of data. Reference can be made to Table 8 for a more detailed comparison of Arizona soils with the other three citrus areas. All the Arizona soil samples are calcareous.

Zinc.—It is significant that the zinc uptake is highest for the seedlings grown in Arizona soils. Since the zinc content of the seedling tops is closely the same as the other soils the difference lies largely in the zinc content of the roots. The fact that the root: top ratio is highest for the Arizona soils shows a good supply of zinc and a good availability. The data may be interpreted as showing a greater uptake by the seedlings than is needed for top growth. In other words, there is no indication of a zinc deficiency in Arizona soils.

Iron.—The seedling tests for iron uptake show a favorable iron content for the roots but a low iron content in the tops. The high, unfavorable, root: top ratio shows a restricted movement of iron from the roots to the tops, especially for the seedlings grown in the soils from severely chlorotic groves.

Manganese.—The seedling tests for manganese availability show a good supply in the Arizona soils. The manganese content of the seedling roots is higher in the Arizona soils than in any of the others. This is not true, however, for the manganese content of the seedling tops. These low values and the high root: top ratio indicate that conditions are unfavorable for the transport of manganese within the plants. In this the manganese data are in agreement with the seedling data for iron in these same soils.

On the whole the data from this experiment show that micro-nutrient element availability is high for Arizona soils; that absorption by the seedling roots is good; that the root: top ratio for all three of the elements studied is highest; and finally, that activity is more restricted than for the seedlings grown in soils from Texas, California, and Florida.

FLORIDA SOILS

This group of soils includes a wide variety of types with respect to reaction, texture, and organic matter content. Two of these soils, No. 1 and 3 (Table 11), are known to have been fertilized with a commercial mix containing micro-nutrient elements. For example, the first soil had been fertilized with an 8-0-12 fertilizer which contains magnesium sulphate to supply 3.0 per cent MgO, manganese sulphate to supply 2.0 per cent MnO, copper sulphate to supply 1.0 per cent CuO, iron sulphate to supply 0.5 per cent Fe_2O_3, and zinc carbonate to supply 0.5 per cent ZnO. It is necessary to consider this in interpreting the data on Florida soils.

TABLE 11.—BARLEY SEEDLING TESTS ON CITRUS SOILS FROM TEXAS, CALIFORNIA, FLORIDA, AND ARIZONA

	Iron (Fe)			**Manganese (Mn)**			**Zinc (Zn)**			**Fe:Mn (ratio)**	
Groves	**Roots**	**Tops**	**Ratio**	**Roots**	**Tops**	**Ratio**	**Roots**	**Tops**	**Ratio**	**Roots**	**Tops**
Texas Soils											
	6.7	.60	11.1	.20	.23	0.9	.140	.180	0.8	33.5	2.6
Engelman A	5.0	.68	7.4	.18	.22	0.8	.135	.170	0.8	27.7	3.1
Engelman B	5.2	.60	8.6	.17	.22	0.8	.100	.185	0.5	30.6	2.7
Bayview A	6.0	.69	8.7	.17	.22	0.8	.117	.195	0.6	35.3	3.2
Bayview B	5.7	.48	11.9	.23	.22	1.0	.133	.192	0.7	24.7	2.2
Goodwin A	6.2	.52	11.9	.17	.22	0.8	.150	.175	0.9	36.8	2.3
California Soils											
Whittier A	7.6	.50	13.2	.21	.17	1.2	.155	.175	0.9	36.0	2.9
Whittier B	6.2	.72	8.6	.17	.17	1.0	.195	.195	1.0	36.0	4.2
Mitchell	14.4	.78	18.4	.28	.22	1.3	.180	.195	0.9	51.0	3.3
Forbes No. 3	5.9	.50	11.8	.15	.14	1.1	.195	.210	0.9	40.0	3.6
Forbes No. 4	6.2	.54	11.4	.20	.18	1.1	.150	.195	0.8	31.0	3.0
Mueller	3.8	.35	10.9	.17	.15	1.1	.150	.170	0.9	22.0	2.3
Steiner	4.6	.27	17.0	.19	.11	1.7	.145	.115	1.3	24.0	2.5
DuBois	4.4	.55	8.0	.21	.31	0.7	.170	.195	0.9	21.0	1.8
Florida Soils											
Oak Hill 1	12.4	.32	38.8	.49	.51	1.0	.215	.220	1.0	25.3	0.6
Sugar Loaf 2	2.4	.25	9.6	.18	.15	1.2	.155	.170	0.9	13.3	1.6
Smith Isld. 3	2.6	.27	9.7	.63	.26	2.4	.275	.175	1.6	4.1	1.0
Sligh 4	2.6	.53	4.9	.16	.13	1.2	.175	.170	1.0	16.2	4.1
Calcareous 5	2.0	.26	7.7	.14	.14	1.0	.155	.150	1.0	14.3	1.8
Arizona Soils											
Severely chl.	4.2	.27	15.6	.42	.16	2.6	.209	.194	1.1	10.0	1.8
Slightly chl.	2.5	.18	13.8	.32	.16	1.4	.181	.160	1.1	7.8	1.1
Nonchl.	2.5	.24	10.2	.32	.18	1.7	.201	.157	1.3	7.7	1.4
Sand	**0.54**	**.16**	**3.4**	**.19**	**.10**	**1.9**	**.237**	**.144**	**1.6**	**22**	**1.6**

Zinc.—The zinc values obtained from the seedling tests in this group of soils are only slightly higher than for the Texas soils. However, the root: top ratios indicate a less active utilization. It is significant that in both the soils which had been fertilized with fertilizer containing zinc salts, the zinc content of both roots and tops of seedlings is highest. These are both acid soils. The high root: top ratio indicates that in these two soils the roots had taken up more zinc than needed by the seedling tops. The fifth soil is a virgin calcareous soil taken near the Oak Hill district on the east coast. This soil and the Sugar Loaf Hill soil, a soil known to respond to zinc fertilization, are lowest in zinc content of roots and tops. A comparison of the seedlings grown in Texas and Florida soils indicates a deficiency in the former, if one considers only the zinc content of the roots. The higher zinc of the seedling tops for the Texas soils indicates that this may be offset, at least in part, by the more active utilization of zinc.

Iron.—Except for the one case, the Oak Hill soil, which had been fertilized with a mixture containing iron sulphate, the seedlings grown in Florida soils are lowest in iron. Since this is true for both acid and calcareous soils, it indicates a low availability and low uptake by plants for this group of soils. However, if we omit the Oak Hill sample because of its abnormally high iron value, the seedlings show a favorable root: top ratio and a better proportionate movement of iron from roots to tops than in any of the other soil groups. We would expect this for the acid soils.

Manganese.—The manganese values obtained by growing barley seedlings in the Florida soils are again indicative of increased uptake for the soils fertilized with micro-nutrient elements. Both the soils known to have been fertilized gave very high manganese values for seedling uptake, both for the roots and tops. The unfertilized Florida soils gave manganese values which are among the lowest, both in roots and tops, for soils from all four states.

The data obtained from the seedling tests with Florida soils are strongly indicative of the value of the seedling technic for studying micro-nutrient element availability in soils. The greater uptake of zinc, iron, and manganese in the two soils that had been fertilized with micro-nutrient elements is highly significant.

DISCUSSION

The physiological disturbance in plants which manifests itself as some type of chlorotic leaf pattern is present in many Arizona citrus groves in varying degrees of severity and to some extent in deciduous fruit orchards, but it is rarely observed in truck or field crops. All the citrus groves in Arizona are located on alkaline-calcareous soils and inherent soil characteristics do not vary greatly. The several different leaf patterns are often present on the same tree. This would indicate that so far as the soil problem is concerned we can recognize its contribution to the trouble as being limited to rather definitely defined soil properties, notably their reaction, their calcareous nature, and their structure. The structure is important in its relation to irrigation practice and moisture control and, therefore, root respiration and activity.

The investigation presented in this bulletin is one phase of a study of the chlorosis and mottle-leaf problem in Arizona. After developing a seedling test for micro-nutrient elements a group of soil samples was collected from representative areas within the state and subjected to such a test. The purpose of these seedling tests was to examine the application of a plant method for determining micro-nutrient element availability in a study of the inherent soil characteristics which contribute to chlorosis and mottle-leaf. The various chlorotic leaf patterns are widely identified as deficiencies of micro-nutrient elements. Important among these are iron, manganese, zinc, and copper. In growing plants these elements are present in greatest abundance in the actively growing tissues. Therefore, seedlings, in which the percentage of actively growing tissue is at a maximum, should be ideally suited for measuring availability and uptake of micronutrient elements in soils.

IRON

Linder and Hartley (12) mention four ways in which iron nutrition may be affected and chlorosis developed in plants: (a) true iron deficiency; (b) upset in phosphate: iron balance; (c) upset in iron: manganese balance; and (d) lime-induced deficiency symptoms.

True iron deficiency.—There is no evidence of a true iron deficiency in Arizona soils. The total iron in the soils of the irrigated valleys will average 4 to 6 per cent expressed as Fe_2O_3, and this iron is thoroughly distributed throughout the soil mass. Iron oxides and hydrates are known to be available forms of iron in soils especially when iron is a component of the colloid fraction which can make intimate contact with the plant roots. Chapman (6) found that magnetite, if finely ground, is an excellent source of iron in plant cultures. This shows the essentiality of intimate contact between the roots and the iron compounds in the soil. The seedling tests on Arizona soils show a good supply of available iron.

Phosphate:iron balance.—There is no evidence of a phosphate: iron upset in plants growing in Arizona soils either on the basis of the seedling tests or in the extensive phosphate studies that have been conducted with these soils. Phosphate is abundant in Arizona soils, but it is present in a form which is not readily available under alkaline-calcareous soil conditions. An excessive uptake of phosphate does not appear probable for plants growing in Arizona soils.

Iron:manganese balance.—Iron and manganese appear to have important reciprocal relations in their effects upon the metabolic processes of plants. Sugar cane leaves retain a normal green color on low manganese if iron is high and on low iron if manganese is high (14). In the study of this phase of the chlorosis problem the work of Somers and Shive (17) is outstanding. Their investigations have shown that an iron deficiency can be an excess of manganese and a manganese deficiency can be an excess of iron. The absolute amount or concentration of these two elements is far less important than the balance between them. This is in large part due to the higher oxidation-reduction potential of manganese. If absorbed in excess by the plant it may produce manganese toxicity, namely, iron deficiency by oxidation of ferrous iron to ferric which is biologically far less active. At low concentrations of each and at high concentrations of each they are usually in balance. At certain unbalanced ratios between these two extremes chlorosis is often manifested. Using soybeans as an indicator plant, they obtained best growth in a substrate of approximate Fe:Mn ratio of 2. Likewise on the analyses of plants for iron and manganese in the tissue fluids, the same approximate Fe:Mn ratio was found. Since only the soluble iron and manganese are considered as active in the plant it is questionable whether total iron and manganese in plants are indicative.

The analyses given in Tables 4 and 5 for seedlings grown in acid and alkaline soils are significantly different even though they represent the total and not the soluble iron and manganese in the tissue fluids. The iron content of seedling tops is lower and the manganese content is higher for the former indicating that a lower total Fe:Mn ratio is characteristic of acid soils. This is significant in the light of the fact that chlorosis usually occurs in acid soils only when deficiencies in the soil exist, while in our calcareous soils there is no deficiency but a disturbed activity within the plant. It is doubtful whether an excessive manganese uptake is possible under alkaline-calcareous soil conditions as manganese solubility is more or less limited in alkaline soils, and the roots must utilize their solvent properties for manganese uptake. In fact, an unbalanced Fe:Mn uptake can be corrected in acid soils by liming, and a manganese deficiency created by overliming acid soils.

In Tables 7 and 8 the highest Fe:Mn ratio is noted for the seedling roots grown on soils from the severely chlorotic groves. Table 11 indicates that the Fe:Mn ratio is lowest for the tops of seedlings grown in acid soils and highest in the Texas and California soils.

In Table 10 it is shown that sulphur applications to calcareous soils increase the Fe:Mn ratio in tops of seedlings except where heavy applications are made to the soil. In these two cases the ratio is reduced to the range of that found for acid soils.

There is some evidence in the seedling studies that a high Fe:Mn ratio is the principal micro-nutrient element relationship existing in plants grown in these soils, and excessive applications of sulphur will be required to reduce this,

Lime-induced chlorosis.—In the West micro-nutrient element deficiency symptoms are often found in crops growing on calcareous soils. This is the major factor contributing to deficiency chlorosis in Arizona. The manner in which the calcium carbonate contributes to the development of chlorosis is not entirely clear. There is evidence that calcium carbonate under certain conditions will stimulate citrus root growth (2). However, the effect of the hydrolytic product of $CaCO_3$, namely, $Ca(OH)_2$, is a different story. Breazeale (2) studied the relative toxicity of $Ca(OH)_2$, NaOH, and Na_2CO_3 for grapefruit and lemon seedlings. Signs of distress were noted at 80 p.p.m. $Ca(OH)_2$ and at 250 p.p.m. for NaOH. It was necessary to increase the concentration of Na_2CO_3 to 550 p.p.m. to stop growth of seedlings. Apparently we must look for some indirect effect if we are to consider $CaCO_3$ as a major contributing factor in iron deficiency chlorosis and mottle-leaf.

In Arizona there is evidence that overirrigation or keeping the subsoil zone too continuously wet contributes to citrus chlorosis (3). This has been mentioned as a contributing factor also in California and Texas citrus groves. Chapman (6) even found that flooding citrus in sand cultures often produced chlorosis. Flor (7) found a chlorotic disease of flax to be more severe on wet soils (calcareous soils). Similar observations were made by Wann (19) in Utah. Experimental evidence shows that the poorly aerated soil conditions, probably anaerobic, created by overirrigation or keeping the soil too continuously moist definitely contributes to development of chlorosis. Under such conditions $CaCO_3$ will hydrolyze to $Ca(OH)_2$ which may accumulate when there is not sufficient aeration to convert $Ca(OH)_2$ to $Ca(HCO_3)_2$. This may be the reason why chlorosis is confined almost entirely to deep-rooted crops on our irrigated calcareous soils. It is of interest in this connection that unaerated solution cultures of plants will frequently develop chlorosis.

There are many excellent citrus groves growing in calcareous soils in Arizona, Texas, California, and Florida. A calcareous soil is, therefore, potentially but not necessarily always productive of chlorotic plants.

The seedling tests show an excessive accumulation of iron in the roots of plants grown in alkaline-calcareous soils. Also the root: top ratio for iron is higher (Tables 7 and 8) for soils from severely chlorotic groves. Furthermore, the data in Table 9 show a greater uptake of calcium by seedlings grown in the soils from the severely chlorotic groves. This indicates that both wet soils and active calcium as $CaCO_3$ may be contributing factors.

There is no evidence in the seedling tests that there is a deficiency of available iron in these calcareous soils but that there is a failure of the seedling to utilize rather than a failure to absorb.

MANGANESE

Arizona soils appear to be well supplied with manganese. Analyses made in this laboratory show a variation of 0.3 to 1.0 per cent in the soil expressed as Mn_3O_4. This is equivalent to 6 to 20 tons per acre-foot of soil. Also, there is little or no danger of loss by leaching or weathering. In acid soils oxidation-reduction conditions favor the reduction to the manganous form which is more available than the manganic but is readily leached from acid soils. The opposite relation exists in alkaline-calcareous soils, namely, manganic manganese and practically no loss from leaching. Thus, most cases of manganese deficiency exist on acid soils. Since most acid soils are very low in manganese content, a deficiency chlorosis will often develop when such soils are overlimed or limed to a pH above 6.7. There is lime-induced manganese deficiency as well as lime-induced iron deficiency on many acid soils. Calcareous soils must have a higher manganese content than acid soils to supply crops, and Arizona soils appear to meet this requirement.

There is no evidence of any manganese deficiency in the seedling tests (Tables 7 and 8). The distribution of manganese in the plant is similar to that for iron, namely, it is present in largest amounts in the roots. The soils are grouped into severely chlorotic, slightly chlorotic, and nonchlorotic. It is significant that the roots of seedlings growing in soils from the severely chlorotic groves are highest in manganese but the tops are lowest. This indicates a retarding influence on translocation of this element as being a contributing factor in calcareous soils when uptake of calcium is excessive (Table 9 and Figure 1). Further evidence of this will be found in Tables 4 and 5 where the manganese root: top ratio is shown to be lower for acid soils. The data show quite conclusively that if manganese deficiency is a contributing factor in the chlorosis of plants grown in alkaline-calcareous soils in Arizona, it is a case of an unbalanced Fe:Mn ratio or failure of the plant to utilize the manganese rather than a failure to absorb. The behavior of manganese is thus in agreement with that of iron.

ZINC AND COPPER

The zinc deficiency leaf pattern, commonly called mottle-leaf when present on citrus trees, is quite prevalent in citrus groves in Arizona, but copper deficiency has not been observed. The zinc content of Arizona soils has not been determined chemically, but a number of copper determinations have been made and all have shown the presence of this element. Soil alkalinity is known to be a contributing factor in zinc and copper deficiency symptoms on alkaline-calcareous soils. That factors other than soil reaction are involved in mottle-leaf is shown by the fact that it occurs both on highly calcareous soils and the gravelly, stony types which are not highly calcareous. Alkaline soils are usually higher in zinc than acid soils, but solubility and availability are greater in acid soils. Because of this, acid soils which contain just enough zinc to support normal plant growth will exhibit zinc deficiency symptoms if they are overlimed. The exchange complex of the soil has a great affinity for zinc and copper as shown by the fact that much better re-

spouse is obtained with zinc and copper sprays than with applications to the soil. This fact together with the alkalinity of calcareous soils means that alkaline soils must contain more of these elements than acid soils in order to support normal plant growth.

The seedling tests do not indicate a deficiency of either zinc or copper in Arizona soils, although there is no information in the literature on plant nutrition as to just what constitutes a sufficiency of either of these elements in plants. The great affinity of the soil for zinc and copper is indicated by the number of seedling tests in which the seedlings grown in soils had less zinc and copper in the roots than those grown in sand. This shows that some of the zinc and copper in the seed was absorbed by the soil during the changes taking place in germination.

There is apparently less restricted movement of zinc and copper from roots to tops than of iron and manganese. At least the interpretation of the root: top ratios indicate this. This ratio is less for the soil-grown seedlings than for the sand-grown seedlings and is just the opposite of the root: top ratio noted for iron and manganese. As a whole, the data on uptake of zinc and copper by seedlings indicate that there is no deficiency of available forms in Arizona soils. Seedlings grown in alkaline-calcareous soils actually contain more zinc than those grown in acid soils.

Ca:K RATIO

Throughout our work on citrus chlorosis and mottle-leaf the most consistent difference in chemical composition between green and chlorotic leaves has been the Ca:K ratio (13). With only rare exceptions it is lower in the chlorotic leaves than in the green leaves, indicating a high potassium uptake and a low calcium uptake in the former. This low Ca:K ratio has also been noted for chlorotic citrus leaves in California. Linder and Hartley (12) found the same low Ca:K ratio for pear leaves and they venture the opinion that the lesser uptake of calcium and greater uptake of potassium may be a cause of iron chlorosis. This hardly seems true for Arizona citrus leaves. New leaves are high in potassium and decrease in potassium content toward maturity. It is not a question of greater uptake but a failure of calcium to move into the leaves and of potassium to move out as the leaves mature.

The seedling tests show a definite Ca:K relationship for the grouping of soils in Tables 7, 8, and 9. The calcium and potassium determinations were made on the whole plant. However, the calcium potassium relationship for seedlings is just the opposite of that found for citrus leaves. The average Ca:K for the soils from the chlorotic groves is 0.64; for the soils from the groves that were only slightly chlorotic it is 0.18; and for the nonchlorotic groves it is 0.17. Barley seedlings, therefore, do not exhibit the same type of calcium potassium balance as citrus in alkaline-calcareous soils, but it is definitely indicative and about what one would expect when roots and tops are not analyzed separately.

SOIL TYPES

The soils on which the trees were severely chlorotic were Mohave fine gravelly, sandy loam, Mohave loam, and Laveen loam. The former is composed of sharp angular fine gravel and coarse sand fragments and contains slightly over 2 per cent calcium carbonate which by the seedling test shows a high availability. While iron deficiency patterns are present on trees growing in this soil the zinc deficiency is more dominant. The Laveen loam and Mohave loam are more highly calcareous, 4.5 and 2.5 per cent $CaCO_3$ in one case, and 10.45 and 6.7 in the other. It is underlain with regular layers of caliche which are much higher in CaCOa than the above analyses. The iron deficiency pattern is more dominant in this soil type.

SULPHUR

There are a number of reasons why sulphur is useful for Arizona soils, and especially for the soils of citrus groves. It will reduce alkalinity, help to maintain good soil structure, and correct poor soil structure. It will also correct micro-nutrient element deficiency symptoms. Some success has followed the use of sulphur for citrus soils in Arizona (13), California (10,18), and Texas (9).

Chandler and Scarseth (4) found that sulphur applications to the soil tended to reduce chlorosis of peanuts. Piper (16) corrected manganese deficiency in three different soils by increasing the acidity. Alben and Hammar (1) found that sulphur alone lessened rosette condition in pecans, a zinc deficiency disease, and when in combination with zinc sulphate and manure, brought about complete recovery.

The seedling experiment with soils incubated for thirty days with sulphur shows a definite increase in micro-nutrient uptake as well as an improvement in root: top ratio. The experiment shows a sound theoretical basis for recommending sulphur applications for alkaline-calcareous soils where crops are chlorotic.

CALCIUM CARBONATE

Calcium carbonate is so widely prevalent in soils where chlorotic leaf patterns exist on plants that the condition is usually referred to as "lime-induced" chlorosis. Soil surveys and field observations have demonstrated quite conclusively that destruction of chlorophyll occurs extensively on plants growing in calcareous soils. The fact remains, however, that in calcareous soils some plants which are susceptible to chlorosis often bear entirely normal foliage. This fact lends credence to the idea that it is not the calcium carbonate itself that brings about the loss of chlorophyll, but that some other associated factor is present to induce chlorosis.

The seedling tests conducted during this investigation showed an excessive absorption of calcium from soils taken from seriously chlorotic groves. Gile (8) has shown that excessive uptake of calcium from calcium sulphate does not induce chlorosis, while excessive uptake from calcium carbonate does.

The seedling tests also show that there is a reduction in micro-nutrient availability in soils from groves that are seriously chlorotic, and a greater retention of iron in the roots of seedlings grown in this group of soils. Since the chemical analyses of both seedlings and citrus leaves do not show a definite deficiency of micro-nutrient elements in leaves, this suggests two theories. First, the uptake of micro-nutrient elements is reduced in alkaline-calcareous soils, but the magnitude of this reduced uptake does not appear to be sufficient to represent an actual deficiency. Second, the root: top ratios show that the cause of the deficiency symptoms is a failure to utilize the elements after they have been taken up by the roots from the soil.

This is well illustrated by the following seedling experiment conducted with a Florida soil in which a highly calcareous marl, 46.1 per cent $CaCO_3$, is blanketed with 4 inches of a highly organic muck containing 13.1 per cent $CaCO_3$. Seedling tests with this surface muck and the marly subsoil yielded the data given in the following table.

	Zinc (Zn)*			Iron (Fe)*			Manganese (Mn)*		
	Roots	Tops	Ratio	Roots	Tops	Ratio	Roots	Tops	Ratio
Surface	.175	.170	1.07	2 6	.53	4.9	.16	.13	1.2
Subsoil	.140	.145	0.97	2.2	.43	5.1	.13	.11	1.2

* Mg. per two seedling cultures.

These data show that an increase from 13 per cent $CaCO_3$ to 46 per cent reduced the uptake of all three elements in both roots and tops.

From the seedling tests conducted in this study of chlorosis, the data definitely show that the uptake of micro-nutrient elements is reduced by alkaline-calcareous soils. Added to this evidence is the all too frequent occurrence of chlorosis on plants grown in calcareous soils, and the fact that the chlorosis usually occurs when acid soils are overlimed. On the other hand, this seedling study, as well as many other analyses made in this laboratory, shows appreciable amounts of all the elements in seedlings and other plants grown in Arizona alkaline-calcareous soils. As already mentioned, therefore, the calcium carbonate contributes to chlorosis, in most part, by creating a physiological condition within the plant itself which reduces utilization.

SUMMARY

Data have been presented to show the value of seedling tests for studying the availability of micro-nutrient elements in soils and the soil conditions which influence their absorption and utilization.

Both rosen rye and Sacramento barley seedlings are suitable for the test. The analytical data obtained from the tests are more useful if roots and tops are ashed and analyzed separately. A calculation of root: top ratio is more illuminating than analyses representing the plant as a whole.

Seedling tests with a group of soils representing a fair cross section of the state show a good supply of available zinc, copper, manganese, and iron in all cases.

The root: top data are significant in that they are lowest for zinc and copper, and highest for iron, while the manganese values are intermediate. This shows a greater retention of iron in the roots. The roots of seedlings grown in soil frequently contain less zinc and copper than the roots of seedlings grown in sand controls containing no soil. This is true for both acid and alkaline soils. The root:top ratios for seedlings grown in acid soils are surprisingly close in agreement with those in alkaline-calcareous soils.

Seedling tests were conducted with soils from severely chlorotic, slightly chlorotic, and nonchlorotic citrus groves. In an examination of the data on the basis of the distribution of micro-nutrient elements between roots and tops, there is evidence of a greater fixation or retarded movement of these elements in the seedlings grown in the soils from severely chlorotic groves. This is particularly true for iron and manganese in roots and tops of seedlings grown in the soils from severely chlorotic groves. Since there is no available data on what represents a sufficiency in plants, it is not possible to draw any final conclusions on whether a deficiency exists. However, the data indicate that all the seedlings contained a sufficiency of iron, zinc, and manganese, and further indicate the chlorosis of plants grown in Arizona alkaline-calcareous soils is largely a failure of the plant to utilize these elements.

Chemical and nutritional studies with the soils from these groves show a definite relation between calcium carbonate, active calcium in the soil, and chlorosis. This inherent soil characteristic induces a physiological condition within the plant which prevents a proper utilization of micro-nutrient elements.

Studies in which seedling tests were conducted with soils that had been previously incubated with sulphur showed that sulphur increases the uptake of micro-nutrient elements and stimulates a more effective utilization within the plant. This latter is proved by the more favorable root: top ratio for seedlings grown in sulphur treated soils.

Seedling tests with soils from citrus groves from Texas, California, Florida, and Arizona showed that Arizona citrus soils give as good or better availability of micro-nutrient elements as those from the other states. The root: top ratios for zinc, iron, and manganese are highest for Arizona soils. This shows a more restricted movement from roots to tops.

This investigation of the inherent characteristics of alkaline-calcareous soils which contribute to chlorosis shows that the nutritional disturbance involved is within the plant itself, and the soil contributes only indirectly to chlorosis. Further knowledge of the plant mechanism involved in chlorosis should be sought within the plant itself. It is interesting to note in recent literature that in research on chlorosis more emphasis is being placed in the study of the physiology of the plant itself.

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