tively unaffected by nitrogen or phosphorus fertilization although the potassium content of the fruit is increased by fertilization with that element. The potassium content of the peel is affected to a larger extent than that of the juice.

LITERATURE CITED

1. Bryan, O. C. The quality of citrus fruit as affected by cultural practice. Proc. Fla. State Hort. Soc. 53: 98-100. 1940.
2. Chapman, H. D., and W. P. Kelley. The mineral nutrition of Citrus. Chap. VII. The Citrus Industry, Vol. 1, Univ. of Calif. Press. 1943.
3. Fudge, B. R. Relation of magnesium deficiency in grapefruit leaves to yield and chemical composition of fruit. Bull. 331. Fla. Agr. Exp. Sta. 1939.
4. Reuther, W., and P. F. Smith. Relation of fertilizer treatment to fruit quality of Valencia oranges. Proc. Fla. State Hort. Soc. 64: 29-35. 1951.
5. Reuther, W., F. E. Gardner, P. F. Smith, and W. R. Roy. A progress report on phosphate fertilizer trials with oranges in Florida. Proc. Fla. State Hort. Soc. 61: 43-60. 1948.
6. Roy, W. R. Effect of potassium deficiency and of potassium derived from different sources on the composition of the juice of Valencia oranges. Jour. Agr. Res. 70: 143-169. 1945.
7. Smith, P. F., and W. Reuther. Seasonal changes in Valencia orange trees. I. Changes in leaf dry weight, ash, and macro-nutrient elements. Proc. Amer. Soc. Hort. Sci. 55: 61-72. 1950.
8. Smith, P. F., W. Reuther, and G. K. Scudder, Jr. Effect of differential supplies of nitrogen, potassium and magnesium on growth and fruiting of young Valencia orange trees in sand culture. Proc. Amer. Soc. Hort. Sci. 61: 38-48. 1953.
9. Smith, P. F., W. Reuther, and A. W. Specht. Seasonal changes in Valencia orange trees. II. Changes in micro-elements, sodium, and carbohydrates in leaves. Proc. Amer. Soc. Hort. Sci. 59: 31-35. 1952.
10. Smith, P. F., W. Reuther, and A. W. Specht. Mineral composition of chlorotic orange leaves and some observations on the relation of sample preparation technique to the interpretation of results. Plant Physiol. 25: 496-506. 1950.

MINERAL COMPOSITION OF VALENCIA ORANGE SEEDLINGS GROWN IN SOLUTION WITH VARYING AMOUNTS OF COPPER, ZINC, MANGANESE, AND IRON

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Last year a report was made to this Society on various aspects of the toxic effect of heavy metals on citrus seedlings (3). The present paper gives some chemical analyses of the plants grown in the solutions described in table 1 of last year's report. Pictures of some of the plants involved and discussed here were published last year as figures 2 and 3 (3).

The plants were grown in nutrient solutions (pH near 4) by placing newly germinated seeds in corks in 1-quart fruit jars. Supplementary amounts of copper, zinc, and manganese were added to a complete nutrient solution. Chelated iron (K-Fe EDTA) was added to each of the treatments at a low rate (0.5 p.p.m. Fe) or a high rate (5 p.p.m. Fe). After 75 days in the greenhouse the plants were harvested, divided into tops and roots and analyzed for 12 elements. The tops were detergent-washed before drying, while the roots were only rinsed in distilled water. There were four replicates for each treatment, but these were combined in order to have sufficient dry matter to analyze. Even so, certain treatments were so toxic that the amount of growth made did not provide enough material for chemical analysis. This accounts for certain missing treatment numbers in the present tables as compared with those in table 1 of the previous report (3).

It is felt that the comparative values obtained will be helpful primarily in planning future studies of heavy-metal toxicities and in elucidating the behavior of the metals within the plant.

RESULTS

The data in tables 1 and 2 are arranged in 3 groups on the basis of plant size. The mean dry weights indicate the degree of stunting. The plants which made normal growth tended to have lower nitrogen concentrations than those in the two stunted groups. The differences are not large and are possibly of no particular significance. Phosphorous concentrations tend to be similar regardless of treatment. The same is true of boron. Sodium and aluminum were not intentionally supplied, but apparently were present as contaminations in the salts used or came into solution from the glass culture jars. Neither shows any definite trend in relation to treatment.

The three main base elements, potassium, calcium, and magnesium, showed variations that are apparently related to the heavy metals. In the moderately stunted plants, potassium concentrations were unchanged but calcium and magnesium showed lowered concentrations in plant tops when compared with the

Table 1. Mean dry weight and macro-element composition of tops and roots of 75-day-old Valencia seedlings grown in nutrient solutions with different heavy-metal treatments as indicated.

No.	Treatment	Plant Part	Dry Wt. (mgm.)	Percent in dry matter N	P	K	Ca	Mg
			Normal Plants					
1	Check--low metals	Tops	450	1.82	0.17	1.20	2.00	0.40
1	Check--low metals	Roots	350	1.42	0.17	0.85	0.34	0.37
7	5 ppm. Fe	Tops	480	1.92	0.16	1.60	1.50	0.36
7	5 ppm. Fe	Roots	300	2.10	0.22	1.40	0.40	0.33
20	5 ppm. Fe + 5 ppm.Mn	Tops	500	1.58	0.13	1.40	1.75	0.37
20	5 ppm. Fe + 5 ppm.Mn	Roots	430	1.30	0.14	0.85	0.35	0.33
	Average for Tops		477	1.77	0.15	1.40	1.75	0.38
	Average for Roots		360	1.61	0.18	1.03	0.36	0.34
			Moderately Stunted Plants					
2	0.1 ppm. Cu	Tops	315	2.62	0.19	1.50	2.00	0.38
2	0.1 ppm. Cu	Roots	250	2.00	0.25	1.10	0.45	0.33
8	0.1 ppm.Cu + 5 ppm.Fe	Tops	340	2.00	0.16	1.60	1.35	0.35
8	0.1 ppm.Cu + 5 ppm.Fe	Roots	255	2.05	0.18	1.40	0.40	0.30
11	3 ppm. Zn	Tops	240	2.25	0.22	1.50	1.35	0.33
11	3 ppm. Zn	Roots	160	2.20	0.23	1.10	0.40	0.32
14	3 ppm.Zn + 5 ppm.Fe	Tops	340	2.10	0.19	1.60	1.25	0.35
14	3 ppm.Zn + 5 ppm.Fe	Roots	270	1.85	0.16	1.40	0.40	0.40
17	5 ppm. Mn	Tops	230	2.20	0.18	1.60	1.50	0.34
17	5 ppm. Mn	Roots	180	2.15	0.26	1.00	0.40	0.26
21	25 ppm.Mn + 5 ppm.Fe	Tops	375	1.75	0.14	1.20	1.15	0.25
21	25 ppm.Mn + 5 ppm.Fe	Roots	300	1.55	0.19	0.95	0.35	0.31
	Average for Tops		307	2.15	0.18	1.50	1.43	0.33
	Average for Roots		236	1.97	0.21	1.16	0.40	0.32
			Severely Stunted Plants					
3	0.25 ppm. Cu	Tops	130	2.02	0.15	1.10	0.90	0.28
3	0.25 ppm. Cu	Roots	140	1.80	0.11	0.45	0.40	0.17
4	0.5 ppm. Cu	Tops	100	---	0.15	1.00	1.00	0.25
4	0.5 ppm. Cu	Roots	120	1.80	0.12	0.50	0.35	0.15
9	0.5 ppm.Cu + 5 ppm.Fe	Tops	115	2.00	0.16	1.30	1.15	0.25
9	0.5 ppm.Cu + 5 ppm.Fe	Roots	140	2.00	0.16	0.80	0.45	0.19
12	15 ppm. Zn	Tops	45	1.60	0.17	1.20	0.70	0.30
12	15 ppm. Zn	Roots	50	2.05	0.14	0.55	0.30	0.20
15	15 ppm.Zn + 5 ppm.Fe	Tops	50	---	0.16	1.20	0.70	0.27
15	15 ppm.Zn + 5 ppm.Fe	Roots	80	2.00	0.13	0.65	0.30	0.21
18	25 ppm. Mn	Tops	110	2.00	0.17	1.00	0.80	0.27
18	25 ppm. Mn	Roots	110	2.45	0.25	0.55	0.30	0.22
22	50 ppm.Mn + 5 ppm.Fe	Tops	180	2.10	0.17	1.10	1.15	0.26
22	50 ppm.Mn + 5 ppm.Fe	Roots	130	2.04	0.31	0.70	0.45	0.24
	Average for Tops		104	1.94	0.16	1.13	0.91	0.27
	Average for Roots		110	2.02	0.17	0.60	0.36	0.20

Table 2. Concentrations of heavy metals, boron, and sodium in the tops[a] and roots of 75-day-old Valencia seedlings.

No.	Treatment	Plant Part	Parts per million in dry matter						
			Cu	Zn	Mn	Fe	B	Na	Al
			Normal Plants[b]						
1	Check--low metals	Tops	9.3	48	40	110	70	170	20
1	Check--low metals	Roots	50	225	440	550	15	440	300
7	5 ppm. Fe	Tops	6.8	64	40	200	60	180	14
7	5 ppm. Fe	Roots	33	180	240	1,000	16	330	640
20	5 ppm. Mn + 5 ppm. Fe	Tops	7.3	48	910	90	50	150	14
20	5 ppm. Mn + 5 ppm. Fe	Roots	35	185	7,800	820	14	270	250
			Moderately Stunted Plants						
2	0.1 ppm. Cu	Tops	11.0	40	38	110	65	190	18
2	0.1 ppm. Cu	Roots	260	225	350	500	18	550	190
8	0.1 ppm.Cu + 5 ppm. Fe	Tops	8.9	59	34	150	60	190	20
8	0.1 ppm.Cu + 5 ppm. Fe	Roots	170	200	190	940	15	300	190
11	3 ppm. Zn	Tops	15.0	380	40	75	80	190	17
11	3 ppm. Zn	Roots	50	3,600	590	700	14	---	250
14	3 ppm.Zn + 5 ppm. Fe	Tops	8.7	350	35	120	70	180	15
14	3 ppm.Zn + 5 ppm. Fe	Roots	30	3,100	330	1,100	14	---	220
17	5 ppm. Mn	Tops	16.0	62	1,350	50	70	180	11
17	5 ppm. Mn	Roots	47	205	18,000	690	14	220	300
21	25 ppm.Mn + 5 ppm.Fe	Tops	6.5	46	8,700	120	50	130	13
21	25 ppm.Mn + 5 ppm.Fe	Roots	25	120	23,000	1,100	17	270	250
			Severely Stunted Plants						
3	0.25 ppm. Cu	Tops	14.0	80	21	45	60	200	16
3	0.25 ppm. Cu	Roots	500	150	140	640	12	210	220
4	0.5 ppm. Cu	Tops	15.0	66	21	45	65	220	22
4	0.5 ppm. Cu	Roots	600	130	70	560	11	190	220
9	0.5 ppm.Cu + 5 ppm.Fe	Tops	14.0	43	26	140	60	220	15
9	0.5 ppm.Cu + 5 ppm.Fe	Roots	340	160	120	1,300	16	230	280
12	15 ppm. Zn	Tops	6.8	275	25	60	60	400	30
12	15 ppm. Zn	Roots	32	3,800	260	900	12	---	520
15	15 ppm.Zn + 5 ppm. Fe	Tops	8.8	240	25	110	70	410	50
15	15 ppm.Zn + 5 ppm. Fe	Roots	32	4,450	150	1,200	11	---	310
18	25 ppm. Mn	Tops	15.0	46	11,000	40	70	170	14
18	25 ppm. Mn	Roots	70	140	42,000	680	15	180	280
22	50 ppm. Mn + 5 ppm.Fe	Tops	7.6	43	18,000	150	50	180	15
22	50 ppm. Mn + 5 ppm.Fe	Roots	35	150	80,000	1,900	19	330	350

normal plants. In the severely stunted plants, all base-element concentrations except root calcium were substantially lower than in the normal plants. Copper, zinc and manganese appear to behave similarly in this regard; therefore the values for the various elements in table 1 are averaged for easy comparison.

All the heavy metals accumulated in the roots. The root concentrations also increased with increased supplies. Copper ranged from 33 to 50 p.p.m. in roots of normal plants, 170 to 260 in moderately stunted plants, and 340 to 600 in severely stunted plants. Zinc ranged from 180 to 225 p.p.m. in normal roots, 3100 to 3600 in moderately stunted roots, and 3800 to 4450 in severely stunted root systems. Manganese ranged from 240 to 7,800 p.p.m. in normal roots, 18,000 to 23,000 in moderately stunted roots, and 42,000 to 80,000 in severely stunted plants. Iron varied from between 550 and 900 in roots given the low-iron treatments to between 820 and 1900 in the 5 p.p.m. iron treatments (considered irrespective of plant size or other metal treatment.)

The tops of the plants showed lower concentrations of all metals than the roots. Copper increased slightly (from 9.3 to 15 p.p.m.) as the rate of supply was increased. Zinc concentrations in tops showed a rather large increase when the plants were given supplementary zinc but they were not proportional to the degree of stunting. Zinc supplied at 3 p.p.m. produced foliage concentrations of 350 and 380 p.p.m., while 15 p.p.m. gave values of 240 and 275 p.p.m. in the foliage. Manganese was found in concentrations of 40 and 910 p.p.m. in tops of normal plants, 1,350 and 8,700 in moderately stunted plants, and 11,000 and 18,000 in severely stunted plants. Increasing the iron supply alone increased the iron concentration in tops from 110 to 200 p.p.m. Similar proportionate increases in iron with increased rate of iron supply are seen in the various matched treatments containing excess copper, zinc or manganese.

Discussion

The lowering effect of copper, zinc, and manganese on the base elements is probably indirect since there is little indication of direct replacement by the metals. The effect also cannot be logically associated with carbohydrate starvation since some of the severely stunted plants were not chlorotic. Those with 5 p.p.m. iron were mostly of normal green color and presumably able to carry on photosynthesis. Their base element composition was affected in the same general way as in the low-iron (chlorotic) plants. It seems probable that impaired root function, such as possible interference in respiratory processes, results in reduced base-element absorption.

The present results support the previous observations (3,4) that copper is much more toxic than zinc and zinc in turn is more toxic than manganese. Citrus roots may adsorb much more copper than the 600 p.p.m. shown here, but the roots are killed when copper exceeds about 800 p.p.m. (5). Dead roots, however, may continue to adsorb copper up to several thousand p.p.m. (5). This should be taken into consideration when root analysis is used for diagnostic purposes.

The increases in the concentrations of copper in the foliage shown here are all within the range of normal copper content. The low-copper treatments tend to show slightly low foliage values. The results thus confirm previous observations (4) that excess quantities of copper may accumulate in roots without inducing excess quantities in the foliage.

The ranges of zinc concentrations shown by both tops and roots are similar to those found previously (3,4). However, the marked stunting and toxic effect on roots shown here were not as evident in plants grown in vermiculite cultures with dosages inducing comparable zinc concentrations in roots.

Manganese concentrations in both tops and roots exceed any previously reported for citrus. There is no way of judging from the present study to what extent this element occurred on the outside of the roots. The roots grown in the high-manganese solutions were slightly darker brown than those grown in other solutions. It seems unreasonable to assume that 80,000 p.p.m. manganese (8.0% of dry weight) was entirely inside the roots. Even so, there is no doubt that very large amounts of manganese entered the tops (1.8% extreme value) and that this element is much less toxic to citrus than is copper. As previously described (4,5) plants given treatment 22 showed brown necrotic leaf spots, a symptom of manganese toxicity. They also made stubby root growth. The plants were harvested 15 days after those observations were made and by that time several of the plants in treatment 18 (1.1% Mn in tops) showed the necrotic spots. The previous study (3, 4) indicated that young stems are somewhat lower in manganese concentration than the corresponding leaves. Thus the present values would be a conservative estimate of leaf manganese concentrations. The copper and zinc values in young stems and leaves, however, are comparable (3, 4).

The present data are insufficient to support extensive speculation as to possible mechanisms by which these metals retard growth. Which comes first, induced chlorosis or a direct toxic effect? Hewitt (1) stated than manganese-induced chlorosis and toxicity can be distinguished as separate phenomena, chlorosis being the initial effect of the excess metal. The following observations suggest that manganese and copper may behave differently in this respect.

All three of the metals in the present study, under certain conditions, caused both stunting of growth and iron chlorosis. Sufficient quantities of chelated iron prevented chlorosis in the presence of excessive amounts of the other three metals. None of these elements reduced the iron concentrations in roots. In these respects the effects are similar regardless of which metal is involved. On the other hand differences were evident. The higher level of iron, which prevented chlorosis, improved growth in the excess-manganese cultures (compare numbers 20 and 17; 21 and 18) without necessarily lowering the manganese concentration in the tissues (compare 17 and 21). Within a few days after the start of the experiment chlorosis was prominent in the plants of treatment 17 and was so pronounced that even the buds were chlorotic. Root growth was reduced, but stubby roots did not develop. These observations suggest that it is also possible to induce iron chlorosis of citrus (and consequent stunting) with sub-toxic levels of manganese. The importance of iron-manganese ratios in other plants has been indicated by numerous workers. The work of Hopkins et al. (2) is outstanding in this regard.

Copper, however, appears to function in a different manner even though the end result may appear to be the same as that of manganese toxicity. It has been repeatedly observed that excess copper, in soil, sand, vermiculite and solution cultures, causes chlorosis (if at all) mostly after stunting of growth has been evident for some time. Plants of treatment 2 showed no chlorosis for several weeks and then only the 1 or 2 youngest leaves were affected out of an average of 19 leaves per plant (3). Toxicity to roots (manifested by stubby, malformed laterals) was evident from the beginning of the culture period. Furthermore, little or no improvement in growth resulted at any copper level when chlorosis was prevented by 5 p.p.m. of iron. Thus, the possibility exists that toxicity to roots is the initial effect of excess copper and not chlorosis as with manganese. Iron chlorosis thus may be a secondary response, just like the disturbed base-element nutrition which appears to accompany a debilitation of the root system. If so, it may be somewhat comparable to iron chlorosis induced by poor aeration or water damage.

This distinction between the effects of mild excesses of copper and manganese may not be borne out by more critical experiments but, from data now available, it appears unlikely that excesses of all heavy metals depress growth through exactly the same mechanism.

Summary

Chemical analyses were made for 12 elements in Valencia orange seedlings grown in solution culture with excessive quantities of copper, zinc or manganese. These strengthen previous observations in regard to the toxic effect of these metals.

The relative tolerance of the plant for these metals is reflected by the concentrations of the metals in the roots and tops. Thus, copper, which is the most toxic of the three, occurs in the lowest concentrations in the plant. Manganese, the least toxic, shows the highest concentrations in the plant parts.

Large excesses of all three metals lowered the absorption of the base elements. All three metals lowered the iron concentration in the tops but not in the roots. Adequate chelated iron maintained a normal supply of iron in the foliage, which parallels its prevention of chlorosis.

LITERATURE CITED

1. Hewitt, E. J. Resolution of factors in soil acidity; Some effects of manganese toxicity. Ann. Rept. Agr. and Hort. Rsch. Sta., Long Ashton, Bristol. pp. 50-61. 1946.
2. Hopkins, E. F., V. Pagan and F. J. Ramirez Silva. Iron and manganese in relation to plant growth and its importance in Puerto Rico. Jour. Agr. Univ. Puerto Rico 28(2): 43-101. 1944.
3. Smith, P. F. and A. W. Specht. Heavy-metal nutrition in relation to iron chlorosis of citrus seedlings. Proc. Fla. State Hort. Soc. 65: 101-108. 1952.
4. Smith, P. F. and A. W. Specht. Heavy-metal nutrition and iron chlorosis of citrus seedlings. Plant Physiol. 28: 371-382. 1953.
5. Smith, P. F. Heavy-metal accumulation by citrus roots. Bot. Gaz. 114: 426-436. 1953.

PRELIMINARY STUDIES OF APHID TRANSMISSION OF TRISTEZA VIRUS IN FLORIDA

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The tristeza, or quick-decline, virus of citrus can be transmitted by budding or grafting (1, 6), or by insect vectors (3, 9). Searches for probable insect vectors had been carried on in South America (1, 3), Africa (8), and California (5) for a number of years before the mild form of the disease was discovered in Florida (7), where it now has been reported in all the citrus-producing counties (2).

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