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Zinc in Soil and Citrus Trees Showing Declinio Symptoms

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ABSTRACT. The content of different forms of zinc in soil samples and the zinc content of leaves and trunk wood were determined in a citrus orchard showing different degrees of declinio symptoms. The samples were taken five times at 4-month intervals. In general, the zinc leaf content was greater in declinio-affected trees which were at the last stage of the disease and the element accumulated in the trunk wood of declinio-affected trees. The greatest value for soil zinc was observed in the humin fraction. The soluble soil zinc and the zinc of fulvic and humic acid fractions tended to be greater in samples taken under plants showing declinio symptoms. At only one sampling time there was a significant difference between treatments for zinc in the humic matter. The content of zinc in the humin was similar in all treatments. In twelve of the possible correlations with the analyzed parameters the t test was significant.

Index words. blight, soil organic matter, humic matter, humin, fulvic acid, humic acid.

The citrus market is one of the greatest Brasilian sources of export earnings from fruit or juice. The producers are now facing a great problem with respect to citrus culture which is a disease called declinio. Declinio of citrus trees in Brasil is similar to citrus blight in Florida, both are of unknown cause and characterized by wilt and dieback of the canopy (1, 2, 13, 14). The cause of blight is not very obvious and the internal reallocation of nutrients within the plants can be due to a nutritional inbalance (20).

Plants affected by declinio have showed accumulation of zinc in trunk wood (16, 19), reduced water uptake and translocaton (2, 6) and the presence of occlusions in xylem vessels (2, 3). Amorphous plugs consisting of solid material are found in the lumen of xylem vessels and may completely block them (2). In blight or declinio-affected citrus trees the amorphous plugs react positively for callose, lignin, pectic substances, gums, proteins and lipids (1).

Some studies have shown that the appearance of citrus blight is influenced by soil conditions (4, 5, 21, 22). According to some authors declinio may be a disease caused by multiple factors (8).

In this paper we present the data obtained in a trial carried out to study the content of zinc in some fractions of the soil organic matter and their correlation to zinc content of leaf and trunk wood samples of healthy and declinio-affected citrus trees.

MATERIAL AND METHODS

The trial was carried out in an orchard at São Paulo Farm, near Bebedouro city, São Paulo State, Brazil, with 18-yr-old Pera sweet orange on Rangpour rootstock. The soil was a Dark Red Latosol-medium texture.

The experimental design was a randomized complete block with four treatments (healthy plants and plants in stages 1, 2, and 3 of declinio, with symptom severity increasing from stages 1 to 3) with six replications. Each replication (experimental plot) involved four plants so that the trial was consisted of 96 plants. During the experiment citrus trees received the same care as the commercial orchard.

At five different dates, samples of soil, leaves and trunk wood were collected for zinc analysis. Four single soil samples were taken from each plant at 0-20 cm deep at the dripline, which were combined with the single samples of the other plants of the same plot to form a composite sample representing the replication (24 composite samples per sampling time).

Leaf samples were collected from the spring flush without fruit about 1.50 m high in the four quadrants of the plant. The four leaves of each plant were combined with the others of the same replication to form the composite sample. Trunk wood samples were taken 50 cm above the soil line with 0.6 cm drill and to 2.5 cm deep on oppoosite sides of the plant. The other procedures were the same for leaf samples.

The different fractions of the soil organic matter were obtained according to the method of Dabin (7). Air-fried soil samples (20.0 g, 2 mm sieve) were extracted with 100 ml of 0.1M sodium pyrophosphate by shaking for 24 hr. The organic fraction that was not extracted was called humin. The soluble organic matter (humic matter) was fractionated by acidifying to pH 1-2 with H_2SO_4. The precipitated organic matter fraction was called humic acid and the soluble one fulvic acid.

Samples of plant (0.5 g for leaf and 1.0 g for trunk wood) were digested with a nitric-perchloric acid solution and zinc was determined in the extract by atomic absorption spectroscopy.

Soil, humic matter and fulvic acid were digested with a nitric-perchloric acid solution and the zinc content was determined in the extract by atomic absorption spectroscopy. The content of zinc in humic acid and humin was calculated by difference.

Soluble zinc was extracted with 0.05N HCl in a 0.23N H_2SO_4 solution in the proportion of 1:5. The content of zinc in the extract was then determined by atomic absorption spectroscopy.

RESULTS AND DISCUSSION

The content of leaf zinc varied from 19 to 147 ppm (Table 1), and was always greater than the critical level of 16 ppm proposed by Nagai *et al.* (11). The highest values were observed in February when the leaves were about 5 months old. The F-test for treatments was significant at all sampling dates and in all cases the content of zinc was greater in the trees showing symptoms at stage 3 of declinio, which is in agreement with previous data. Wutscher and Hardesty (18) found that blighted, 16-yr-old Valencia orange trees had higher zinc levels in the leaves and in the trunk wood than healthy trees. The leaf zinc correlated only with the content of zinc in the nitric-perchloric extract of the soil (r = 0.47**). There was no correlation between leaf zinc and trunk wood zinc which means that there were problems with the transport of the element in declinio-affected citrus trees.

TABLE 1
ZINC IN LEAVES OF DECLINIO-AFFECTED AND HEALTHY CTIRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	93b[y]	19a	42a	58a	69bc
Stage 1	124a	27a	46a	49c	59b
Stage 2	110a	25a	53ab	58a	85a
Stage 3	147a	50b	63b	67b	81ac
F	7.62(**)[x]	18.99(**)	10.58(**)	18.74(**)	15.77(**)
C.V. (%)	17.07	25.38	14.14	7.24	9.95
LSD (5%)	34	16	15	9	15

[z]Symptoms increasingly severe from stage 1 to stage 3.
[y]Mean separation by Tukey test, P<0.05.
[x]** = significant at P<0.01.

TABLE 2
ZINC CONCENTRATION IN TRUNK WOOD OF DECLINIO-AFFECTED AND HEALTHY CITRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	12ac[y]	10	9bc	11	13a
Stage 1	11bc	9	11ac	12	12a
Stage 2	12ac	11	12ac	15	18ac
Stage 3	16a	14	13a	13	22bc
F	5.55(**)[x]	2.61	3.89(*)	1.15	12.71(**)
C.V. (%)	16.55	26.59	17.13	29.33	20.19
LSD (5%)	4	—	4	—	7

[z]Symptoms increasingly severe from stage 1 to stage 3.
[y]Mean separation by Tukey test, $P<0.05$.
[x]*, ** = significant at $P<0.05$ and $P<0.01$, respectively.

In the trunk wood, the content of zinc fluctuated from 9 to 22 ppm (Table 2) similar to the data obtained by Wutscher (20), who found 14.22 ppm in blighted trees and 3.8 ppm in healthy trees. At three of the sampling dates the F-test was significant for treatment. The plants in an advanced stage of declinio showed the greatest values for zinc. As the content of zinc was high in the trunk wood even in healthy plants we believe that all the plants of the trial were in a potential stage of declinio. The content of zinc in the trunk fluctuated as a function of the sampling date which may be caused by the availability of zinc in the soil, by the stage of plant development or by the fact the samples were taken in different points around the trunk. Zinc in the trunk wood correlated with soluble zinc ($r=0.57$**), zinc in the nitric-perchloric extract of humic matter ($r=0.51$*), and in humin ($r=0.63$**). This suggests that the content of zinc in the humin nitric-perchloric acid extract can be the best one to evaluate the absorption of zinc by citrus trees.

The soil soluble zinc (Table 3) ranged from 2.7-7.95 ppm. There were observed significant differences between treatments at four sampling dates. In most cases, zinc content was greater in plants showing advanced symptoms of declinio. Wutscher and

TABLE 3
SOLUBLE ZINC IN SOIL SAMPLES TAKEN UNDER DECLINIO-AFFECTED AND HEALTHY CITRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	3.74	3.78a[y]	3.64b	3.61ab	6.88ac
Stage 1	4.73	3.74a	3.51b	3.78ab	5.46bc
Stage 2	4.45	3.53a	2.88b	3.15b	5.66bc
Stage 3	3.68	2.70b	5.95a	4.42a	7.95a
F	2.82	5.58(**)[x]	10.69(**)	6.90(**)	5.35(*)
C.V. (%)	18.33	15.23	25.24	13.07	18.94
LSD (5%)	—	0.87	1.68	0.82	2.05

[z]Symptoms increasingly severe from stage 1 to stage 3.
[y]Mean separation by Tukey test, $P<0.05$.
[x]*, ** = significant at $P<0.05$ and $P<0.01$, respectively.

TABLE 4
ZINC IN NITRIC-PERCHLORIC ACID EXTRACT OF SOIL SAMPLES TAKEN UNDER DECLINIO-AFFECTED AND HEALTHY CITRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	15.83	13.67	15.17	17.17	17.92bc[y]
Stage 1	16.58	14.83	16.00	15.33	15.33ac
Stage 2	17.25	14.92	14.17	14.25	14.25ac
Stage 3	16.25	14.50	15.83	15.08	19.33b
F	0.25	0.46	1.47	2.04	6.30**[x]
C.V. (%)	17.94	14.21	11.00	13.65	13.61
LSD (5%)	—	—	—	—	3.79

[z]Symptoms increasingly severe from stage 1 to stage 3.
[y]Mean separation by Tukey test, $P<0.05$.
[x]** = significant at $P<0.01$.

Hardesty (18) did not find singnificant differences in soil samples collected under blight-affected or healthy plants and extracted with 1N ammonium acetate. Soluble zinc correlated with zinc in the nitric-perchloric acid extract of soil ($r=0.65$**) and in humic matter ($r=0.80$***). The data obtained for soluble zinc in the soil were very close to that obtained for the element in the nitric-perchloric extract of fulvic acid (3.00-5.93 ppm).

The content of zinc in the nitric-perchloric extract of the soil was between 13.67 and 19.33 ppm (Table 4), which is generally lower than those values of total zinc of 8-300 ppm cited by some authors (10, 17).

In the nitric-perchloric extract of fulvic acid (Table 5), the content of zinc was between 3.00 and 5.93 ppm and on three sampling dates there was a significant difference between treatments. Only in one case did stage 3 of declinio have the highest value for zinc. Zinc in this fraction correlated with zinc in the nitric-perchloric extract of soil ($r=0.52$**), humic matter ($r=0.64$**) and humin ($r=-0.55$*). The data suggest that humic matter is more easily converted to available zinc than humin zinc.

The humic acid zinc content ranged from 0.30-1.66 ppm, the lowest in the organic matter fraction, but significant differences between treatments were observed in most cases. The greatest value of zinc occurred sometimes in the stage 3 and sometimes in the stage 1 of declinio.

TABLE 5
ZINC IN NITRIC-PERCHLORIC ACID EXTRACT OF HUMIC MATTER OBTAINED FROM SOIL SAMPLES TAKEN UNDER DECLINIO-AFFECTED AND HEALTHY CITRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	4.83	4.48	4.17	4.92	7.75
Stage 1	4.40	4.40	5.02	4.48	5.21
Stage 2	5.29	4.71	3.25	4.17	5.10
Stage 3	6.27	4.69	9.10	5.50	8.29
F	1.65	1.81	2.29	0.98	2.71
C.V. (%)	29.49	6.13	37.68	30.09	37.71

[z]Symptoms increasingly severe from stage 1 to stage 3.

TABLE 6
ZINC IN NITRIC-PERCHLORIC ACID EXTRACT OF FULVIC ACID OBTAINED FROM SOIL SAMPLES TAKEN UNDER DECLINIO-AFFECTED AND HEALTHY CITRUS TREES

Treatment[z]	Date of sampling				
	02/13	06/01	09/08	03/22	06/21
	Zn (ppm)				
Healthy	3.83	4.20	3.46ab[y]	3.80b	5.54ab
Stage 1	3.54	3.82	3.82a	3.80b	4.39b
Stage 2	3.85	4.33	3.00b	3.87b	4.57ab
Stage 3	4.19	3.93	3.80a	4.96a	5.93a
F	0.69	0.71	3.98(*)[x]	5.38(*)	4.46(*)
C.V. (%)	20.22	17.10	13.33	14.71	16.88
LSD (5%)	—	—	0.78	1.00	1.43

[z]Symptoms increasingly severe from stage 1 to stage 3.
[y]Mean separation by Tukey test, $P<0.05$.
[x]* = significant at $P<0.05$.

Humin content of zinc varied from 8.96 to 11.60 ppm, the greatest values in the studied fractions of the organic matter. Only at one sampling date was observed a significant difference between treatments, when samples obtained from plants in stage 3 of declinio were higher in zinc content. In a general way the content of zinc tended to be greater in samples taken from healthy plants.

CONCLUSIONS

From the data obtained in this trial the following conclusions can be drawn: 1) the content of zinc in the leaves tended to be greater in the plants in the final stages of declinio; 2) zinc accumulated in the trunk wood of plants showing symptoms of declinio; 3) the content of zinc of the fulvic acid was similar to the content of soluble zinc; 4) the content of zinc of the organic matter fractions and the content of soluble zinc tended to be greater when the samples were taken under declinio-affected citrus trees; 5) the soil zinc predominated in the humin fraction of the soil organic matter.

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