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INDUCTION OF CITRUS BLIGHTLIKE ZINC ACCUMULATION IN THE WOOD AND BARK OF 3-YEAR-OLD 'HAMLIN' ORANGE TREES IN SOLUTION CULTURE

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Abstract. **Three-yr-old 'Hamlin' orange,** Citrus sinensis **(L.) Osbeck, trees on rough lemon,** C. limon **Burm. f., and sour orange,** C. aurantium **L., rootstock were grown in 2 solutions for 8 months, one high in sulfate and low in silicon (solution 1), the other high in silicon and low in sulfate (solution 2). Trees on rough lemon accumulated higher Zn levels in the wood, especially the outer layers of the wood and the bark when grown in solution 1. Trees on sour orange accumulated Zn only in the outer wood. The affected trees had blotchy leaves, poor and abnormal root growth and a higher wood pH.**

The debate about the nature of citrus blight has been underway for at least 50 yr (9, 10). Contradictory evidence of its transmissibility has been published recently (11, 20). Work on explaining blight has been hampered by the lack of reliable visible symptoms, but water injection into the trunk (4, 6) and analysis of the outer wood for Zn (13) are accepted as diagnostic tests in areas where citrus blight occurs (2, 5, 15). The level of Zn in the trunk wood fluctuates seasonally (16) and Zn accumulation occurs simultaneously or precedes visual symptoms by no more than 1 yr in most cases (18). Lower sulfate and chloride levels in the soil under blight-affected than under healthy trees have been reported (17, 18), but the view that blight is a nutritional disorder is far from universally accepted in spite of evidence for this (3, 14, 17, 18, 19). Because anions seemed to be involved in blight, a solution culture experiment was set up to test the hypothesis that anion nutrition has an effect on blightlike symptoms.

Materials and Methods

Seedlings of rough lemon and sour orange were grown in flats in peat moss/perlite (50:50, v:v) in a greenhouse and transplanted into 23-cm-diameter pots containing sterile sand-peat moss-perlite mix (50:25:25) in 1980. When the seedlings were pencil size, they were budded with virus-free 'Hamlin' orange buds from a mother tree which had grown in the greenhouse for 4 yr. When the trees were 18 months old, they were transplanted into 27 cm-diameter pots containing a 2:1 sand-red subsoil clay mix limed to pH 6.8 with calcitic limestone. In September 1983, when the trees were 32 months old, 6 trees on rough lemon and 6 trees on sour orange rootstock were removed from the pots and their root systems washed clean. They were then grown in a greenhouse in aerated solution culture in 10-liter crocks for 8 months. In May 1984, the trees were harvested and divided into leaves, bark, wood and roots. After washing and drying at 65°C, the various tissues were analyzed by standard methods (14). Stem segments were checked for plugs in the xylem and for water uptake by the syringe test (6). The pH of fresh wood was measured as described recently (18). There were 2 solution treatments, each with 3 trees on rough lemon and 3 trees on sour orange. The composition of the solutions (actual analysis values) is shown in Table 1. Both solutions were made with ammonium nitrate and triple superphosphate as N and P sources, using tap water (37 ppm Ca, 8 ppm Mg, 14 ppm Cl, 5 ppm S, 6 ppm Si). Solution 1 (high SO_4, low Si) contained langbeinite (18% K, 11% Mg, 22% S), a natural mineral sold as a soluble K and Mg source. In solution 2 (low SO_4, high Si) potassium silicate was the K source, with Mg supplied as magnesium sulfate; 4.1 g of water-insoluble calcium silicate was also added to each 10-liter culture at each solution change. Equal amounts of minor elements in form of a stock solution devised by Smith (7) and 1 ml/culture of metalaxyl, a fungicide, were added to both solutions. The solutions were changed every 2 weeks. The solution pH was measured at the beginning of the experiment, 10 days before and at harvest time. In an effort to increase leaf symptoms, the concentrations of N, P, K, Mg and S were doubled in solution 1 for the last 38 days, with no change in minor element addition. Solution levels were maintained by daily additions of tap water and the trees were checked frequently for visual symptoms. The *F*-test was used to compare treatments and rootstocks; the t-test was used to analyze the wood pH data.

Results and Discussion

Equal amounts of N, P, and minor elements were added to both solutions, yet there were some differences in the levels found by analysis (Table 1). This could be due to ionic interferences or precipitation. The only large differences between the 2 solutions were in S and Si, however. The pH of both fresh solutions was 7.6; over 10 days the pH of double strength solution 1 fell to 4.1, the pH of solution 2 to 7.1. A sharp drop in soil pH under blight-affected trees has been found in Argentina (personal communication, Juan Pedro Agostini).

Table 1. Mineral element concentrations in nutrient solutions (ppm).

	N			P	K	Ca	Mg	S	Si
	Total	NH_4	NO_3						
Solution 1	81	43	38	8	82	39	52	125	6
Solution 2	72	39	33	3	110	43	43	71	85

	Fe	Mn	Zn	Cu	Cl	B	Mo	Na
Solution 1	1	1	0.2	0.1	15	0.3	0.1	10
Solution 2	4	2	0.1	0.1	19	0.5	0.1	16

There were no sharp differences in visual appearance of the trees and there were no significant differences in fresh and dry weight at the end of the experiment. About 30% of the leaves of the trees growing in solution 1 showed blotchiness, some terminal leaves were small and completely yellow. There were no distinct Zn deficiency symptoms; the leaf patterns were similar to S toxicity symptoms. The root systems were easily distinguishable. The trees growing in solution 2 were completely healthy, with many vigorously growing white, normal root tips. Trees in solution 1 had dull brown roots, with little new growth. The root tips were thickened and the new roots were coarse (Fig. 1). The wood pH was lower with solution 2 (SiO_3) than with solution 1 (SO_4), but with only 3 replications the difference was significant only in trees on rough lemon (P = 0.05, t = 5.75) (Table 2). When whole trunk sections, including the heart wood, were ground and analyzed, trees on rough lemon growing in solution 1 (SO_4) had more Zn in the wood than those growing in solution 2, with no significant difference in trees on sour orange. When only the outermost (3 mm) layers of the wood were analyzed, there were higher Zn levels in trees on both rootstocks grown in solution 1; in trees on rough lemon Zn was twice as high with solution 1 (SO_4) as with solution 2 (SiO_3). This indicates a Zn gradient toward the center of the trunk as found by Smith (8) in blight-affected trees in the field. There also was a twofold difference in bark Zn (1) in trees on rough lemon, but no significant difference in trees on sour orange (Table 2). Trees on both rootstocks accumulated Mn in the bark when grown in solution 1, an effect found in blighted trees in the field (12). There were no significant differences in Zn in roots and in the leaves, but Mn was higher in the roots of trees on both rootstocks and in the leaves on rough lemon. The only effect of the 2 solutions on Fe were higher levels in the bark of trees on sour orange in solution 1. Copper levels in the roots of both types of trees and in the bark and leaves of trees on sour orange were higher with solution 1. A check of the trunk xylem showed elevated numbers of filamentous plugs (12/200 vessels) in 1 'Hamlin' orange/rough lemon tree and of amorphous plugs (16/200 vessels) in 1 tree on sour orange which had been grown in solution 1 (SO_4). Water uptake in syringe injections was slow in 2 of 3 trees on both rootstocks grown in solution 1, fast in 2 of 3 trees on rough lemon and 3 of 3 trees on sour orange grown in solution 2 (SiO_3).

The greater Zn accumulation in the wood of trees on blight-susceptible rootstocks (21) was reflected in differences between trees on rough lemon and sour orange in the experiment. While Mn accumulated in the bark as in blighted trees (12), it also was higher in the roots and the leaves of trees in solution. The accumulation of Zn was confined only to the wood and the bark. The results of the experiment show that some aspects of citrus blight can be duplicated in solution culture.

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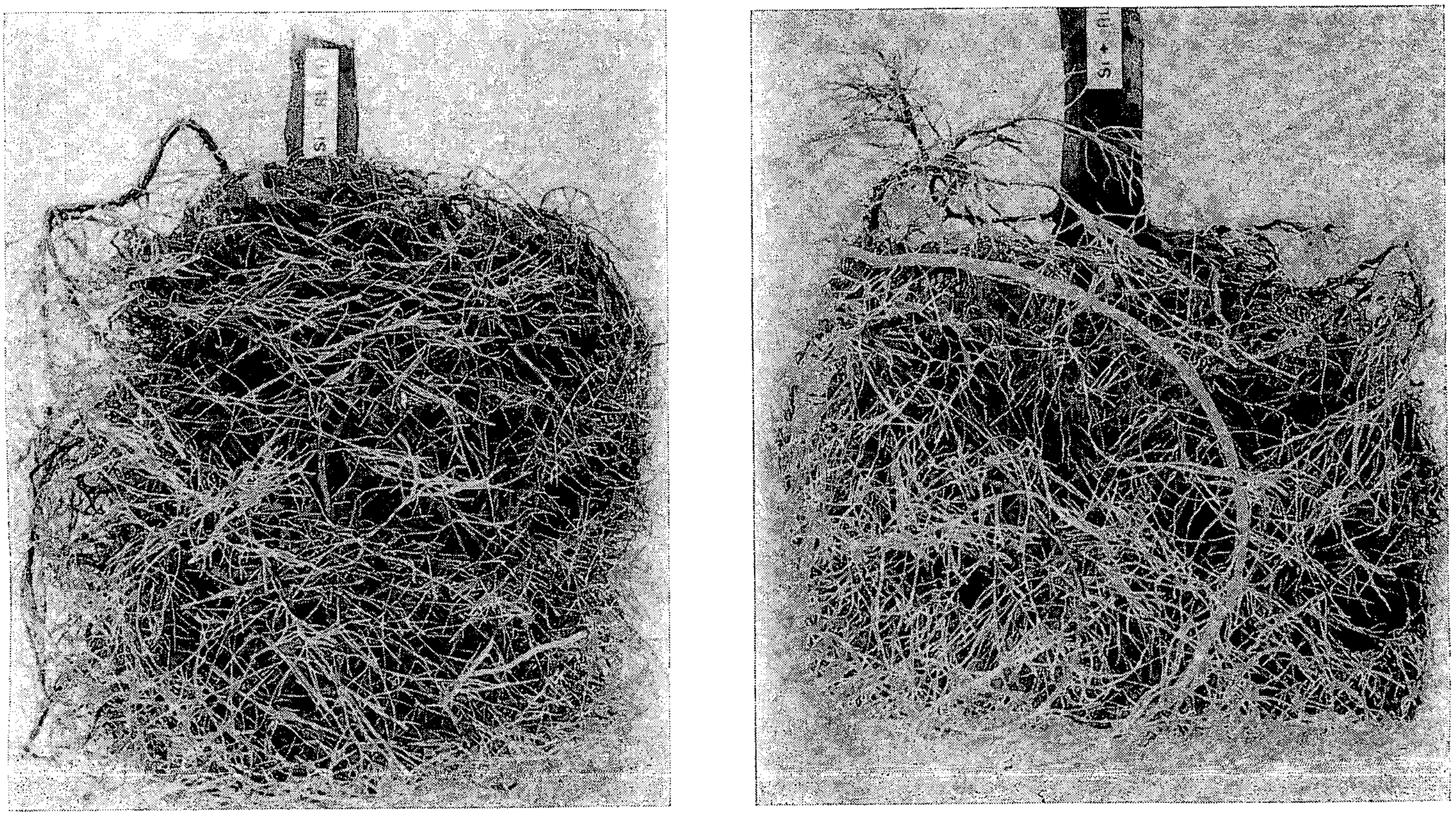

Fig. 1. Rough lemon root systems after 8 months in solution 1 (high SO_4, low Si) (left) and in solution 2 (right) (high Si, low SO_4).

Table 2. Wood pH, Mn, Zn, Fe and Cu levels in the leaves, bark, whole trunk wood, outer (3 mm) trunk and feeder roots of 'Hamlin' orange trees on rough lemon and sour orange rootstock grown for 8 months in 2 nutrient solutions.

			Leaves	Bark	Trunk wood	Outer trunk wood	Roots
Wood pH	Sol. 1[z]	RL[y]	—	—	6.06[x,w]	—	—
		SO[v]	—	—	6.24	—	—
	Sol. 2	RL	—	—	5.94[v]	—	—
		SO	—	—	5.86	—	—
ppm Mn	Sol. 1	RL	64 a[x,u]	59 a	7 a	7 a	2445 a
		SO	25 b	38 b	5 a	5 ab	2894 a
	Sol. 2	RL	39 b	17 bc	4 a	4 ab	738 b
		SO	32 b	15 c	2 a	2 b	509 b
ppm Zn	Sol. 1	RL	26 a	113 a	7 a	23 a	655 a
		SO	16 a	63 b	5 b	12 b	616 a
	Sol. 2	RL	22 a	57 b	5 b	10 bc	205 a
		SO	18 a	37 b	4 b	8 c	182 a
ppm Fe	Sol. 1	RL	132 a	99 ab	22 a	35 a	993 a
		SO	100 a	114 a	21 a	40 a	696 a
	Sol. 2	RL	97 a	63 a	33 a	34 a	966 a
		SO	82 a	72 b	23 a	35 a	438 a
ppm Cu	Sol. 1	RL	9 b	12 ab	3 a	4 a	105 a
		SO	14 a	16 a	3 a	3 a	121 a
	Sol. 2	RL	7 b	9 b	3 a	4 a	67 b
		SO	7 b	7 b	3 a	4 a	57 b

[z]Solution 1 = high SO_4, low Si; solution 2 = high Si, low SO_4.
[y]Rough lemon.
[x]Means of 3 trees.
[w]Difference between trees on rough lemon significant at the 5% level, $t = 5.75$.
[v]Sour orange.
[u]Mean separation by Duncan's multiple range test, 5% level.

Lake Alfred, for checking stem segments for xylem plugs and water uptake by syringe injection.

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