Cadmium-Zinc Interrelationships in Tomato Plants

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ABSTRACT

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A possible interaction between various concentrations of cadmium and zinc was investigated in intact and split-root tomato plants in sand culture. The addition of cadmium to the substrate enhanced zinc uptake into tomato foliage, resulting in increased phytotoxicity. Zinc additions to the substrate did not affect cadmium uptake. There was no evidence that the elements acted competitively. Inasmuch as the enhancement effect was observed when Cd and Zn solutions were added separately to plants, the interaction must have occurred within rather than outside the plant. There was also reason to speculate that the uncontaminated root in split-root plants could continue to function normally.

Additional key words: Lycopersicon esculentum.

The presence of cadmium (Cd) in the environment is normally linked to zinc (Zn), an essential micronutrient (12,15), because of their association in nature and their chemical similarity. Studies with rats have shown that cadmium and zinc will compete for certain enzyme sites, and the behavior of these elements in animals has been described as mutually antagonistic (13,15). It has been hypothesized that the phytotoxicity of cadmium is due in part to the fact that Cd competes successfully with Zn for similar active sites but does not functionally substitute for it (17). A Cd/Zn concentration ratio $\leqslant 1\%$ has been proposed as a regulatory device to allow safe use of sewage sludge on agricultural land (1,2). Although many researchers have focused on the relationship between Cd and Zn uptake by plants (3–10,13,14,16,18), no consensus has been reached. Some of the variability in results might be attributed to the lack of uniformity in experimental designs, the particular plant species tested or plant fraction analyzed, the relative concentrations of zinc and cadmium employed, or to reactions occurring between the two elements in the substrate.

In an attempt to clarify the Cd/Zn relationship in tomato plants we adopted a split-root technique. Cadmium and zinc were introduced in separate parts of the root system, thus preventing the occurrence of any interactions between the two elements in the substrate or at the root surfaces. Any effect observed in the tomato shoots would have to occur within the plant per se. A series of intact plants given similar Cd and Zn treatments was included in the experiment for comparison. Cd and Zn treatment levels were selected to permit visual as well as elemental analysis of the response of tomato plants to treatment.

MATERIALS AND METHODS

Tomato seeds (*Lycopersicon esculentum* L. 'Rutgers') were germinated in vermiculite, and after 11 days uniform seedlings were transplanted to 5-L plastic pots of washed sand and given a complete nutrient solution. The nutrient solution used in the experiment had the following composition: 1.0 mM KH_2PO_4, 2.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.0 mM K_2SO_4, 1.0 mM $MgSO_4$, 0.5 mM $(NH_4)_2SO_2$, plus 1.0 ppm Fe as $FeSO_4$, 0.1 ppm B as H_3BO_3, 0.25 ppm Mn as $MnCl_2 \cdot 4H_2O$, 0.32 ppm Cl as $MnCl_2 \cdot 4H_2O$, 0.1 ppm Zn as $ZnSO_4 \cdot 7H_2O$, 0.01 ppm Cu as $CuSO_4 \cdot 5H_2O$, and 0.01 ppm Mo as $NaMoO_4 \cdot 2H_2O$. When the seedlings were 20 cm tall, half were carefully removed from the pots and made into split-root cultures by dividing the roots into equal volumes and cutting into the base of the root crown approximately 1.2 cm and distributing the root system between two 5-L plastic pots of washed sand. The remainder of the plants was maintained intact in the original pots. Both sets of plants were randomly assigned treatments as described below.

Nutrient solution amended with either 0, 0.8, or 2.0 ppm Cd as $CdCl_2$ was added to one side of the split-root cultures. These levels represent normal, moderate, and toxic levels of cadmium for tomato as determined in preliminary studies. To the opposite side was added nutrient solution amended with either 0.1, 1.0, or 2.5 ppm Zn and $ZnSO_4$, which also represent normal, moderate, and toxic levels for tomato (10). Zinc was excluded from the cadmium-amended nutrient solutions to eliminate interaction between these two elements in the substrate or at the root surface. Preliminary experiments provided evidence that phosphorus did not significantly influence cadmium uptake when the two elements were supplied daily in soluble form in the same nutrient mix. Therefore, phosphorus was maintained at normal levels in the cadmium-amended nutrient solutions.

Intact plants received nutrient solution with the same concentrations of cadmium or zinc as the split-root plants. Each level of cadmium was combined with each level of zinc in split-root or intact plants for a total of 18 ($3 \times 3 \times 2$) treatments. The statistical design was a randomized block of five replicates with one plant per replicate.

Plants received 500 ml of the appropriate solution every day for 3 wk. Every third day the sand was flushed with deionized water to prevent a potentially toxic buildup of nutrient salts in the substrate. Plants were observed daily for foliar symptoms of cadmium or zinc toxicity. At harvest plants were divided into root or half root, stem, and leaf fractions. Root tissue samples were rinsed twice in deionized water to remove surface contaminants. Plant samples were air-dried in a forced draft oven at 70 C for 48 hr and then ground through a 0.5-mm (40-mesh) screen in a micro Wiley mill. One-gram samples were ashed at 550 C in a muffle oven for 8 hr, digested in 3N HNO_3, and analyzed for Cd and Zn using a Perkin-Elmer model 303 atomic absorption spectrophotometer. Data were subjected to an analysis of variance following a log transformation, and means were separated by Duncan's new multiple range test at a significance level of $P = 0.05$. The entire experiment was completed twice, the first time with only two replicates per treatment. Only the results of the second experiment, with five replicates per treatment, are reported here.

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RESULTS

Symptom development. A dose of 0.8 ppm Cd for 3 wk caused a slight interveinal chlorosis of middle-aged leaves such that approximately 15% of the leaves of split-root plants and 30% of the leaves of intact plants were affected. The higher dose of 2.0 ppm Cd caused a moderate chlorosis to 45 and 60% of the leaves of split-root and intact plants, respectively. Plants treated with 1.0 ppm Zn remained symptomless, whereas the higher dose of 2.5 ppm Zn caused a foliar chlorosis indistinguishable from that caused by 2.0 ppm Cd. A combination of Cd and either 1.0 or 2.5 ppm Zn resulted in a 15–30% increase in foliar chlorosis in both split-root and intact plants. The young as well as the middle-aged leaves were chlorotic on those plants receiving high levels of both Cd and Zn, and leaf curl accompanied chlorosis. Both cadmium and zinc toxicity symptoms were more severe on intact than split-root plants.

TABLE 1. Cadmium concentration in split-root and intact tomato plants that received various levels of Cd and Zn in the nutrient solution

Cd treatment (μg/ml)	Root treatment	Cd concentration (μg/g)			
		Leaf	Stem	Root (+Cd)[x]	Root[y]
0.0	Split	1.3 a[z]	1.2 b	3.0 a	3.9 a
	Intact	1.1 a	0.9 a	1.4 a	···
0.8	Split	45.7 b	19.6 c	549.9 b	2.5 a
	Intact	60.9 c	44.4 d	486.7 b	···
2.0	Split	134.3 d	51.9 d	871.1 c	3.9 a
	Intact	207.5 e	155.6 e	922.8 c	···

[x]Values for cadmium content of the roots of split-root plants are from the half-root receiving cadmium-amended nutrient solution.
[y]Values shown are from the half-root of split-root plants not exposed to cadmium.
[z]Mean cadmium concentrations, expressed as the antilogs of the transformed data, combining all levels of zinc at each cadmium treatment level for a total of 15 replicates per mean. Means within a column, and followed by the same letter, are not significantly different according to Duncan's multiple range test, $P = 0.05$.

TABLE 2. Zinc concentrations in split-root and intact tomato plants that received various levels of zinc and cadmium in the nutrient solution

Zn treatment (μg/ml)	Root treatment	Zn concentration (μg/g)			
		Leaf	Stem	Root (+Zn)[x]	Root[y]
0.1	Split	71.4 b[z]	402.9 a	379.5 a	327.9 a
	Intact	52.5 a	426.2 a	371.5 a	···
1.0	Split	125.9 cd	532.6 b	677.6 b	370.5 a
	Intact	101.8 c	619.4 b	715.8 bc	···
2.5	Split	132.6 d	620.9 b	841.4 c	382.2 a
	Intact	128.2 cd	660.7 b	831.8 c	···

[x]Values for Zn content of the roots of split-root plants are from the half-root receiving Zn-amended nutrient solution.
[y]Values shown are from the half-root of split-root plants not exposed to zinc.
[z]Mean Zn concentrations, expressed as the antilogs of the transformed data, combining all levels of cadmium at each Zn treatment level for a total of 15 replicates per mean. Means within a column and followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

Cd concentration in plants. As the cadmium treatment level was increased, the concentration of cadmium in the leaves, stems, and roots increased significantly in both split-root and intact plants (Table 1). However, a statistically significant interaction between root treatment (Rt) and cadmium treatment (CdT) with respect to leaf and stem concentration data, showed that Cd movement from the root to the shoots was much less efficient in split-root than intact plants. Table 1 shows the nature of the RT/CdT interaction. Cadmium values for the untreated half-root ranged from 2.5 to 3.9 ppm (Table 1), indicating that little Cd was translocated down from the top into unexposed root tissues.

Zn concentration in plants. As the zinc treatment level was increased, the concentration of Zn in the leaves, stems, and roots increased significantly in both split-root and intact plants (Table 2). Contrary to the case with Cd, the zinc concentration in the leaves and stems of split-root and intact plants was generally similiar at a given zinc treatment level. Apparently, the movement of zinc from the roots to the shoots was not altered in split-root plants. Zinc concentrations up to 382.2 ppm were found in the half-root not supplied with zinc in the nutrient solution (Table 2).

Cadmium effect on zinc concentration. The addition of 2.0 ppm Cd to the substrate significantly increased zinc uptake of tomato leaves in intact plants receiving 1.0 ppm Zn and in split-root plants receiving 2.5 ppm Zn (Table 3). The effect of increasing cadmium treatment on leaf zinc concentrations at 0.1 and 2.5 ppm Zn in intact plants and at 0.1 and 1.0 ppm Zn in split-root plants was variable and insignificant.

Cadmium treatment had no significant effect on the zinc content of tomato stem tissues in split-root or intact plants (Table 3).

Zinc effect on cadmium concentration. In contrast to the observed enhancement of zinc uptake by cadmium treatment, as described above, increasing zinc treatment did not have a statistically significant effect on the cadmium content of tomato leaf and stem tissues in either intact or split-root plants (Table 4).

DISCUSSION

In this experiment both cadmium treatments (0.8 and 2.0 ppm) and the highest zinc treatment (2.5 ppm) caused chlorosis of tomato foliage. The combined effect of the two elements tended to be additive, and indeed even at the 1.0 ppm Zn treatment level there was increased injury to Cd-treated plants. Phytotoxic cadmium levels significantly increased the zinc concentration of the leaf tissue. This effect was observed in both intact and split-root plants

TABLE 3. Effect of cadmium treatment on the Zn content (μg/g) of tomato leaf and stem tissues at normal, moderate, and high Zn levels in solution for intact and split-root plants

Cd treatment (μg/ml)	Zn content (μg/g) of					
	Intact plants that received (μg/ml)			Split-root plants that received (μg/ml)		
	0.1 Zn	1.0 Zn	2.5 Zn	0.1 Zn	1.0 Zn	2.5 Zn
Leaves						
0.0	42.3 a[z]	88.7 bcde	97.7 cdef	59. 8 abc	117.8 def	106.6 def
0.8	55.8 ab	72.9 bcd	132.9 ef	83.7 bcde	139.9 ef	139.1 ef
2.0	61.2 abc	163.4 f	162.2 f	72.6 bcd	122.2 ef	157.3 f
Stems						
0.0	426.6 ab	570.7 de	666.6 e	391.6 ab	572.8 de	607.4 de
0.8	377.0 a	614.7 e	635.3 e	447.7 abc	550.8 cde	609.4 de
2.0	486.8 bcd	677.6 e	682.2 e	372.8 a	478.6 bcd	645.6 e

[z]Values shown are the means of five replicates. Means within a leaf or stem section and followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

TABLE 4. Effect of Zn treatment on the cadmium content (μg/g) of tomato leaf and stem tissues at normal, moderate, and high Cd levels in solution for intact and split-root plants

Zn treatment (μg/ml)	Cd content (μg/g) of					
	Intact plants that received (μg/ml)			Split-root plants that received (μg/ml)		
	0 Cd	0.8 Cd	2.0 Cd	0 Cd	0.8 Cd	2.0 Cd
Leaves						
0.1	0.9 a[z]	59.2 cd	210.2 f	1.3 ab	44.4 c	128.2 e
1.0	1.1 ab	63.9 d	211.3 f	1.4 b	44.8 c	134.9 e
2.5	1.2 ab	60.1 cd	201.6 f	1.2 ab	49.2 cd	139.7 e
Stems						
0.1	0.9 ab	41.2 de	164.3 f	1.2 b	18.9 c	50.5 de
1.0	0.9 ab	53.5 de	163.2 f	1.2 b	19.9 c	48.3 de
2.5	0.7 a	39.7 d	140.6 f	1.0 ab	19.8 c	57.2 e

[z]Values shown are the means of five replicates. Means within a leaf or stem section and followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

where cadmium and zinc had no contact in the substrate or at the root surface. Therefore, the interaction must have occurred within the plant shoot.

The enhancement of zinc uptake by cadmium depended on the levels of the element used and also on the fraction of the plant analyzed. For example, in intact plants, an increase in cadmium from 0 to 0.8 ppm at 0.1, 1.0, and 2.5 ppm Zn had no effect on zinc uptake, whereas a further increase in cadmium from 0.8 to 2.0 ppm significantly enhanced zinc uptake at 1.0 ppm Zn, but not at 0.1 and 2.5 ppm Zn. Furthermore, the enhancement effect was observed in the leaves, but not the stems. There are reports in the literature that both support and refute these findings. For example, Root et al (14) found that in corn the zinc concentration decreased as cadmium concentration increased. Hawf and Schmid (7) found that cadmium inhibited zinc uptake in bush bean plants but did not interfere with the translocation of zinc within the plants. Zinc amendments have been shown to suppress cadmium uptake in ryegrass (8) as well as in oats and lettuce (9). In radish, increasing concentrations of zinc suppressed cadmium uptake at low cadmium concentrations in solution, but increased cadmium uptake at high cadmium concentrations (13). Turner (16) found that cadmium treatment tended to increase total zinc uptake in beet root, lettuce, radish, and tomato, but decreased it in carrot and Swiss chard. In soybean, the addition of zinc from the 5–50 ppm range increased plant cadmium concentration due to a decrease in plant growth (6). Zinc additions significantly increased Cd concentrations in the stem and leaf tissues of bush beans, but significantly lowered Cd concentrations in the roots (5). In a sand culture experiment, increased cadmium treatment enhanced zinc uptake in tomato plants (10). Finally, Czuba and Ormrod (4) found no interaction between cadmium and zinc in cress and lettuce plants.

The present study provides additional clues as to the relationship between cadmium and zinc in tomato plants. For example, both Cd and Zn were readily translocated from the root to the shoot, but Cd accumulated in the leaves whereas Zn accumulated in the stems (Table 1 and 2). Zinc was freely translocated from the shoot to the unexposed roots in split-root plants (Table 2), but Cd was not recirculated (Table 1). Kirkham (11) observed no movement of Cd into untreated roots of wheat when the roots were split between two pots filled with soil and one half of the root system was treated with a Cd-amended solution. However, he did observe Cd recirculation when the roots were split between soil and solution or solution and solution. Kirkham (11) suggested that the amount of Cd transferred from exposed to unexposed roots was dependent on the relative capacity of plants to take up water, and therefore Cd, from the different substrates.

In addition, the decreased sensitivity of split-root compared to intact plants is an interesting phenomenon. Although split-root plants concentrated 35% less cadmium in the leaves than intact plants, they still contained approximately 140 ppm Cd, a concentration sufficient to cause toxicity in tomato according to Kim and Motto (10) and Turner (16). We suggest that the unexposed half root may play a role in avoiding or alleviating Cd toxicity. This could have practical importance in contaminated environments such as urban or sludge-treated soils. To the extent that some functioning roots escape contamination, a plant will have more normal cadmium and zinc levels and consequently fewer foliar symptoms.

This study, as well as others (9,10,13), illustrates the complexity of the relationship between Cd and Zn in plants. Certainly, no evidence was found to support the concept that these two elements act competitively in plants. In future studies, the split-root technique may prove to be particularly useful in elucidating cadmium interrelationships with other elements in plants.

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