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Differential Cultivar Tolerance in Soybean to Phytotoxic Levels of Soil Zn. II. Range of Zn Additions and the Uptake and Translocation of Zn, Mn, Fe, and P[1]

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ABSTRACT

This investigation was part of a general characterization of Zn tolerance among cultivars of an agriculturally important plant species. Greenhouse studies were conducted to further evaluate the effect of soil Zn additions on soybean (*Glycine max* L. Merr.) cultivar differences in Zn tolerance, as well as plant Zn, Mn, Fe, and P contents. Four cultivars selected from a previous Zn tolerance screening trial ('Wye' and 'Hudson Manchu', tolerant; 'York', sensitive; 'Columbus', normal) were grown for 4 weeks on a fertilized Sassafras sandy loam (Fragiudult) amended with a wide range of Zn additions at pH 5.5 (nine additions between 1 and 131 ppm Zn) and 6.5 (10 additions between 1 and 524 ppm Zn).

Leaf dry weight reductions were curvilinear across the Zn additions at each pH, with yield increases occurring at the lower Zn treatments. For all cultivars, root dry weight was unaffected by the Zn additions at the lower pH and significantly reduced at the higher pH by Zn additions greater than 262 ppm. Significant cultivar differences in leaf dry weight yields were observed at the higher Zn treatments.

Leaf and root Zn contents increased linearly with increasing soil Zn at each soil pH (root $r^2 > 0.92$, leaf $r^2 > 0.93$ for all cultivars). The soil pH strongly influenced plant Zn contents; at an equivalent Zn addition, tissue values at pH 6.5 were approximately one-third as great as at pH 5.5. Differential absorption and translocation of Zn was shown by the cultivars; at both pH levels, Hudson Manchu absorbed more Zn but translocated less than the other cultivars.

Generally, leaf yield reductions of 20% corresponded to leaf Zn contents greater than 620 ppm at both pH levels; however Hudson Manchu had only 370 ppm Zn in its leaves at 20% yield reduction at pH 6.5. Cultivar differences in both soil and leaf Zn contents at a 20% reduction for leaf dry weight illustrate the inherent weakness of foliar analysis as a monitoring tool for available metals at toxic levels.

Root Mn was increased linearly by soil Zn additions at both pH levels, while leaf Mn increases were curvilinear at pH 5.5 and linear at pH 6.5. Leaf Mn concentrations at both pH levels reached reported toxic levels (> 500 ppm) illustrating the complex nature of Zn phytotoxicity. DTPA-extractable Mn was unaffected by soil Zn additions at both pH levels.

Foliar (combined trifoliolate leaves) Fe, at pH 5.5 and 6.5, and P at pH 6.5, decreased with increasing soil Zn, but did not approach reported deficiency levels. Similar amounts of Fe and P were found in Zn-tolerant and Zn-sensitive cultivars.

These results illustrate the complex nature of Zn phytotoxicity and the importance of cultivar selection for cropping soils amended with metal-containing wastes.

Additional index words: Metal tolerance, Heavy metal, *Glycine max* L. Merr.

CROP selection for agricultural soils used for the disposal of excessive amounts of industrial sludges and refuse composts must include considerations of the relative metal tolerances of both plant species and cultivars. Studies of edaphic ecotypes have shown that heavy metal tolerance tends to be metal specific and associated with a given metal's presence in the soil (1, 11). Although this tolerance is heritable, dominant, and easily insolated by Darwinian selection (10, 11, 22, 25), it is complex and can be modified by environmental factors (3, 9). Many species are unable to evolve metal tolerance (10).

Zinc phytotoxicity in higher plants and the ameliorative effects of increased soil and nutrient solution pH have been noted by a number of investigators (8, 15, 16, 17, 23, 24). Because the work of Earley (8) suggested possible differential cultivar tolerance to Zn in soybean (*Glycine max* L. Merr.) grown in sand culture, an extensive screening trial was conducted to determine the extent of Zn tolerance among soil-grown soybean cultivars. The results of that trial, reported in a companion paper (24), show that significant cultivar differences in Zn tolerance can be demonstrated in a greenhouse pot study. However, the Zn additions, though adequate for screening for Zn

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Table 1. Yield reductions and Zn contents of the trifoliolate leaves of four soybean cultivars selected from a Zn-tolerance screening trial (24).

Cultivar	Yield reduction† (% of control)	Trifoliolate zinc†
		μg/g
Wye	12	403
Hudson Manchu	9	642
York	33	700
Columbus	31	726

† Data is from a pH 6.5, 262 mg Zn/kg soil treatment.

Table 2. Dry weight of the roots and trifoliolate leaves of soybean grown at two soil pH levels and a range of soil Zn additions. Each value is the mean of four cultivars.

Added soil Zn	Plant part: Roots	Plant part: Trifoliolate leaves
mg/kg	g	g
		pH 5.5
1.31	0.86 a*	2.29 a
16	0.93 a	2.24 ab
33	0.99 a	2.15 abc
49	0.89 a	2.11 bc
65	0.85 a	2.16 abc
82	0.93 a	2.14 abc
98	0.86 a	2.01 cd
115	0.80 a	1.93 d
131	0.82 a	1.61 e
		pH 6.5
1.31	0.94 ab*	1.90 bc
33	0.89 abc	2.04 a
65	0.90 abc	2.01 ab
131	0.96 ab	1.94 abc
196	0.96 ab	1.85 c
262	1.03 a	1.87 c
327	0.78 cd	1.52 d
393	0.77 cd	1.27 e
458	0.69 d	1.05 f
524	0.81 bcd	0.68 g

* For each soil pH, means within a column having the same letter are not significantly different at the 5% level, according to Duncan's Multiple Range Test.

tolerance, were insufficient for accurately characterizing a total soybean Zn response. More information is needed, for example, about possible fertilizer or growth stimulation effects by lower soil Zn additions, Zn uptake patterns across a range of additions, and further clarification of an observed Zn-induced Mn uptake (24). Additionally, prominent symptoms of Zn phytotoxicity include an interveinal chlorosis similar to that observed for Fe-deficiency and severe stunting, with or without chlorosis (2, 4, 8, 15, 20, 24). Because deficient P levels have been associated with severe stunting (7) and excessive P levels with induced Zn deficiency (5, 19), analysis for Fe and P over a range of Zn additions was desirable.

The study reported here was conducted to examine more fully, at two pH levels, the effects of wider and more complete soil Zn addition ranges on the growth responses and Zn, Mn, Fe, and P contents of four differentially Zn-tolerant soybean cultivars.

MATERIALS AND METHODS

Details of the experimental soil, pot size, fertilizer rates (except B) and sources, growth period, mixing, harvesting, and analyses techniques have been reported previously (24). Additional modifications and techniques for this study were: B was added at 1.0 ppm B from H_3BO_3; Zn additions (as $ZnSO_4 \cdot 7H_2O$) at pH 5.5 were: 1.31 (control), 16, 33, 49, 65, 82, 98, 115, and 131 ppm; at pH 6.5: 1.31 (control), 33, 65, 131, 196, 262, 327, 393, 458, and 524 ppm. At each pH and Zn treatment, $CaCO_3$ additions necessary for pH adjustment to 5.5 and 6.5 were determined from $CaCO_3$ additions vs. pH curves established for each Zn treatment. At pH 5.5 the required amounts of $CaCO_3$ were: 0.32, 0.35, 0.37, 0.38, 0.40, 0.43, 0.45, 0.48, and 0.50 g; at pH 6.5 the amounts were: 1.95, 2.17, 2.38, 2.80, 3.00, 3.30, 3.35, 3.40, 3.50, and 3.60 g. The increasing amounts correspond to the increasing Zn levels listed above. The cultivars selected for this study are listed along with their pertinent Zn-tolerance data in Table 1.

Although run simultaneously, the two pH levels were treated as separate experiments and placed on separate greenhouse benches in completely randomized blocks with three replications. The average daily temperatures ranged between 18 and 24 C, while the average daily humidity ranged between 48 and 83%.

Zinc and Mn levels were analyzed by atomic absorption spectrophotometry. Plant Fe was determined by the 0-phenanthroline method as described by Saywell and Cunningham (21). Plant P was determined by the molybdivanadophosphoric acid method described by Kitson and Mellon (13).

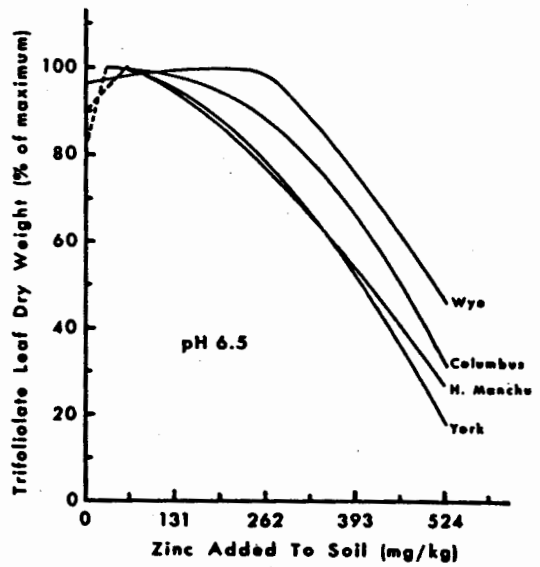

Fig. 1. The influence of added soil Zn on the trifoliolate leaf dry weight (as percent of maximum) of four soybean cultivars grown at pH 6.5.

RESULTS AND DISCUSSION

Dry Weight. The dry weight yields of the roots and trifoliolate leaves are given in Table 2. At pH 5.5, there was no significant change in root dry weight over the range of nine soil Zn additions. At pH 6.5, additions of soil Zn up to 262 ppm did not significantly alter the root dry weight; further additions at this pH depressed root growth slightly, with an unusual increase occurring at the highest Zn treatment. Although there were some differences in root growth among the four cultivars, there was no clear pattern of the phytotoxic effects of Zn on root growth.

Trifoliolate leaf dry weight yields decreased progressively with each soil Zn addition at pH 5.5, but, other than a slight decrease at the 49 ppm treatment, were not significantly lower than the control until the 98 ppm addition (Table 2). At pH 6.5, the trifoliolate leaf dry weight was not significantly reduced below the control until the 327 ppm Zn level (Table 2). To examine cultivar differences more clearly, the

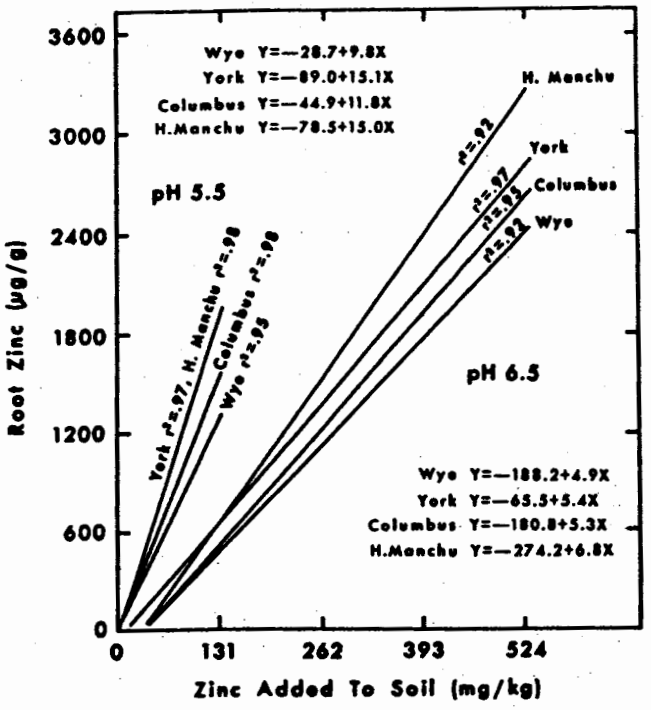

Fig. 2. Regression of root Zn content of four soybean cultivars on added soil Zn at pH 5.5 and 6.5.

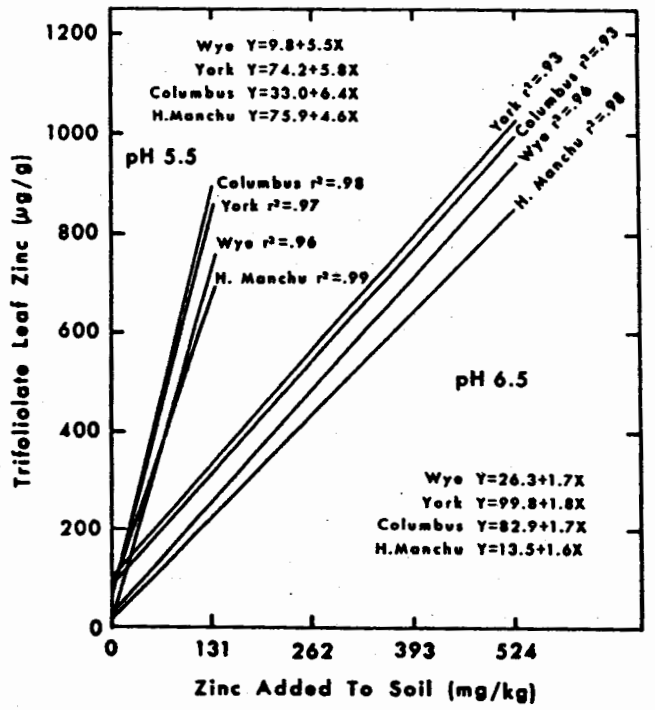

Fig. 3. Regression of trifoliolate leaf Zn content of four soybean cultivars on added soil Zn at pH 5.5 and 6.5.

leaf yields, as a percentage of each cultivar's maximum yield, were plotted as a function of added soil Zn (Fig. 1). Only the curves (drawn by inspection) for the higher pH are shown because cultivar responses were similar at each soil pH.

Soybean, in general, seems capable of tolerating moderate amounts of soil Zn during seedling growth without substantial reductions in trifoliolate leaf dry weight. A plot of the average response of the four cultivars at each Zn treatment would give a curve similar to that of Columbus (Fig. 1). This shows non-significant yield responses at Zn treatments up to 262 ppm, with rapidly declining yields resulting from further soil Zn additions (Fig. 1, Table 2).

The differential sensitivities or tolerance found among these cultivars was moderate: all were severely injured by high soil Zn additions (524 ppm Zn at pH 6.5). Wye and York represented the response extremes. Although York yield peaked at the 33 ppm Zn treatment, its leaf yield reductions were more severe than Wye at the higher Zn additions. Significant leaf dry weight increases above the control were observed for York, Columbus, and Hudson Manchu at the lower Zn levels (33, 33, and 65 ppm, respectively), while Wye had non-significant yield differences through 262 ppm added soil Zn (Fig. 1). Columbus and Hudson Manchu had intermediate responses to the Zn treatments, with Columbus slightly more tolerant than Hudson Manchu.

The soil pH, however, had a strong influence on the relative phytotoxicity of the Zn. At pH 5.5, all of the cultivars had yield reductions greater than 23% below their controls at the 131 ppm Zn treatment (not shown). This same addition at pH 6.5 resulted in yield increases above the controls (Fig. 1).

Tissue Zn Contents. There was a strong, positive, linear relationship between soil Zn additions and root and trifoliolate leaf Zn contents at both pH levels (Fig. 2, 3). As indicated by the high coefficients of determination (r^2) for each cultivar, the Zn increases for both the roots and leaves were a direct result of the added soil Zn. At an equivalent soil Zn addition the Zn contents of the tissues at pH 6.5 were approximately one-third as great as those at pH 5.5.

Differential absorption of Zn was shown by the cultivars (Fig. 2). Hudson Manchu absorbed more Zn from the soil than the other cultivars at both pH levels while Wye absorbed the least. Zinc entering the roots was readily translocated to the trifoliolate leaves, and soil pH had a strong influence on the amount of Zn found in the leaves (Fig. 3). At the 131 ppm soil Zn treatment, for example, the average trifoliolate leaf Zn of the four cultivars was reduced from approximately 800 ppm at pH 5.5 to 250 ppm at pH 6.5. Although York, Columbus, and Wye translocated Zn in proportion to that absorbed, Hudson Manchu behaved differently: across the Zn addition range it absorbed the most Zn, but translocated the least (Fig. 2, 3).

Leaf and Soil Zn Levels at Specific Yield Reductions. As mentioned above, York, Columbus, and Hudson Manchu showed positive growth responses to lower soil Zn additions at both pH levels, with greater additions being harmful, while Wye showed an ability to withstand greater Zn additions before it too became injured. Because of these response differences across the Zn addition ranges, it was desirable to determine, for each cultivar, the soil and tissue levels of Zn at which significant yield reductions occurred. Table 3 lists these values.

Table 3. Trifoliolate leaf, root, and soil Zn contents at which a 20% yield reduction occurred in the trifoliolate leaves of four soybean cultivars at two soil pH levels.

Cultivar	Soil†	Trifoliolate leaf‡	Root§
	mg Zn/kg		
	pH 5.5		
Wye	120	740	1,125
York	91	620	1,265
Columbus	117	860	1,320
H. Manchu	117	620	1,645
	pH 6.5		
Wye	373	680	1,630
York	255	730	1,320
Columbus	315	710	1,470
H. Manchu	244	370	1,380

† Values are from inspection curves of leaf yield reduction vs. added soil Zn. ‡ Values are from inspection curves of leaf yield (percent of maximum) s. leaf Zn. § Values are from linear regression curves of root Zn vs. added soil Zn.

At pH 5.5, serious yield reductions (>20%) were associated with the leaf Zn levels greater than 620 ppm. These values were similar to those reported by Boawn and Rasmussen (2) for several field crops (but not soybean) suffering a 20% yield reduction when grown in a growth chamber in Zn-amended alkaline soil. But they were higher than the 380 ppm leaf Zn content that Lee and Craddock (14) reported as being associated with a 28% reduction in actual bean yield in field-grown soybean (12 ppm Zn added to an acid loamy sand).

Although Wye, Columbus, and Hudson Manchu had 20% leaf yield reductions occurring at similar soil Zn levels at pH 5.5, they had slightly different leaf Zn contents (Table 3). These cultivar differences did not correspond to the differences in Zn content found in the roots. For example, York and Hudson Manchu had similar leaf Zn levels but differed by 400 ppm in their root Zn contents.

At pH 6.5, Wye (680 ppm Zn), York (730 ppm), and Columbus (710 ppm), had similar leaf Zn contents at the 20% reduction level (Table 3), while Hudson Manchu had a very different level (370 ppm). The Zn tolerance reaction of Hudson Manchu seems to suggest a complex pH effect, because this cultivar had considerably more Zn in its leaves at the lower pH for the same yield reduction. Root Zn levels did not reflect the cultivar differences in leaf Zn content (Table 3). Leaf yield reductions of 20% occurred at considerably higher soil Zn levels at the higher pH, with cultivar differences observed at both pH levels.

Zn, Mn Interaction. As reported in the first paper of this study (24), increasing soil Zn additions resulted in considerable increases in plant Mn. Examination of this phenomenon over a more inclusive range of soil Zn additions shows that root Mn increased linearly at both pH levels (Fig. 4, 5), while leaf Mn increases were curvilinear at pH 5.5 and linear at pH 6.5.

At the lower pH, translocation of Mn from the roots to the leaves did not occur beyond the 65 ppm Zn treatment, although absorption by the roots continued. At pH 6.5, increases in leaf Mn continued across the Zn addition range, unlike the curvilinear response observed at pH 5.5. Because root Mn concentrations at the higher Zn additions were very similar to those at pH 5.5, the lack of continued translocation at the lower pH is unclear. At both pH levels DTPA-extractable Mn was unaffected by the soil Zn additions (Fig. 4, 5), and harvest pH measurements showed no pH changes.

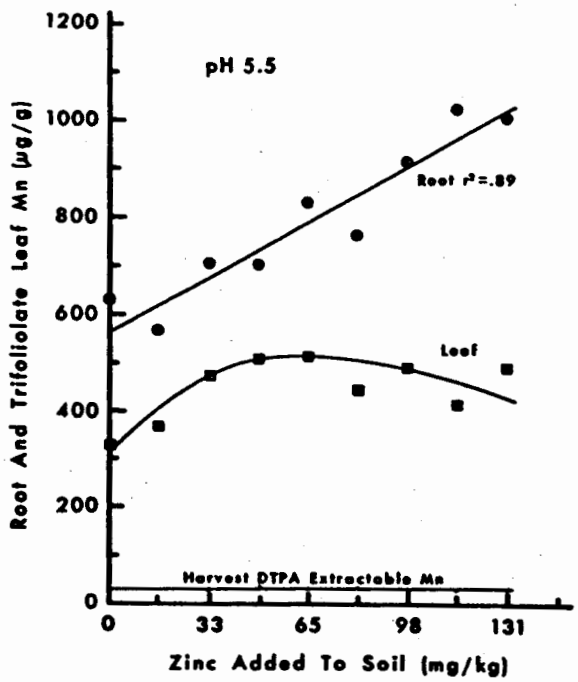

Fig. 4. The influence of added soil Zn on the Mn content of soybean root and trifoliolate leaves, and DTPA-extractable Mn. Each data point is the mean of four cultivars grown at pH 5.5.

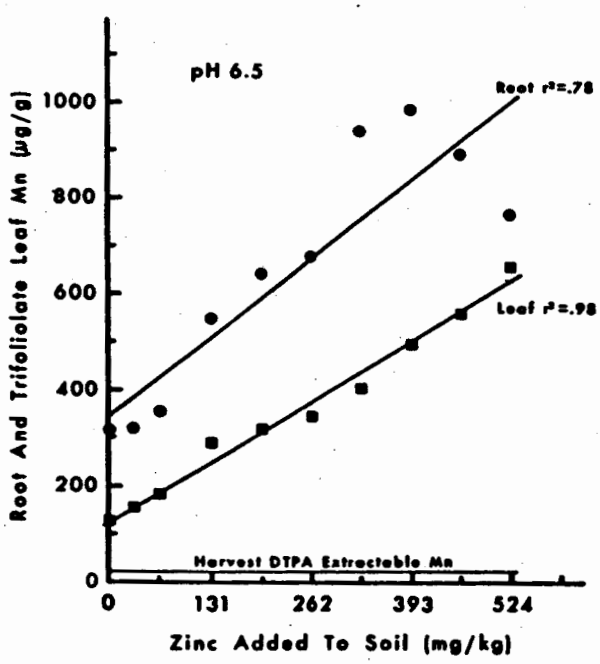

Fig. 5. The influence of added soil Zn on the Mn content of soybean root and trifoliolate leaves, and DTPA-extractable Mn. Each data point is the mean of four cultivars grown at pH 6.5.

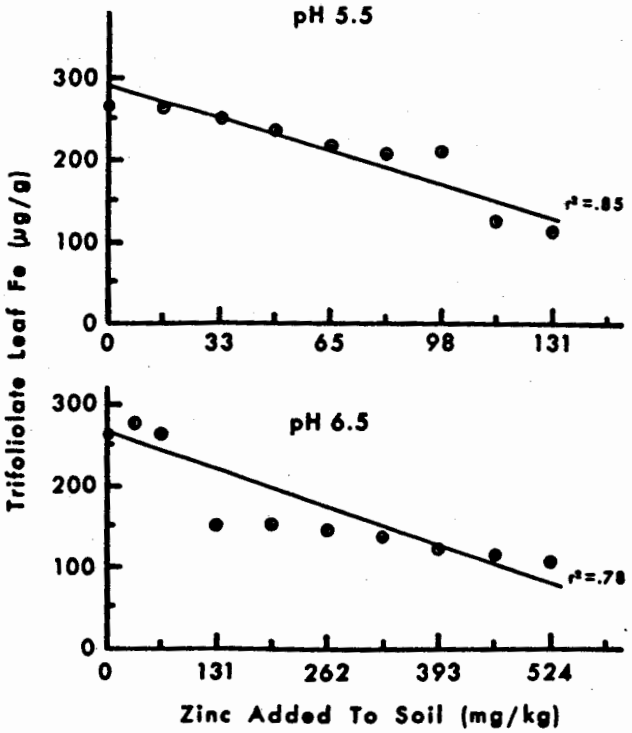

Fig. 6. Regression of trifoliolate leaf Fe content of soybean on added soil Zn at two soil pH levels. Each data point is mean of four cultivars.

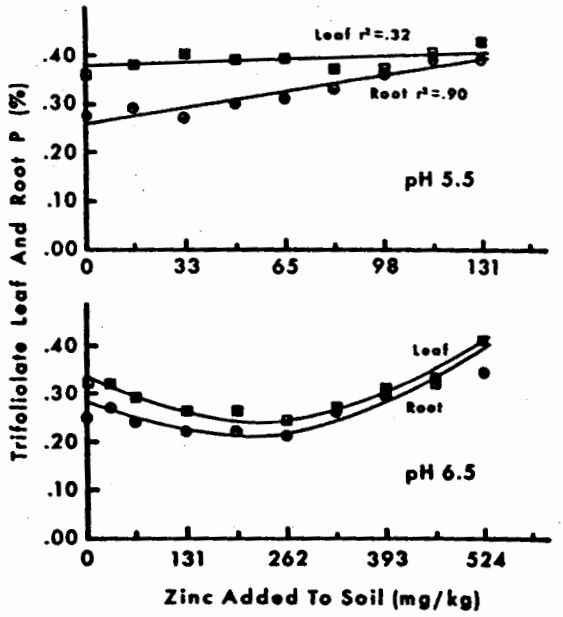

Fig. 7. The influence of added soil Zn on the P content of soybean trifoliolate leaves and roots at two soil pH levels. Each data point is the mean of four cultivars.

Manganese concentrations greater than 500 ppm in the leaves have been cited as being injurious to soybean (18), and as shown in Fig. 4 and 5, this concentration was exceeded slightly and quite possibly contributed to the phytotoxicity observed at the higher Zn treatments. Toxicity may have been related to the interaction of high levels of both Zn and Mn.

Variations in root and leaf Mn were found among the cultivars (data not shown). The effect of these variations in Mn content on the intercultivar comparisons of Zn tolerance is unclear. Recently Carter et al. (6) reported the results of a study in which they screened 30 cultivars of soybean for their relative tolerance to excess Mn. The plants were grown in nutrient solutions and showed a wide range of tolerance. However, the authors did not report the Mn content of any plant tissue.

The Zn-induced increases in tissue Mn illustrate the complex nature of Zn phytotoxicity. More work on the interaction is necessary to clearly establish the effects of excessive soil Zn per se, as well as induced effects such as the enhanced tissue Mn.

Trifoliolate Leaf Iron. The Fe content of the trifoliolate leaves was reduced by soil Zn additions at both pH levels (Fig. 6). All the cultivars had reduced Fe concentrations, with only moderate differences observed among the cultivars. Although the leaf Fe contents decreased, they did not approach 40 ppm, a concentration usually considered indicative of simple Fe deficiency in soybean (7). Severe interveinal chlorosis usually occurs in the younger trifoliolate leaves of soybean grown at phytotoxic levels of soil Zn (8, 20, 24). Because both the younger and older trifoliolate leaves were combined and analyzed, the data shown in Fig. 6 represents only the general decrease in leaf Fe content with increasing soil Zn, and not the actual Fe content of the chlorotic leaves. The Fe content of the chlorotic leaves could have been masked by the inclusion of leaves with both larger dry weights and Fe contents. Nevertheless, the trifoliolate leaf Fe contents were substantially reduced by soil Zn additions at both pH levels for all four cultivars.

Trifoliolate Leaf and Root Phosphorus. At pH 5.5, the P content of the roots increased with increasing soil Zn, while the content of the leaves remained basically unchanged (Fig. 7). At pH 6.5, however, there was a steady decrease in the P content of both the roots and leaves, until the 262 ppm soil Zn treatment, where further Zn additions caused an increase in tissue P (Fig. 7).

The translocation of P was unaffected by Zn treatments up to 131 ppm at the lower pH, whereas the translocation of P at pH 6.5 remained proportional to that absorbed by the roots (Fig. 7).

A prominent symptom of Zn phytotoxicity in soybean is a general stunting of the plants, sometimes accompanied by a reddish-brown or purple pigment in the stems, petioles, and veins (20, 24). This is very similar to general symptoms of P deficiency found in many crops (7). At both pH levels, the concentration of P in the leaves remained within or above the sufficiency ranges for soybean cited by other workers: 0.26 to 0.27% at bloom (12). Because the P content of the leaves declines with age (7), the values observed in the present experiments (before bloom) seem adequate for normal growth.

Even though the P content of the leaves at pH 6.5 did not decrease to reported (12) deficiency levels (<0.19%), there was a definite decline in leaf and root P with soil Zn additions up to 262 ppm (Fig. 7). Yet, leaf yields were not significantly affected by soil Zn addition until they were greater than 262

ppm (Fig. 1). The increases in leaf and root P by Zn additions greater than 262 ppm probably reflect the injured plant's unregulated uptake and translocation. Although the data indicate possible Zn/P interactions, they do not support a correlation between the observed stunting and P deficiency.

CONCLUSIONS

Soybean seedlings, in general, seem capable of tolerating moderate amounts of Zn added to a Sassafras sandy loam (approximately 90 ppm at pH 5.5, and 250 ppm at pH 6.5) before substantial reductions (15 to 20%) in leaf dry weight occur. However, cultivar differences do exist and can account for significant differences in tolerance (> 10% difference in yield reduction) among the cultivars at higher Zn additions. The soil pH has a complex influence on the relative tolerance of a cultivar, and comparative classifications of cultivar tolerance seem valid only within specific pH treatments. The use of foliar analysis as an absolute indicator of relative Zn phytotoxicity may be impractical because of differential Zn uptake and translocation. Compounding the pH effect is the complex nature of Zn phytotoxicity, as illustrated by the induced increases in tissue Mn and the Zn/Fe, Zn/P interactions. An imbalance of other elements may also be occurring while the plants are under a stress imposed by the excess Zn. However, major effectors such as plant species, soil type, and native macro- and micronutrient levels must be considered in any investigation of these interactions.

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