ABSTRACT

Zinc toxicity and Zn-Fe interactions were studied in corn (Zea mays L. var. Barbecue hybrid) grown in hydroponic culture. High Zn greatly reduced the root and shoot fresh weights; increasing Fe largely, but not completely, restored normal growth. Correlation analyses of root and leaf Zn and Fe contents suggested that Zn may interfere with the translocation of Fe; however, Zn toxicity was not associated with a diminished leaf Fe content. Fe did appear to retard both the absorption and the translocation of Zn. The chlorosis of Zn-toxic plants is not attributable to diminished total leaf Fe; however, this chlorosis is relieved by increasing nutrient Fe. Zn and Fe probably do interact at some site.

While examining plant response to high soil Zn levels at the site of an abandoned zinc mine, we observed that affected plants of corn and other grains often showed interveinal chlorosis in the new growth comparable to that of Fe deficiency. Our field studies in corn suggested that Zn inhibited Chl production by interfering with Fe metabolism, but not by lowering the Fe content of the leaves (J. A. Rosen, C. S. Pike, M. L. Golden, J. Freedman, in preparation). These findings were in accord with Giordano and Mortvedt's conclusion that Zn did not block Fe transport in soil-grown corn (9). Hydroponic studies using several species, including soybeans (1, 13) and navy beans (17), suggested that high nutrient Zn did interfere with Fe uptake and/or translocation, resulting in manifestations of Fe deficiency. The addition of Fe to the growth medium of soybeans could overcome the effects of high nutritent Zn, suggesting a competition between Zn and Fe at some site (1).

Examination of competition between Zn and Fe necessitates separate analysis of the absorption of metals by the roots and translocation to the leaves, using carefully controlled nutrient conditions. This report describes a hydroponic system designed to determine possible mechanisms of Zn toxicity at the level of these transport processes. The effect of Zn on Chl was also studied.

MATERIALS AND METHODS

Plant Material. Seeds of corn (Zea mays L. var. Barbecue hybrid, W. Atlee Burpee) were surface-sterilized in 50% Clorox for 30 sec and rinsed with distilled H2O. Seedlings were grown in flats of vermiculite in Percival E-54U growth chambers using a day temperature of 27 C, a night temperature of 20 C, a 16-hr photoperiod, and a light intensity of 800 ft-c. Plants were given deionized H2O daily.

After 6 days seedlings were transplanted to foil-covered, quart glass jars lined with polyethylene bags, using two plants/jar and at least four plants/treatment. The basic growth medium was 1/4 strength Hoagland solution (pH 5.75), excluding the Fe salt (2). Varying levels of Zn and Fe were added as ZnSO4·7H2O and ferrous tartrate, respectively, as indicated in the tables. The control treatment contained 1.4 mg/l Fe and 0.01 mg/l Zn. Nutrient solutions were made with deionized H2O and were changed every 2 days. Plants were grown in the growth chambers as above. Each jar received filtered air (20).

Plants were harvested after 21 days in solution and rinsed in deionized H2O to remove surface contamination. The plants were blotted dry, and roots and shoots were separated and weighed. Randomized replicate samples were prepared as described by Vickery (21). Leaf samples were taken from the third, fourth, and fifth leaves. Leaves were deribbed and macerated; following randomization, a portion (0.5 g) of each sample was used immediately for Chl determinations. The remaining leaf tissue and the root tissue were dried at 55 C for 12 hr.

Determination of Chlorophyll and Metal Content. Chl was extracted in 80% (v/v) aqueous acetone and absorption measured in a Beckman DU spectrophotometer at 645 and 663 nm (3). Dried leaf and root samples (0.2 g) were boiled for 40 min in 20 ml of 6 n HNO3. Then 2.2 ml of HClO4 was added and boiling continued for 10 min. The extracts were filtered and levels of Zn and Fe determined in a Perkin-Elmer model 303 atomic absorption spectrometer. Chl and metal data were analyzed by means of partial correlation coefficients, in which the influence of one metal is analyzed independently of the influence of the other (19).

RESULTS

Growth Parameters. Control plants appeared green and healthy and showed considerable lateral root development. With Fe held at 1.4 mg/l, as nutrient Zn increased, three toxic symptoms were observed: depressed plant height, depressed lateral root development, and interveinal chlorosis of the new growth. Increasing nutrient Fe at constant, high nutrient Zn appeared to alleviate totally the chlorosis but had only a slight effect on the other two symptoms. Table I shows fresh weight data from one experiment; other experiments gave comparable results. Root and shoot weights decreased with increasing nutrient Zn at constant nutrient Fe. The weight decrease was not completely restored by increasing the nutrient Fe concentration with high nutrient Zn, even though, as shown later, the Chl content was restored to normal.

Metal Content. Table II indicates that both root and shoot Zn/Fe ratios increased with increasing nutrient Zn and decreased with increasing nutrient Fe. However, the latter trend appears to be more marked. Statistical analysis (Table III) provides additional information. Metal uptake was dependent upon the solution.
metal content. Significant positive correlations existed between nutrient Zn and root Zn, nutrient Zn and leaf Zn, and root Zn and leaf Zn, as well as between nutrient Fe and root Fe, nutrient Fe and leaf Fe, and root Fe and leaf Fe. To assess competitive effects, we considered the effects of one metal upon the other. Nutrient Fe showed significant negative correlations with both root Zn and leaf Zn, and root Fe showed a significant negative correlation with leaf Zn. Root Zn and leaf Fe were significantly negatively correlated, but the correlations of nutrient Zn with root Fe and nutrient Zn with leaf Fe were not significant.

Effects of Metals on Chlorophyll Content. As shown in Table III, total Chl concentration was significantly positively correlated with nutrient Fe, with root Fe, and with leaf Fe, and negatively correlated with nutrient Zn, with root Zn, and with leaf Zn. However, none of the Zn/Chl correlations were significant.

**DISCUSSION**

High nutrient Zn led to stunting and interveinal chlorosis of the new growth. The chlorosis, but not the stunting, has been attributed to an interference with Fe metabolism by Zn (6). Some of the inhibition of plant growth by Zn (Table I) is reversed by increased nutrient Fe. Since some inhibition remains, Zn may also affect some growth processes not involving Fe.

The uptake of Zn and Fe by corn was correlated with the respective concentrations of the metals in the solution. The Zn-Fe competition experiments showed that each metal affects the movement of the other, but not in a strictly reciprocal way. Increasing nutrient Fe while maintaining a high nutrient Zn level progressively overcame the effects of high Zn (Table II). There were significant negative correlations between nutrient Fe and root Zn, nutrient Fe and leaf Zn, and root Fe and leaf Zn, as well as between root Zn and leaf Fe. However, nutrient Zn was not significantly correlated with either root Fe or leaf Fe. These results are compatible with our field study (J. A. Rosen et al., in preparation), in which we found a significant inverse correlation between soil Fe and leaf Zn but no correlation between soil Zn and leaf Fe.

These results indicate the importance of examining the component processes of absorption by the roots and translocation to the leaves in order to assess the effects of one metal upon the movement of the other. From our hydroponic work it seems that Fe can affect both absorption and translocation of Zn. However, Zn appears to act only (and not very strongly) on the translocation of Fe. Although the translocation of Fe may be influenced by Zn while absorption is not, when the two processes were lumped together (in calculating the correlation of nutrient or soil Zn with leaf Fe), no over-all significant correlation was seen. Therefore, high nutrient (or soil) Zn does not act by lowering the total Fe content of the leaf. Our results can provide information on the nature of the carriers that are involved in ion movements. These carriers apparently differ in the ease with which one metal can interfere with the transport of the other.

The Chl content of corn grown in high Zn was inversely related to the Zn/Fe status of the medium and the plant. In our field study Chl was significantly positively correlated with leaf Fe.
and soil Fe, and significantly negatively correlated with leaf Zn and soil Zn. Table III shows that nutrient Fe, root Fe, and leaf Fe were all significantly positively correlated with Chl. Fe-deficient plants (Table II) showed the typical chlorosis (9, 15). Although all of our hydroponic work (reported here and unpublished) showed negative and usually significant correlations between Chl and nutrient Zn, root Zn, and leaf Zn, the present experiment did not reveal a significant correlation. One possible explanation for this lack of significance lies in the experimental design. Table II shows that Chl content leveled off as nutrient Fe was increased while holding Zn constant. The “plateau” occurred at different nutrient Fe concentrations, depending on the nutrient Zn concentration. Presumably at the plateau level, the leaf has enough Fe to fulfill the needs of Chl biosynthesis. Any additional Fe represents excess, and a further increase will not yield a corresponding increase in Chl.

An alternate approach is to consider, e.g. only the four samples grown at 1.4 mg/l Fe. Then we obtain the following correlations: nutrient Zn/chl, r = -0.83; root Zn/chl, r = -0.96; leaf Zn/chl, r = -0.82. The root Zn correlation is significant at the .05 level; but, because of the small sample size, the others lie in the range 0.10 < P < 0.20. But based on our laboratory and field data, we propose that Zn does exert a strong, negative influence on Chl. The strength of a statistical association is given by the square of the correlation coefficient (19); using the above correlation coefficients, the variation in Zn concentration (nutrient, root, or leaf) accounts for at least two-thirds of the variation in Chl content.

Giordano and Mortvedt (9), using soil-grown corn, did not find evidence for the inhibition of Fe transport to plant tops by Zn, even though the toxicity symptoms were typical of Fe deficiency. In contrast, increasing soil Fe did lower the Zn content of tops. Their results are thus compatible with our laboratory and field observations. However, Brown and Tiffin (5) found that the addition of large amounts of Zn to soil caused a large drop in the total plant Fe content in corn and millet but not in several other species. Both of these previous studies (5, 9) used a Zn-deficient soil in pots, with the soluble Zn and Fe salts added; our field study used a completely natural system. None of these soil studies examined absorption and translocation separately. In a hydroponic study on soybeans, Ambler et al. (1) suggested that Zn toxicity involves an interference with the translocation of Fe. The responses to high levels of Zn may differ depending on the species and the type of soil or nutrient conditions (5, 18).

High Zn did interfere with Chl metabolism, but the results on metal content argue against an interference with the amount of Fe reaching the leaf. Other possibilities include: (a) Zn may compete with Fe for a site on a particular Chl biosynthetic enzyme, as discussed below. (b) Zn may influence the Fe2+/Fe3+ ratio in the plant. In a hydroponic study Zn retarded the reduction by the roots of Fe3+ to the favored Fe2+ form (1). (c) Zn may alter the subcellular or cellular distribution or availability of Fe in the leaf. Preliminary attempts to isolate organelle fractions by differential centrifugation were hampered by the low yield of Zn-toxic tissue.

Increasing the nutrient Fe while holding Zn constant, either in the experimental design or statistically by the procedure of partial correlation, led to an increased Chl, up to a point. Fe also relieved Zn-induced chlorosis in soybeans (1). Fe can thus overcome the deleterious effects of Zn on Chl, arguing against an action of Zn on Chl by a mechanism not involving Fe. This finding cannot differentiate among the three models presented above. Returning to the metal content data, it is also possible that increasing nutrient Fe relieves Zn toxicity by diminishing the amount of Zn that reaches the leaves.

If we hypothesize a Zn-Fe competition for binding at some site (such as a fixed site, an enzyme, an ion carrier in a membrane, or a carrier in the vascular tissue), the following points should be considered. The mutual substitution of Zn2+ and Fe2+ is plausible given their identical ionic radii (0.83 A) (10). The order of activity of heavy metals in inducing chlorosis is similar to the order of the strengths of their bonding to certain chelating agents, such as ethylenediamine (7, 16). By this view, then, Zn could successfully compete with Fe2+ for certain binding sites, and, once bound, Zn would not easily be displaced by Fe2+.

Several tests of model (a) above are possible. The conversion of coproporphyrinogen to protoporphyrinogen requires Fe and is impeded in Fe-deficient plants (8, 11, 14). Fe is required for the activity of ALA synthetase, the initial step of the porphyrin pathway in animals and photosynthetic bacteria (12). Beale et al. (4) have shown that barley leaves may synthesize ALA by an entirely different mechanism, using glutamate; the effects of Fe and Zn on this process need to be explored.

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5 Abbreviation: ALA: δ-aminolevulinic acid.

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