Copper Toxicity in *Phaseolus vulgaris* L. as Influenced by Iron Nutrition. II. Elemental and Electron Microprobe Analyses[1,2]

Roland R. Daniels[3], B. Esther Struckmeyer[4], and L. A. Peterson[4]
University of Wisconsin, Madison

Abstract. In plants grown without Fe or with Fe tartrate, 0.50 ppm Cu reduced Fe concn only in the roots. When FeEDTA was used, Fe concn were higher in the roots at 0.04 and 0.50 ppm Cu than other treatments. Concentrations of Cu in the roots increased with the increase in Cu in the nutrient solution; in the shoots there was little difference. With FeEDTA, the Cu concn in the roots and shoots at both levels of Cu was considerably less than with Fe tartrate or without Fe. Microprobe analysis showed accumulations of Fe in wall areas of some xylem elements in the midrib of leaves of plants grown with Fe tartrate-0.50 ppm Cu only. Iron did not accumulate in comparable tissues with Fe tartrate-0.04 ppm Cu and with FeEDTA-0.04 and 0.50 ppm Cu. Copper was distributed uniformly throughout the tissues but P accumulated only in wall areas of the cells. The localization of Fe and P in the same cell suggested that high Cu induced chlorosis by precipitation of Fe as Fe phosphate.

A high concn of Cu in the growth medium has been associated with low Fe concn in the shoots of rice (3), clover (6), soybeans (10), citrus (14), and tobacco (17). However, no differences in concn of Fe in tobacco shoots with increased levels of Cu in the nutrient solution have been reported (16). Mueller and Wallace suggested (12) that high Cu levels interfered with Fe translocation in soybeans. Brown et al. (1), demonstrated that it was impossible to induce Fe chlorosis with high concn of microelements using the split-root technique, indicating that the effects occur as exterior, competative interactions of Cu and Fe for absorption sites. When Fe chelates were used, the effects of high Cu on the Fe in plants were diminished (11, 17). We studied the effects of high Cu on the concn and distribution of Fe, Cu, Mn, Zn, and P in beans, and attempted to determine differences associated with the Fe source in the nutrient cultures.

Materials and Methods

Seeds of *Phaseolus vulgaris* L. 'Topcrop' were germinated in horticultural perlite until the primary leaves were approximately 5 cm long. Uniform seedlings were placed 3 per pot, in 10-l pots of aerated nutrient solutions. The treatment solutions for 1 week were 1) Hoagland's (8) complete nutrient to which 1 ml/l 0.5% Fe tartrate was added the first and second week, 2) Hoagland's complete nutrient plus a single addition at the beginning of the experiment of 4 ml/l of an FeEDTA solution, and 3) Hoagland's minus Fe. Cu levels were maintained at 0.04 ppm for 1 week (control), then raised to 0.50 ppm Cu in half of the solutions (high Cu concn). Each treatment was replicated 3 times. One week later, the experiment was terminated because of severe chlorosis in the Fe tartrate-0.50 ppm Cu and both minus Fe treatments.

Tissue samples from 1 plant in each pot were taken from the second cm from the root tip, the second internode from the stem tip, a midvein portion, and the midportion of a petiole of the second leaflet from the stem tip and killed in isopentane over liquid nitrogen (12). Sections 16 μ in thickness were cut on a cryostat microtome set at -16°C, attached to purified carbon discs, and air dried at room temp. The sections were analyzed for Fe, Cu, and P on the microprobe using an accelerating voltage of 25 kv and 0.03 μA sample current (13). Scanning electron micrographs were taken of the areas analyzed. Separated root and shoot portions were dried, weighed, ground, and analyzed for elemental composition by direct reading emission spectroscopy (4). Analyses from 2 experiments were similar. Dry wt data were analyzed according to Steel and Torrie (15) and means were compared by Waller and Duncan's LSD (18).

Results and Discussion

Dry wt. Roots and shoots of bean plants grown with FeEDTA-0.04 ppm Cu weighed more than plants from other treatments (Table 1). Majumder and Dunn (11) reported greater dry wt of plants grown with 5 μM EDTA compared to those without EDTA. With 0.50 ppm Cu, roots and shoots weighed less than those grown with 0.04 ppm Cu except when grown without Fe. Shoots and roots of tobacco plants weighed less than controls when grown in high Cu levels (16). Bean roots grown with FeEDTA-0.50 ppm Cu weighed less than with Fe tartrate-0.04 ppm Cu, however, shoots weighed approximately the same. The growth depressing effects of high Cu were not overcome by EDTA since the total dry weight of plants grown with FeEDTA-0.50 ppm Cu was less than those grown with FeEDTA-0.04 ppm Cu, which agrees with reports of Majumder and Dunn (11). It did prevent chlorosis associated with Cu toxicity.

Composition. With Fe tartrate or without Fe, 0.50 ppm Cu in the nutrient solution resulted in lower concn of Fe, Zn, and Mn in the roots compared to those grown in 0.04 ppm Cu (Table 2). When EDTA was used, Fe and Mn concn were increased at the high concn of Cu but Zn remained the same. The Cu concn in the roots varied directly with Cu concn of the nutrient solution but were appreciably lower with both FeEDTA treatments compared to those grown with Fe tartrate or without Fe.

The Fe concn in the shoots (Table 2), at both levels of Cu, were approximately the same with Fe tartrate or without Fe. With FeEDTA, Fe concn were higher at 0.04 ppm Cu compared to 0.50 ppm Cu. Manganese concn were lower in all plants grown in 0.50 ppm Cu, especially when grown with Fe tartrate.

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[3]Department of Horticulture, Pennsylvania State University, University Park.

[4]Professor and Associate Professor respectively, Department of Horticulture.

Table 1. Dry wt of roots and shoots of bean plants after 1 week of differential Cu treatments.

Cu (ppm)	Fe source	Roots[z] (mg)	Shoots[z] (g)
0.04	Fe tartrate	377[bx]	1.49[bx]
0.50	Fe tartrate	300[c]	1.00[c]
0.04	FeEDTA	449[a]	2.06[a]
0.50	FeEDTA	297[c]	1.45[b]
0.04	0 Fe	235[cd]	0.92[c]
0.50	0 Fe	222[d]	0.88[c]
LSD[y]		71	0.23

[z]Values are means of 9 plants.

[y]Waller and Duncan's LSD values are for the 95% confidence level.

[x]Within columns means followed by different letters are significantly different from each other.

Hallsworth et al. (6) reported a depression of Mn and Fe absorption at high Cu levels. In contrast to roots, there were small differences in Cu concn in the shoots grown in both levels of Cu with Fe tartrate and FeEDTA. However, Cu concn in plants grown with minus Fe-0.50 ppm Cu were nearly twice as high as the minus Fe-0.04 ppm Cu ones. With FeEDTA, the Cu concn was lower than in other treatments. With increased concn of Cu in the nutrient solution, concn of Cu in tobacco increased (16).

Electron microprobe analysis. Root sections scanned from the epidermis through the xylem for Fe, Cu, and P showed no differences in distribution as a result of treatment. Phosphorus accumulated in all wall areas at the epidermis and endodermis. Line scans of vascular tissue in stems and petioles did not reveal accumulations of Fe.

Line scans 200μ long made on the xylem in the midrib of leaves (Fig. 1-4), showed peaks and troughs for Cu, but there was no significant accumulation regardless of treatment. Phosphorus accumulated in the wall areas of the xylem elements. The variation in intensity of the P peaks from one wall to the opposite one is unexplainable and the absence of P in the lumen of the cells could possibly be due to shrinking of the cytoplasm to the wall areas. Rasmussen et al. (13) reported that K^+ moved from the middle of the element to the wall and beyond the wall because of knife penetration of the tissue and the air drying of the sample on the carbon discs. There was little variation in intensity and no localization of Fe in the midribs with Fe tartrate-0.04 ppm Cu (Fig. 1), FeEDTA-0.04 ppm Cu (Fig. 3), and FeEDTA-0.50 ppm Cu (Fig. 4). However, with Fe tartrate-0.50 ppm Cu (Fig. 2) considerably higher intensities of Fe, estimated to be 500-1000 ppm, were present in the wall areas of some metaxylem elements in the midribs of the leaves.

Scans made across the xylem elements in different sections on the discs revealed similar accumulations in the same relative positions as those shown in Fig. 2. The absence of Fe accumulations in the midribs of leaves grown in FeEDTA-0.50 ppm Cu was expected since EDTA has been reported to overcome chlorosis associated with Cu toxicity (10). Kartashova (9) reported that EDTA also overcame the chlorosis associated with Mn toxicity.

The accumulation of P and Fe at the same sites suggests that Fe was precipitated as a phosphate. Precipitation of Fe as ferric phosphate, under certain growth conditions, has been reported by Biddulph (2). Kartashova (9) reported that when chlorosis was induced with excess Mn the amount of bipyridyl soluble Fe, considered to be readily available for use by the plant, decreased with a concommitant increase in HCl soluble, unavailable Fe. Possibly, high Cu levels could induce the shift to the relatively unavailable forms by causing precipitation of Fe in the xylem as a slightly soluble phosphate. Hewitt (7) suggested that metal-induced Fe deficiency may be the result of competition by the metal with Fe for sites normally occupied by Fe during some stages of chlorophyll formation. This would be difficult to explain on the basis of the Cu concn in the shoots in our experiments. Copper concn in shoots of beans grown with Fe tartrate-0.50 ppm Cu and Fe tartrate-0.04 ppm Cu were approximately the same and it is difficult to conceive that equal concn could cause such extreme symptoms in 1 plant and not the other. Brown et al. (1) proposed an exterior interaction of Cu with Fe for absorption sites on the roots because toxicity symptoms did not develop when the split-root technique was used. However, our results indicate that Fe concn are approximately the same in shoots of plants grown with Fe tartrate-0.50 ppm Cu and Fe tartrate-0.04 ppm Cu. With exterior competitive interaction, Fe concn in the shoots would be expected to be less when grown with 0.50 ppm Cu.

Table 2. Elemental composition of bean roots and shoots as affected by Fe source and Cu concn.[z]

Treatment	Roots (ppm)				Shoots (ppm)			
	Fe	Cu	Zn	Mn	Fe	Cu	Zn	Mn
Fe-tartrate-0.04 ppm Cu	1300	175	248	1025	110	26	55	303
Fe tartrate-0.50 ppm Cu	1125	345	145	470	105	32	35	158
FeEDTA-0.04 ppm Cu	2400	87	195	825	155	12	53	147
FeEDTA-0.50 ppm Cu	4470	117	190	1210	120	11	46	124
0 Fe-0.04 ppm Cu	105	190	277	740	70	14	28	150
0 Fe-0.50 ppm Cu	80	350	133	282	72	26	30	121

[z]Single sample composite of 9 plants.

It is reasonable to assume that the site of the primary effect of Cu should be associated with high concn of Cu in the tissue. Using this criterion, the only place where these conditions occur is in the roots grown with Fe tartrate-0.50 ppm Cu having concn nearly twice those grown with Fe tartrate-0.04 ppm Cu. The chlorotic conditions in the shoots may be secondary to the effect of Cu on the roots. This possibility is supported by anatomical studies by Daniels et al. (5) showing the greatest differences between Cu toxicity and Fe deficiency were confined to the roots. It is possible that the effectiveness of the split root technique in preventing toxicity symptoms (1) could be explained by the presence of adequate tissue functioning normally to offset any deleterious effects that high Cu has in the roots. The effectiveness of EDTA in alleviating chlorosis agrees with these possibilities because Cu concn in the roots are low in both EDTA treatments compared to Fe tartrate and minus Fe treatments.

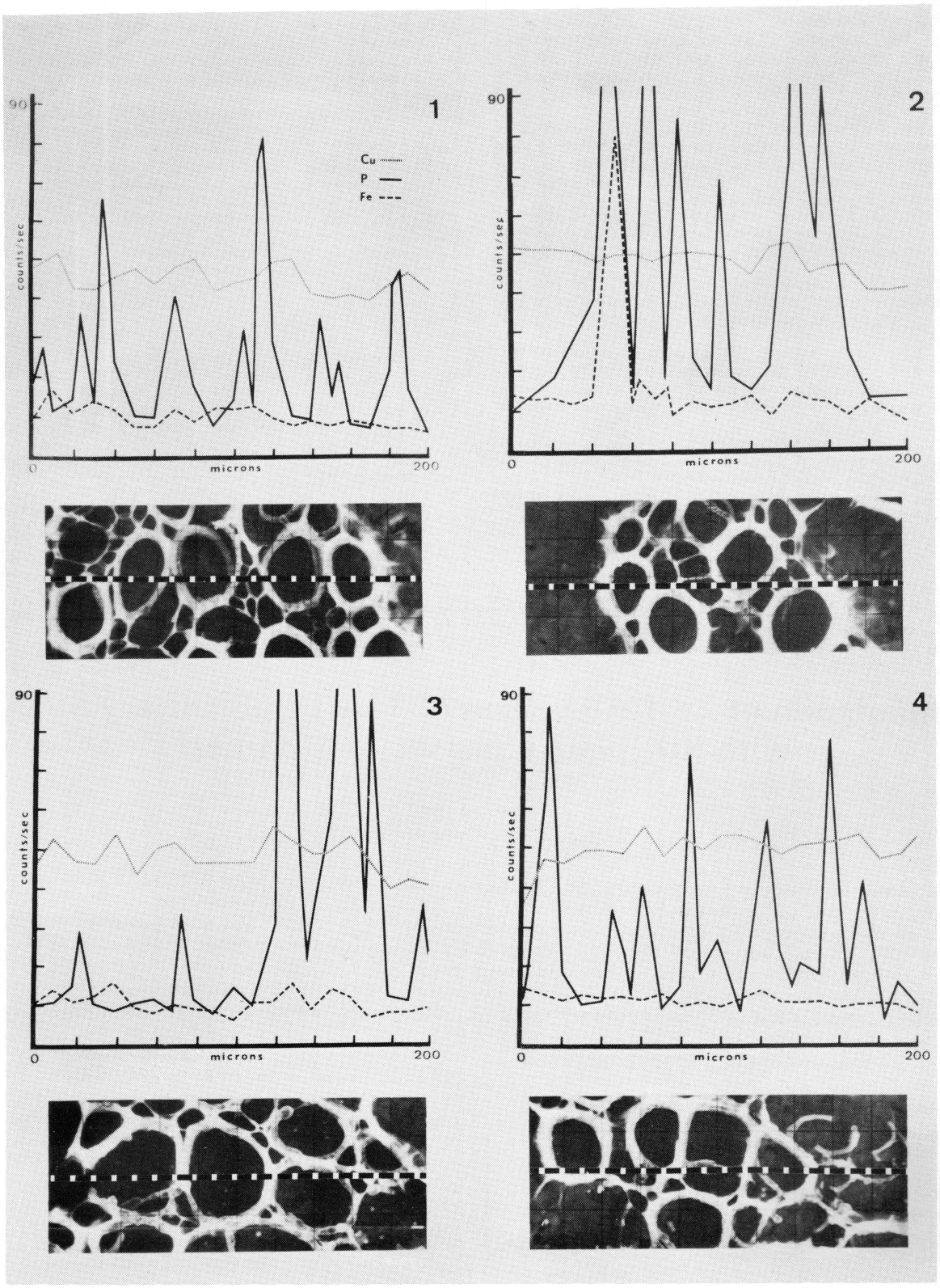

Fig. 1-4. Graphs showing intensity and localization of Fe, Cu, and P in the midrib of leaves. Scanning electron micrographs of the area are directly below the graphs. The dashed line indicates the area traversed by the electron beam. *Fig. 1.* Line scan and scanning electron micrograph; Fe tartrate-0.04 ppm Cu. *Fig. 2.* Line scan and scanning electron micrograph; Fe tartrate-0.50 ppm Cu. *Fig. 3.*. Line scan and scanning electron micrograph; Fe EDTA-0.04 ppm Cu. *Fig. 4.* Line scan and scanning electron micrograph; Fe EDTA-0.50 ppm Cu. All scanning electron micrographs 395X.

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Stimulation of Leaf Abscission of Tree Fruit Nursery Stock With Ethephon - Surfactant Mixtures[1]

Fenton E. Larsen[2]

Washington State University, Pullman

Abstract. Combinations of ethephon and D-WK (Dupont-WK) surfactant were effective in stimulating leaf abscission of 20 cultivars in 5 species of tree fruit nursery stock. Species and cultivars varied considerably in sensitivity to mixtures of these chemicals, but 1 to 3 applications at weekly intervals of 200 to 400 ppm ethephon + 1 to 2% D-WK were generally effective. These treatments caused little xylem, phloem, or bud damage except to 'Early Redhaven' peach and 'Early Italian' prune. 'Rome' apple was sensitive but was not damaged by concn of 200 ppm ethephon + 1% D-WK. D-WK stimulated leaf abscission when used alone at 1 to 2% but acted more slowly than when combined with ethephon. Ethephon alone at 200 to 400 ppm was usually relatively ineffective.

Interest in chemicals for nursery stock defoliation has been previously indicated (4, 8, 11, 12, 13). Ethephon [(2-chloroethyl)phosphonic acid] has been suggested for nursery stock defoliation (5, 7, 9). The role of ethylene in leaf abscission (1, 2, 3) and the ethylene-producing capacity of ethephon (14) has also been discussed. It has been reported that 2000 ppm ethephon + 2% mineral oil produced greater leaf abscission of nursery stock than 2000 ppm ethephon alone (6).

Other workers have suggested that 2000 ppm or more ethephon was necessary for defoliation of nursery stock (5, 6, 7). Previous trials by the writer showed that 1 to 3 applications of 500 to 2000 ppm of ethephon were necessary for nursery defoliation of several tree fruit cultivars (9), but such concn sometimes resulted in bark or bud damage (10). Further experimental work in 1970 and 1971 showed that these concn could be substantially reduced and more rapid defoliation could be obtained by combining 1 to 2% D-WK surfactant (Dupont-WK surfactant with the principal functioning agent being the dodecyl ether of polyethylene glycol) with the ethephon spray. We report the effectiveness of ethephon - D-WK mixtures for defoliation.

Materials and Methods

Ethephon (68-240 acid formulation) and D-WK surfactant were applied alone and in various combinations as sprays during October of 1970 and 1971 at commercial nurseries in central Washington (Hanford area, Quincy, Wenatchee, Brewster) and at the Washington State University Royal Slope Experimental Unit. Sprays were applied to runoff using hand operated trombone sprayers. In 1970, single, double, and triple applications were made at weekly intervals starting October 8 to duplicate plots of 3 or more plants each (0.6 to 1.0 m of row for stoolbeds or seedlings) on 8 apple, 1 pear, and 1 prune cultivar. Concentrations of 500 and 750 ppm ethephon + 2%

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[2]Associate Professor, Department of Horticulture.