Effects of copper on the growth, photosynthesis and nutrient concentrations of *Phaseolus* plants

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Abstract

Bean plants (*Phaseolus vulgaris* L. var. Zargana Kavala) were grown under conditions of increasing Cu concentrations in the growth medium (0.5-160.5 μM). Generally, the Cu concentrations between 0.5-1.5 μM were deficient, 1.5-10.5 μM were optimal, and 10.5-160.5 μM were toxic to plant growth. The Cu toxicity was associated with marked increases in plant tissue Cu concentrations. Under the Cu-deficient and optimal growth conditions, Cu was located primarily in the leaves. Under Cu toxicity, it was primarily sequestered in the roots. With increasing Cu in the growth medium, there was a positive correlation between Cu concentrations in the roots, stems and leaves, Ca in the roots, and K and Mg in the leaves. In contrast, Ca concentrations in the leaves and stems showed a negative correlation. The chlorophyll (Chl) concentration increased with increasing leaf Cu concentration, however, the Chl *a/b* ratio decreased. Since with an increasing leaf Cu concentration the leaf area decreased more markedly than the leaf dry mass, the net photosynthetic rate (P_N) per leaf area increased and per dry mass decreased. The increase in P_N per leaf area was almost entirely accounted for by the increase in Chl concentration. The initial Chl fluorescence (F_0) increased with increasing leaf Cu concentration. The ratio of variable to maximum fluorescence (F_v/F_m) under Cu toxicity decreased. The half-time for the rise from F_0 to F_m ($t_{1/2}$) remained relatively unchanged with increasing leaf Cu concentration. Therefore the Cu-stress caused a small decrease in the efficiency of photosystem 2 photochemistry, but its primary effect was on growth.

Additional key words: chlorophyll; fluorescence induction; leaf area; mineral elements; net photosynthetic rate; photosystems 1 and 2; root; stem.

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Abbreviations: Chl - chlorophyll; DM - dry mass; F_0 - initial fluorescence; F_m - maximum fluorescence; F_v - variable fluorescence; $t_{1/2}$ - the half-time for the rise from F_0 to F_m; LHC - light-harvesting complex; P_N - net photosynthetic rate; PCA - Principal Components Analysis; PS - photosystem; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

Copper is an essential plant nutrient. It is required as a structural and catalytic component of several proteins and enzymes involved in electron transfer and oxygenation reactions, and charge accumulations. Cu deficiency reduces the synthesis of plastocyanin and cytochrome oxidase, which results in growth inhibition and decreased photosynthesis and respiration (Barón-Ayala and Sandmann 1988, Barón-Ayala *et al.* 1992). Cu is essential for photosystem 2 (PS2) activity (Droppa *et al.* 1984, Barón-Ayala *et al.* 1992) in ensuring the correct content and composition of pigments and polypeptides in PS2, and the content and degree of saturation of lipid fatty acids in the thylakoids and PS2 complex (Barón-Ayala *et al.* 1992), and it also serves as a structural component of an active PS2 complex (Arvidsson *et al.* 1993). Yet excess Cu is phytotoxic causing stunted growth, chlorosis, and malformation of the roots (Kabata-Pendias and Pendias 1984, Punz and Sieghardt 1993).

Some controversy exists on the effect of excessive Cu on photosynthesis. Two potential metal sensitive sites in the photosynthetic electron transport chain are indicated: the water-spliting enzyme on the oxidising side of PS2, and the NADPH-oxido-reductase on the reducing side of PS1. A small inhibition of photosynthetic electron transport on the oxidising side of PS2 has been reported in isolated spinach chloroplasts (Shioi *et al.* 1978, Sandmann and Böger 1980, Lidon and Henriques 1991). Chl fluorescence measurements have suggested that a site of Cu inhibition is on the antenna of PS2 (Lidon *et al.* 1993). Sandmann and Böger (1980) also reported a Cu-mediated lipid peroxidation of the chloroplast membrane. However, Baszyński *et al.* (1982) reported that PS2 activity was not affected, and PS1 activity was slightly enhanced (NADP reduction) in Cu-tolerant plants growing under an excess of Cu. An inhibition of photosynthesis may be the result of a direct Cu effect on ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBPCO) activity (Stiborova *et al.* 1986, Lidon and Henriques 1991), however, the RuBPCO activity was expressed on the basis of soluble protein. The ratio of RuBPCO protein to total soluble protein may be different between control plants and plants grown under excessive Cu. In other reports, RuBPCO activity was unaffected by the presence of excessive Cu during growth (Baszyński *et al.* 1982, Lanaras *et al.* 1993). The contents of plastid pigments and lipoquinones in Cu-treated plants decrease (Lidon and Henriques 1991) or increase (Baszyński *et al.* 1982, Maksymiec *et al.* 1994, 1995).

In this study, the effect of Cu deficiency and toxicity on intact *P. vulgaris* plants was examined. Firstly, the effects on growth were established in relation to the accumulation of Cu in the plant tissues. The effect of Cu on the absorption of some other essential nutrients was also investigated. Finally, the effect of Cu on P_N, Chl concentration, and Chl fluorescence was examined in relation to the general strategy of the plants towards confronting conditions of Cu stress.

Materials and methods

Plants and experimental design: Beans (*P. vulgaris* L. var. Zargana Kavala) were

germinated in the dark for 6 d at 24 °C on filter paper saturated with water. The germinated beans were planted in pots (250 cm^3) containing perlite. The plants (7 or 8 at each concentration) were then watered every day with a 1:10 strength Hoagland's solution (Hoagland and Arnon 1950) containing final concentrations of 0.5, 0.6, 1.5, 5.5, 10.5, 20.5, 40.5, 80.5, and 160.5 μM $CuSO_4 \times 5\ H_2O$. The nutrient solution (pH 5.5) was as follows [μM]: KCl 50, H_3BO_3 25, $MnSO_4 \times 7\ H_2O$ 2, $ZnSO_4 \times 7\ H_2O$ 2, $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ 0.5, Fe-EDTA 20, $MgSO_4 \times H_2O$ 0.1, $CuSO_4 \times 5\ H_2O$ 0.5, KNO_3 600, $Ca(NO_3)_2 \times 4\ H_2O$ 400, and $NH_4H_2PO_4$ 200. The plants were kept under a 16/8 h light/dark regime at 23±2 °C for 24 d by which time they had one pair of unifoliate leaves and two trifoliate leaves. They were irradiated with cool-white fluorescence tubes giving an approximate irradiance of 140-200 μmol m^{-2} s^{-1} at the level of the leaves.

Nutrient measurements: Each plant was cut into roots, stem, and leaves (the pair of unifoliate leaves, first trifoliate leaf, and second trifoliate leaf, separately) and the tissue oven-dried at 80 °C to a constant mass. Samples of the plant tissues and soil were digested with a nitric-perchloric acid solution (4:1, v/v) for 4 h at 150 °C. The Cu, Ca, Fe, Mg, Zn, and K concentrations were determined in an atomic absorption spectrophotometer (*Perkin Elmer 2380*) using *SpectrosoL* (*BDH Chemicals*, Poole, England) as a standard solution. All concentrations measured for the samples were within the linear ranges specified by the manufacturer for each element. These ranges are from 0 up to the following values for: Cu, 5.0; Ca, 5.0; Fe, 5.0; Mg, 10.0; Zn, 1.0; K, 20.0 [g m^{-3}]. The detection limits [g m^{-3}] of the absorption spectrophotometer operated with a flow spoiler and using an air-acetylene flame were 0.002, 0.002, 0.004, 0.0001, and 0.002 for Cu, Ca, Fe, Mg, and Zn, respectively.

P_N was measured using a portable infra-red gas analyser (IRGA, *LI-COR* model *6200, LI-COR*, Lincoln, Nebraska) in a closed system, using a 250 cm^3 chamber. The P_N per unit leaf area and stomatal conductance (g_s) were calculated using the equations of Caemmerer and Farquhar (1981). A single, attached leaf was used for each measurement.

Chl fluorescence parameters, initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v, where $F_v = F_m - F_0$), and the half-time ($t_{1/2}$) for the rise from F_0 to F_m, were measured using a portable fluorimeter (Plant Stress Meter, *Biomonitor*, Umeå, Sweden) as described by Öquist and Wass (1988). Measurements were made on the upper surface of leaves which had been dark-adapted for 45 min. Chl was excited for 5 s by actinic radiation with a photon flux of 400 μmol m^{-2} s^{-1}.

Morphometric measurements: The length of the longest root, the length of the stem, and the area of each leaf were measured for each plant. The fresh and dry masses of the roots, stem, and each leaf were also recorded. Leaf area was measured using an *Mk2* Area Meter (*Delta-T Devices*, U.K.) connected to a *TC7000* Series Camera (*Burle Industries*, U.S.A.).

Chl determination: Individual leaf discs (1 cm^2) were pulverised in liquid nitrogen, and Chl was extracted in 90 % acetone for 24 h at -10 °C. After centrifugation of

extracts at 10 000 × *g* for 3 min, the total Chl was determined from the values of absorbance at 664 and 647 nm using the extinction coefficients given by Jeffrey and Humphrey (1975).

Statistical analysis: Principal Components Analysis (PCA) (Gauch 1977) was used to examine the inter-elemental relationships in the plant tissues. Differences between means were assessed using a one-way analysis of variance (ANOVA) followed by a multiple comparison test (LSD) (Sokal and Rohlf 1981).

Results

Plant growth and tissue Cu concentrations: The responses of the roots, stems and leaves to the range of Cu concentrations used in the growth medium (0.5-160.5 μM) indicated that these were deficient, physiological, and toxic to plant growth (Fig. 1*A,B,C*). In the growth medium containing 0.5-1.5 μM Cu the root length and biomass increased, and optimal values occurred at 1.5-5.5 μM Cu for root length and up to 10.5 μM Cu for root biomass. The root Cu concentration increased from 26.5 to 33.6 mg(Cu) kg^{-1}(DM), with a mean value of 28.6±3.4 mg(Cu) kg^{-1}(DM) over the growth medium concentration range of 0.5-5.5 μM Cu. The root length decreased markedly at growth medium Cu concentrations above 5.5 μM, and root biomass at concentrations above 10.5 μM Cu, both of which corresponded to a marked increase in root Cu concentration at a growth medium concentration of 5.5 μM Cu (Fig. 1*A*). A reduction in root length and root biomass occurred at root Cu concentrations exceeding 33.6±2.7 and 55.8±7.4 mg(Cu) kg^{-1}(DM), respectively (Fig. 1*A*, Table 1). Plants grown at 160.5 μM Cu showed a reduction in root length (68 %) and biomass (69 %) compared to optimal values, and had a mean root Cu concentration of 1479.3 mg kg^{-1}(DM) (Table 1), representing a 44-fold increase in root Cu concentration compared to that initiating a decrease in root length.

The stem length of plants growing in up to 1.5 μM Cu increased and remained optimal for those growing in up to 10.5 μM Cu (Fig. 1*B*). The stem length then decreased, slightly for plants growing in 10.5-80.5 μM Cu, and markedly between 80.5 and 160.5 μM Cu. Stem biomass increased for plants growing in up to 5.5 μM Cu, and thereafter decreased with an increasing growth medium Cu concentration. The stem Cu concentration decreased slightly from 23.2 to 19.9 mg(Cu) kg^{-1}(DM) (Table 1) when the growth medium Cu concentration ranged from 0.5 to 5.5 μM Cu, and then increased for plants growing in higher than 10.5 μM Cu. A reduction in stem length and biomass occurred at stem Cu concentrations exceeding 25.3±1.6 and 19.9±1.3 mg(Cu) kg^{-1}(DM), respectively. The stem length proved not to be a good indicator of an increasing Cu concentration in the stem. Plants grown at 160.5 μM Cu showed a reduction in stem length (33 %) and biomass (38 %), compared to optimal values, and had a mean stem Cu concentration of 83.0 mg(Cu) kg^{-1}(DM).

The total leaf area and the leaf biomass per plant increased for plants growing in 0.5-10.5 μM Cu, and were optimal at 10.5 μM Cu (Fig. 1*C*). For this growth medium

Cu concentration range, the mean leaf Cu concentration per plant increased from 22.6 to 62.4 mg(Cu) kg^{-1}(DM) (Fig. 1*C*). A reduction in both leaf area and biomass

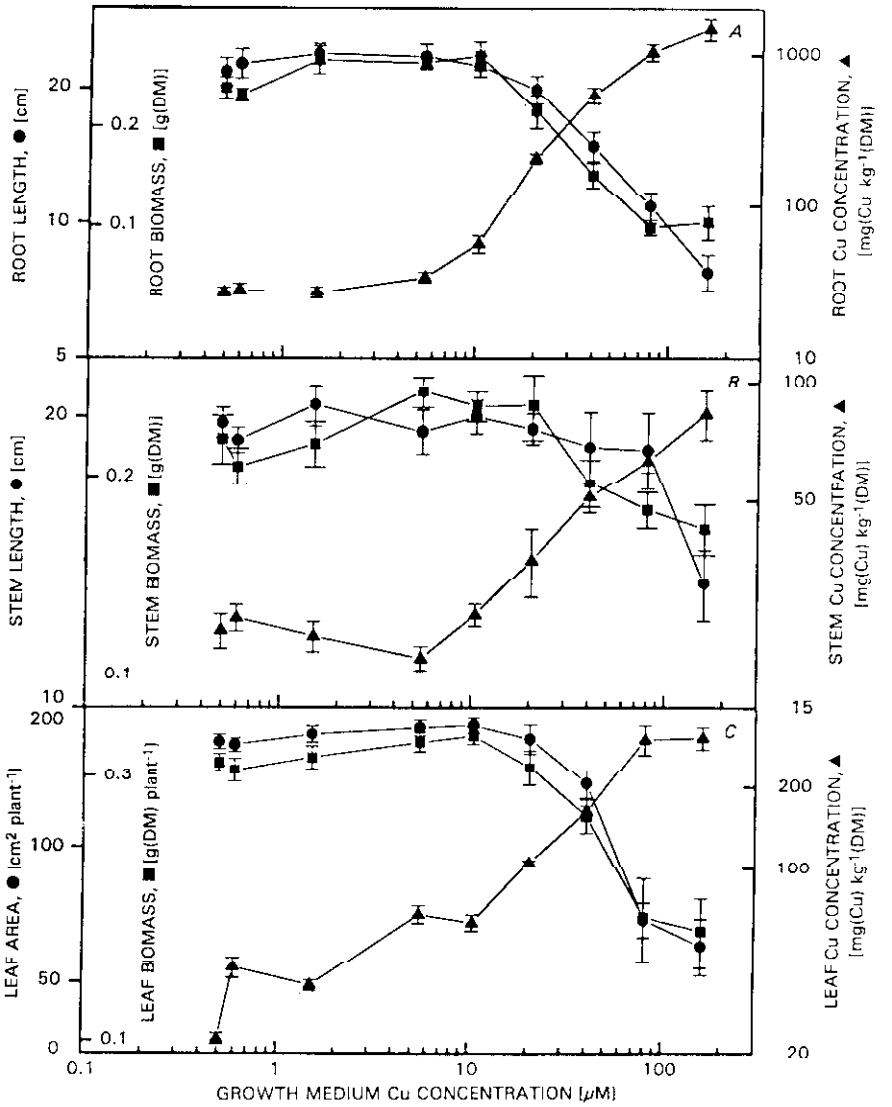

Fig. 1. Nutrient-accumulation curves showing the effect of increasing Cu concentration in the growth medium of *Phaseolus vulgaris* plants on the root, stem, and leaf biomass, and other morphometric parameters (root and stem length, leaf area), and the corresponding Cu concentration in the respective tissues. Root length refers to the length of the longest root. Values are plotted on a log-log scale. Bars represent SE.

occurred at leaf Cu concentrations exceeding 62.4±4.3 mg(Cu) kg^{-1}(DM). Plants growing in concentrations exceeding 10.5 μM Cu showed a marked decrease in both

Table 1. Mean Cu, Ca, Fe, Mg, Zn and K concentrations in the roots, stems, and unifoliate leaves of *Phaseolus vulgaris* plants grown at different Cu concentrations. The element concentrations [mg kg^{-1}(DM)] are expressed as the mean of at least 5 replicates with SE in parenthesis.

	Growth medium Cu concentration [μM]								
	0.5	0.6	1.5	5.5	10.5	20.5	40.5	80.5	160.5
Root									
Cu	26.5	27.8	26.4	33.6	55.8	208.3	544.2	1044.6	1479.3
	(2.1)	(2.6)	(2.5)	(2.7)	(7.4)	(14.1)	(65.6)	(131.0)	(231.6)
Ca	264.9	577.5	504.5	773.8	696.6	1191.8	1155.3	1342.2	1110.9
	(21.5)	(83.5)	(68.5)	(76.7)	(74.5)	(134.1)	(61.3)	(173.8)	(174.6)
Fe	83.8	114.2	99.0	122.2	124.2	106.9	71.7	105.1	346.7
	(14.1)	(15.4)	(19.4)	(17.8)	(25.0)	(16.6)	(5.3)	(11.0)	(120.9)
Mg	3816.3	3265.3	2720.0	2205.9	1643.6	3012.9	2087.5	1889.2	1002.5
	(340.3)	(437.4)	(259.2)	(178.8)	(243.6)	(89.8)	(164.3)	(143.3)	(199.2)
Zn	123.5	131.6	104.3	89.6	112.1	235.5	253.9	178.3	136.0
	(9.4)	(10.9)	(9.5)	(7.8)	(16.3)	(35.5)	(22.7)	(23.2)	(21.0)
K	12314.1	10664.6	8825.9	6980.6	5971.1	10736.7	15353.6	14320.3	11633.6
	(936.4)	(1610.9)	(1246.3)	(896.9)	(1064.4)	(894.4)	(1737.7)	(1638.0)	(2006.2)
Stem									
Cu	23.2	25.0	22.5	19.9	25.3	35.1	51.1	63.5	83.0
	(2.2)	(1.9)	(1.9)	(1.3)	(1.6)	(7.1)	(4.6)	(4.03)	(11.6)
Ca	8694.0	11655.3	10141.2	8464.5	9116.1	6627.3	5408.8	3756.4	3429.9
	(1358.9)	(1295.3)	(822.7)	(879.2)	(750.0)	(783.1)	(692.5)	(712.9)	(1312.1)
Fe	67.7	85.5	78.0	61.5	65.6	53.1	43.1	52.1	51.6
	(5.5)	(6.6)	(3.7)	(4.1)	(2.5)	(6.6)	(3.3)	(6.2)	(6.7)
Mg	3457.3	3840.2	3663.3	3128.8	3101.2	3092.7	2937.0	2803.9	4133.6
	(309.1)	(278.8)	(219.7)	(226.4)	(205.9)	(339.2)	(150.8)	(350.6)	(1557.5)
Zn	72.7	104.5	101.1	92.4	98.6	88.4	75.9	72.6	54.4
	(7.6)	(30.5)	(4.0)	(9.9)	(7.0)	(16.8)	(4.4)	(13.9)	(5.7)
K	15673.5	18624.1	17418.5	14089.5	13494.4	14201.4	18381.1	12434.0	11578.5
	(1937.0)	(1310.4)	(1345.7)	(2801.4)	(705.8)	(2539.8)	(1611.4)	(1014.2)	(1129.5)
Leaves									
Cu	19.1	43.8	31.1	55.4	50.6	82.8	164.4	292.1	364.1
	(1.1)	(4.7)	(4.8)	(1.8)	(5.9)	(1.0)	(10.0)	(62.6)	(45.6)
Ca	15154.6	9290.7	8948.9	9285.8	9268.6	8791.6	6273.5	5111.8	2645.3
	(1558.0)	(1045.9)	(760.8)	(132.6)	(650.2)	(221.4)	(88.3)	(666.0)	(253.0)
Fe	51.3	48.9	85.5	74.7	76.7	71.7	73.9	82.7	48.4
	(8.5)	(2.3)	(3.6)	(3.2)	(3.6)	(3.0)	(3.1)	(16.3)	(3.9)
Mg	1978.3	2710.0	2411.2	2314.6	2567.5	2672.1	3363.7	3456.2	2560.3
	(96.1)	(99.1)	(63.2)	(17.1)	(134.1)	(63.3)	(112.9)	(450.9)	(92.2)
Zn	63.1	65.5	60.9	66.5	98.0	68.0	81.8	94.1	97.1
	(4.9)	(3.1)	(1.2)	(3.6)	(3.5)	(2.0)	(4.9)	(10.5)	(2.6)
K	878.3	1022.0	1008.3	995.0	902.7	1146.2	1263.9	1456.6	1352.7
	(61.0)	(49.8)	(29.9)	(38.4)	(54.9)	(16.1)	(35.6)	(114.3)	(49.7)

leaf area and leaf biomass as the mean leaf Cu concentration increased from 62.4 to

308.9 mg(Cu) kg^{-1}(DM) (Fig. 1*C*). Plants grown at 160.5 μM Cu showed a reduction in leaf area (67 %) and biomass (56 %) compared to optimal values. Leaves of different ages of plants grown on a given Cu concentration generally did not show significantly different leaf Cu concentrations, indicating that Cu was uniformly distributed in all the plant leaves.

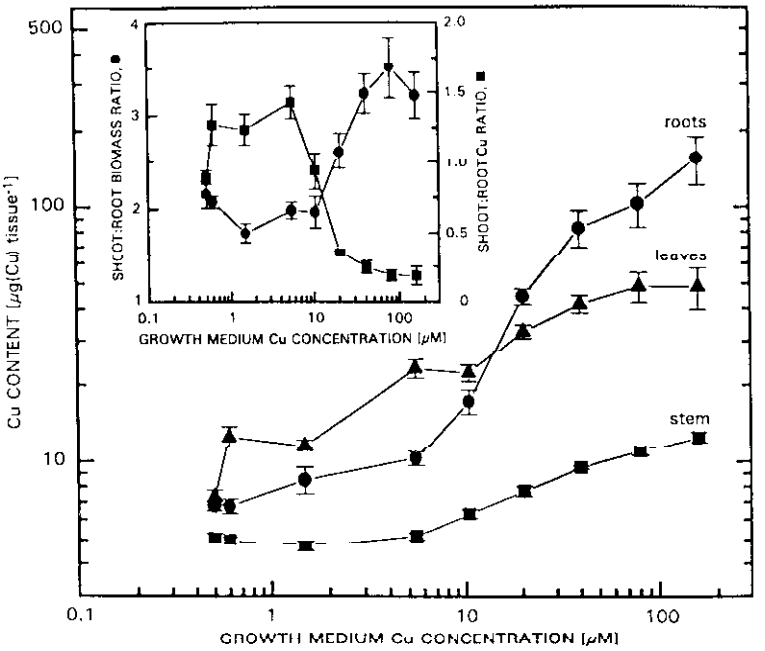

Fig. 2. The amount of Cu accumulated in the roots, stem, and leaves of *Phaseolus vulgaris* plants grown with increasing Cu concentrations in the growth medium. Tissue refers to roots, stem, and leaves. Values are plotted on a log-log scale. *Insert*: The change in the ratio of shoot to root biomass (dry masses), and shoot to root Cu content with increasing Cu concentration in the growth medium. Bars represent SE.

The total amount of Cu accumulated in the roots [μg(Cu) root^{-1}] and stem [μg(Cu) stem^{-1}] increased biphasically, with a marked increase in the content for plants growing in concentrations exceeding 5.5 μM Cu (Fig. 2). Plants grown in 160.5 μM Cu had a mean Cu content of 155.1±33.5 μg(Cu) root^{-1} and 12.6±0.6 μg(Cu) stem^{-1}. However, the total amount of Cu accumulated in the leaves did not show two discrete absorption phases over the range of Cu concentrations used. The plants grown in 160.5 μM Cu had a mean total leaf Cu content of 48.5±8.7 μg Cu leaf^{-1}. In all cases the Cu accumulation saturated at the highest Cu concentrations. This was related to the fact that biomass decreased faster than the increase of the Cu content in plant tissues occurred with increasing Cu in the growth medium. Considering the allocation of Cu in the plants, at growth medium Cu concentrations below 10.5 μM, Cu was located primarily in the leaves, whereas above this concentration Cu was

located primarily in the roots (Fig. 2). At all the growth medium Cu concentrations least Cu was located in the stem. Accordingly, the ratio of the Cu amount in the shoot (stem and leaves combined) to that in the root increased for plants grown in 0.5 5.5 μ M Cu, and then decreased (Fig. 2, *insert*). The ratio was about 1.0 (0.94) at a growth medium Cu concentration of 10.5 μM, equivalent to a mean Cu concentration per plant of 49.0 mg(Cu) kg^{-1}(DM) (Cu concentrations of 55.8 and 47.1 mg(Cu) kg^{-1} (DM) for the roots and shoot, respectively). At mean plant concentrations greater than this, a decrease in total plant biomass was observed (Fig. 1). By comparison, the ratio of shoot to root biomass (determined from DM) decreased slightly, and then increased markedly at growth medium Cu concentrations exceeding 10.5 μM (Fig. 2, *insert*). Therefore, although both shoot and root growth were inhibited by Cu in the growth medium, the root biomass was more adversely affected by Cu than that of the shoot. The leaf biomass represented on average 39 % of the total plant biomass over the range of growth medium Cu concentrations, while the root biomass dropped from 34 to 24 % of the total plant biomass. Correspondingly, an increase in the percentage contribution of the stem to total plant biomass was observed (from 27 to 37 %).

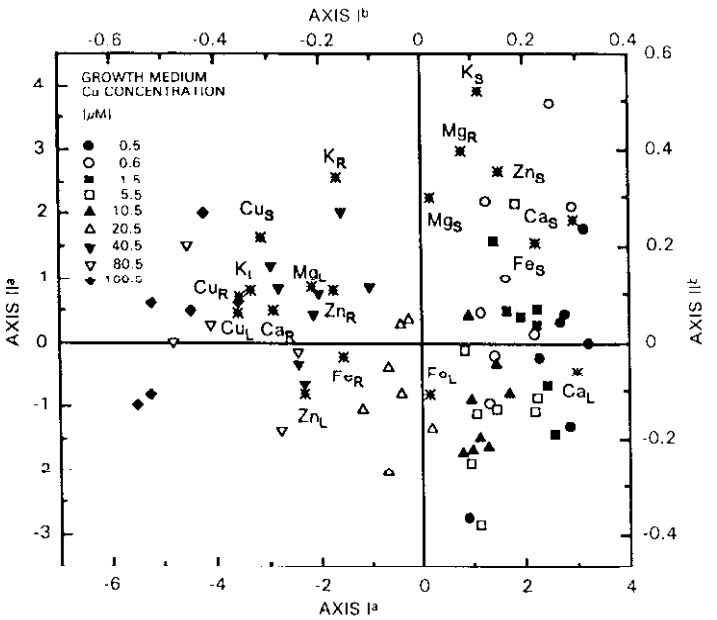

Fig. 3. Principal Components Analysis (PCA) ordination for *Phaseolus vulgaris* plants growing at different Cu concentrations. Analysis of the interrelationships between Cu, Ca, Fe, Mg, Zn, and K in the plant tissues (R: roots, S: stems, and L: unifoliate leaves) growing at nine different Cu concentrations (0.5-160.5 μM). [a], axes refer to the ordination scores for the samples (symbols defined in figure); [b], axes refer to the ordination scores obtained for the variables represented with asterixes.

The relationship between Cu and other nutrient (Cu, Ca, Fe, Mg, Zn, and K) concentrations in the roots, stems and the unifoliate leaves of plants grown at

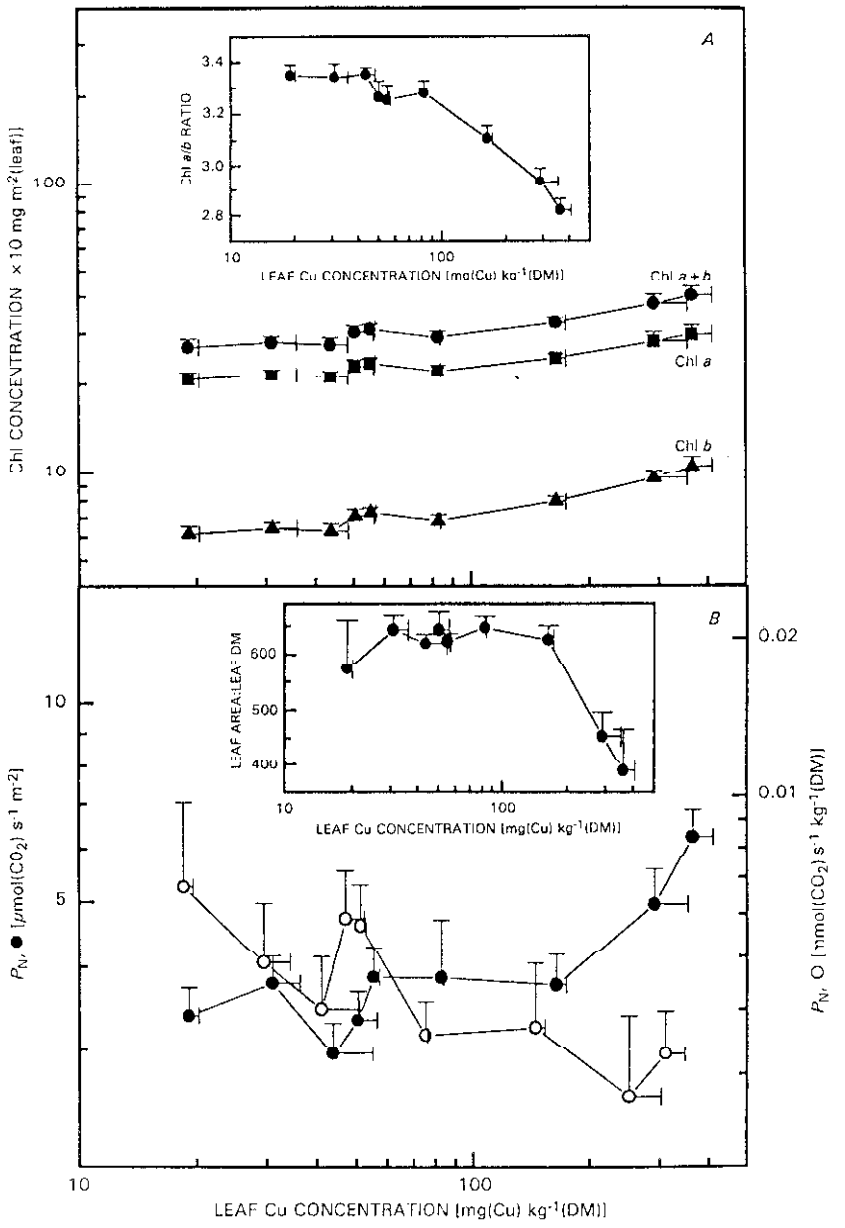

Fig. 4. Changes in chlorophyll (Chl) concentration and the ratio of Chl *a/b* (*insert*) (*A*), and net photosynthetic rate, P_N, and the ratio of leaf area to leaf dry mass, DM (*insert*) (*B*) with increasing Cu concentration in the unifoliate leaves of *Phaseolus vulgaris* plants grown at different Cu concentrations. Values are plotted on a log-log scale. Bars represent SE.

different Cu concentrations (Table 1) was examined using PCA (Fig. 3). The values were separated primarily along AXIS I (representing 34 % of the variance) by the concentrations of Cu in the roots and leaves (*to the left*) and Ca in the stems and leaves (*to the right*). AXIS II, which accounted for 14 % of the variance, was differentiated by the concentrations of K in the stems and Fe in the leaves. Variables in the ordination that are in close proximity indicate parameters showing covariation. For example, the concentrations of Cu in the roots, leaves and stems, and Ca in the roots, and K in the leaves, they all increase with increasing Cu in the growth medium, and altogether will be either high or low in the plants. Opposing variables indicate inverse relationships so that at low concentrations of the above, higher Ca concentrations in the leaf and stem occur. Generally, in the root there was a tendency for the concentration of all the elements measured, with the exception of Mg, to increase with increasing Cu concentration. In the stem there was an inverse relationship between Cu and the other elements measured, and in the leaves an inverse relationship between Ca and the other elements measured.

Photosynthesis and Chl concentration: Total Chl, Chl *a* and *b* concentrations, when expressed per leaf area, increased in the unifoliate leaves as the leaf Cu concentration increased (Fig. 4*A*). This increase was more marked at leaf Cu concentrations greater than 80 mg(Cu) kg^{-1}(DM), as was the relative increase in Chl *b* with respect to Chl *a*, thus the ratio Chl *a/b* decreased (Fig. 4*A*, *insert*). Total Chl concentration in the first and second trifoliate leaves did not show significant variation (overall mean values for plants grown at all Cu concentrations were 262±14 and 292±20 mg m^{-2} for the first and second trifoliate leaves, respectively) with increasing leaf Cu concentration.

The P_N of the unifoliate leaves, expressed per leaf area, remained more or less constant for mean leaf Cu concentrations up to 164.4 mg(Cu) kg^{-1}(DM), and then increased (Fig. 4*B*). However, the total leaf area per plant decreased with increasing Cu in the growth medium and total P_N per plant in fact decreased (see Figs. 1*C* and 4*B*). The P_N expressed per leaf DM decreased with increasing leaf Cu concentration (Fig. 4*B*). These contrasting results for the expression of P_N arose from the fact that the leaves were smaller in the surface area, but thicker, at the highest growth medium Cu concentrations, as indicated by the decrease in the ratio of leaf area to leaf DM (Fig. 4*B*, *insert*). The DM per unit leaf area increased markedly at the two highest growth medium Cu concentrations (see Fig. 1*C*). This increase in DM occurred faster than the increase in P_N, at corresponding leaf Cu concentrations, leading to an observed decrease in P_N expressed per leaf DM. The increase in P_N per leaf area could be accounted for by the increase in total Chl, with the exception of the highest leaf Cu concentration (see Fig. 4).

Chl fluorescence was measured on leaves of different ages. The observed trends were generally more marked in older leaves, and were either lower or non-existent in the youngest leaves. F_0 increased with increasing leaf Cu concentration in the unifoliate leaves (Fig. 5*A*). This increase was more marked at leaf Cu concentrations greater than 55.4 mg(Cu) kg^{-1}(DM). There was no significant difference in F_m (overall mean 2.55±0.04) over the range of leaf Cu concentrations. F_v/F_m decreased at leaf Cu

concentrations greater than 55.4 mg(Cu) kg^{-1}(DM) (Fig. 5*B*). Similar but smaller decreases were observed with younger leaves. The $t_{1/2}$ did not show significant differences with an overall mean value of 50.6±3.2 ms over the range of leaf Cu concentrations. However, there was a slight decrease in $t_{1/2}$ from 51.1±4.0 to 49.5± 4.2 ms over the range 19.1-55.4 mg(Cu) kg^{-1}(DM), and subsequently a slight increase from 49.5±4.2 to 52.8±3.1 ms with higher leaf Cu concentrations.

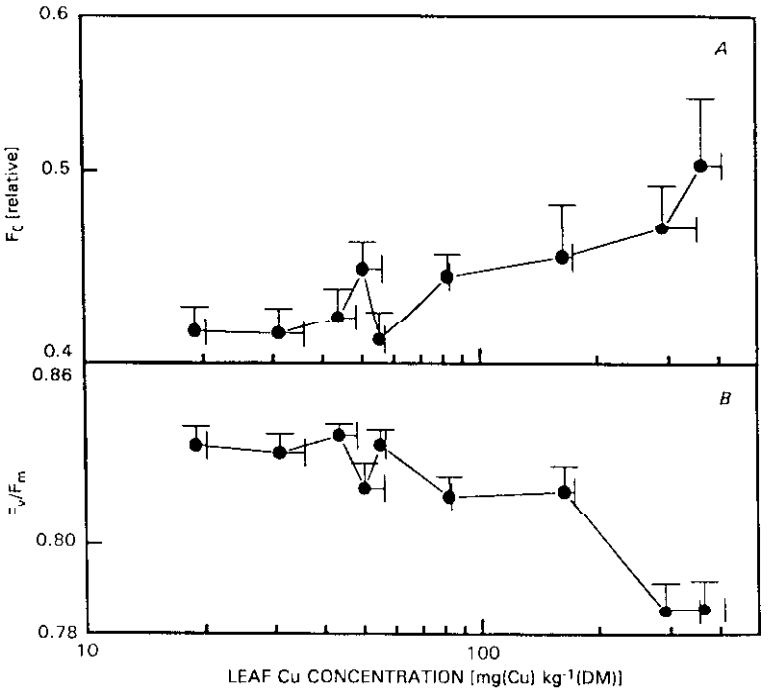

Fig. 5. Changes in chlorophyll fluorescence parameters F_0 (*A*) and F_v/F_m (*B*) with increasing Cu concentration in the unifoliate leaves of *Phaseolus vulgaris* plants grown at different Cu concentrations. Values are plotted on a log-log scale. Bars represent SE.

Discussion

The threshold toxic tissue Cu concentrations (Berry and Wallace 1989) of the roots, stems, and leaves of *P. vulgaris* plants causing a reduction in the respective tissue biomass were 55.8±7.4, 19.9±1.3, and 62.4±4.3 mg(Cu) kg^{-1}(DM), respectively. These concentrations occurred at a growth medium Cu concentration of 10.5 μM, and correspond to a toxic total tissue Cu concentration of 49.0 mg(Cu) kg^{-1}(DM). Root growth was more adversely affected than shoot growth, and consequently the shoot to root biomass ratio increased. Root growth inhibition by Cu has been associated with increasing concentrations of abscisic acid and decreased concentrations of cytokinins (Vizárová and Holub 1994) and a general interference

with cell division (Punz and Sieghardt 1993). The threshold toxic total tissue Cu concentration causing a reduction in root growth was 41.8 mg(Cu) kg^{-1}(DM) for bean [Cu concentration of 33.6 and 46.6 mg(Cu) kg^{-1} (DM) in the roots and shoot, respectively], which compares to 35.1 mg(Cu) kg^{-1} (DM) for rice [Cu concentration of 134 and 21.5 mg(Cu) kg^{-1}(DM) in the roots and shoot, respectively] (Lidon and Henriques 1992, 1993). Biomass yields of rice plants decreased when Cu contents in the roots and shoots were 1683 and 46.5 mg(Cu) kg^{-1} (DM), respectively (Lidon and Henriques 1992, 1993). Bean plants encounter Cu differently from rice in the aspect that Cu is not excluded so rigorously from the shoot, and that Cu is more toxic to the root growth.

The transition point between two absorption phases in a nutrient-accumulation log-log plot defines the threshold latent toxicity level after which higher solution concentrations are potentially phytotoxic (Berry and Wallace 1989). This solution concentration was *ca.* 5.5 μM Cu for the roots and stem of *P. vulgaris* plants. Due to the linear nature of Cu uptake in the leaves, even the lowest growth medium Cu concentrations should be considered potentially phytotoxic. Cu is initially excluded from *P. vulgaris* roots and stem, whereas the leaf Cu concentration is an indicator of the growth medium Cu concentration. Thus, at tissue Cu concentrations below the threshold toxic concentration, Cu is accumulated predominantly in the leaves, and above that, it is accumulated predominantly in the roots. The Cu content increase in the stem may have arisen through lateral transport, resulting from Cu diffusion into the xylem parenchyma (Bidwell 1979). These uptake characteristics may be indicative of the following. Firstly, that the Cu uptake into the shoot is controlled by transport across the endodermis. Cu ions are able to diffuse or flow unrestricted *via* the root apoplast through the cortex to the endodermis (Bidwell 1979). The uptake, either by active or passive transport at the endodermis, would thus be dependent on the external Cu concentration. Continual translocation of Cu to the shoot would maintain maximal uptake rates at the endodermis. Secondly, that when the threshold latent toxicity level for the root is attained, Cu is taken up into the root cortex parenchymal cells due to a Cu-induced breakdown in the permeability barrier of the root cell plasmalemma. This permits the loss of ions and solutes from the root cells, and allows the passive uptake of others, for example Cu. Altered membrane permeability may occur through Cu binding to integral membrane proteins or Cu-induced lipid peroxidation (Sandmann and Böger 1980, De Vos *et al.* 1991).

The observed covariance in root Ca, Zn, Fe, and Cu concentrations is in agreement with a change of root cell plasmalemma permeability since these ions can be immobilized or compartmentalized in the cells, whereas K and Mg can leak out. The increased Cu concentration in the stem was associated with a decrease in K, Zn, Ca, Mg, and Fe concentrations, though with the exception of Ca these were not lower in the leaves, and Cu might be responsible for a displacement of these elements rather than an altered uptake. However, the Ca concentrations were inversely correlated with Cu concentrations in the leaves suggesting that Cu interfered with the uptake of Ca into the shoot. Antagonistic effects exist between Ca and heavy metals in absorption and metabolism (Kabata-Pendias and Pendias 1984, Berry and Wallace 1989). The reduced Ca levels in the *P. vulgaris* leaves may adversely affect cell

division and elongation, and be responsible for the observed reduction in leaf surface area. Runner bean plants (*Phaseolus coccineus*), treated with excess Cu at the beginning of primary leaf growth, had leaves with a reduced surface area, an increased leaf density, smaller palisade parenchyma cells, and reduced mesophyll cell volume (Maksymiec *et al.* 1995).

Physiological Cu concentrations in mature leaf tissue range from 5 to 30 mg(Cu) kg^{-1}(DM) (Kabata-Pendias and Pendias 1984). The majority of this Cu (50-80 %) is present in plastocyanin, the electron donor of PS1 (Hewitt 1983), and it is also an intrinsic component of PS2 (Arvidsson *et al.* 1993). Decreases in *P. vulgaris* leaf biomass occurred at mean total leaf Cu concentrations exceeding 62.4 mg(Cu) kg^{-1} (DM) and the highest leaf Cu concentration was 364.1 mg(Cu) kg^{-1}(DM). However, since Chl synthesis and P_N were not inhibited it would appear that Cu in the leaf was sequestered, and that Cu concentrations in the cytoplasm and chloroplast were physiological. Cu may be sequestered by binding to apoplastic ligands or metallothioneins (Lolkema *et al.* 1984) or by compartmentalisation in the cell vacuole. In rice plants, the P_N and photosynthetic capacity were inhibited when shoot Cu concentrations exceeded 21.5 mg(Cu) kg^{-1}(DM) (Lidon *et al.* 1993), though shoot biomass did not decrease until 46.5 mg(Cu) kg^{-1}(DM) (Lidon and Henriques 1993).

Cu affects the pigment content and composition of the pigment-protein complexes of PS2 (Droppa *et al.* 1984, Barón-Ayala *et al.* 1992). The observed increase in Chl content per leaf surface area in the *P. vulgaris* plants could arise if the leaf cells were smaller and contained a normal number of chloroplasts. In runner bean plants treated with Cu at an early growth stage, the leaf cells were smaller and contained numerous chloroplasts (Maksymiec *et al.* 1995). Also the antenna size or the pigment content of both photosystems might have increased (Anderson *et al.* 1988), which would tally with the decrease of the Chl *a/b* ratio in the *P. vulgaris* plants. The growth of Cu-tolerant spinach in excess Cu has also led to a slight, concomitant increase in total Chl, carotenoids, and lipoquinones content, though the Chl *a/b* ratio was unchanged (Baszyński *et al.* 1982). Yet the growth of rice in excess Cu causes chlorosis (Lidon and Henriques 1991). In runner beans treated with Cu at an early growth stage, the Chl content increased on a leaf area basis, while chlorosis was observed in plants treated with Cu at the final stage of primary leaf growth (Maksymiec *et al.* 1995). The regulatory effect of Cu on Chl synthesis seems to be dependent on the plant species, the capacity to sequester Cu, and the plant growth stage.

Chl fluorescence indicated that a slight reduction in PS2 photochemistry efficiency occurred in *P. vulgaris* plants. The F_0 is considered to represent the fluorescence emission by excited antenna Chl *a* molecules before the migration of excitons to the PS2 reaction centres (Krause and Weis 1991). Increases in F_0 arise after the destruction of reaction centres (Krause 1988) due to impedence of the electron flow from the antenna Chl *a/b* LHC to P_{680} (Krause and Weis 1991), and due to an increase in the size of the Chl *a/b* LHC (Lichtenthaler 1988, Öquist and Wass 1988). An increase in antenna size would be in agreement with the decrease of the Chl *a/b* ratio observed with increasing leaf Cu concentration in this study, but the other possibilities given above can not be ruled out. A decrease in F_v/F_m has been widely used as a measure of the physiological state of the photosynthetic apparatus after

stress, and it indicates a decrease in the efficiency of the primary photochemistry of PS2 (Bolhàr-Nordenkampf *et al.* 1989, Krause and Weis 1991). A significant change in $t_{1/2}$, which is considered to be proportional to the pool size of electron acceptors on the reducing side of PS2 (Krause and Weis 1991), was not observed in the *P. vulgaris* plants. Cu-induced changes in the lipid composition of the thylakoid membrane may affect membrane integrity (Barón-Ayala *et al.* 1992), and adversely affect the microenvironment of some electron transport components leading to changes in PS2 photochemistry efficiency. This reduction in efficiency was not sufficient to be rate limiting for *P. vulgaris* CO_2 fixation. Decreases in F_0 and F_v/F_m have also been observed in wheat growing in excess Cu, suggesting that Cu affected primarily the light reactions of photosynthesis (Lanaras *et al.* 1993), in Cu-treated rice (Lidon *et al.* 1993), and in runner bean plants treated with Cu at the final stage of primary leaf growth (Maksymiec *et al.* 1994).

Photosynthetically, *P. vulgaris* plants demonstrate a high tolerance to Cu even though plant growth is significantly inhibited. The maintenance of high P_N would provide photosynthate for cell metabolism and ATP production, to compensate for extra energy requirements, and to keep the cell electrochemically neutral. The increase in total Chl and a relative increase in Chl *b* suggest that Cu-stressed plants have a higher light-harvesting capacity. However, Cu does slightly reduce the efficiency of PS2 photochemistry.

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