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Effect of Copper on the Iron Uptake of Plants

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There are some reports on copper-caused-chlorosis in green plants. Willis and Piland [1] observed the unfavorable effect of copper on the iron content of corn and stressed the action of copper on the oxidation-reduction potential in soil. Chapman and co-workers [2] found in *Citrus* that copper in nutrient solutions sometimes causes chlorosis. They also suggested that the action of copper may be due to reactions affecting the oxidation of iron and hence the absorbability of iron by plant roots. Ødelien [3] made similar observations of copper-caused-chlorosis on oats.

On the other hand, according to Maquenne and Demoussy [4], the poisonous effect of ferrous ion can be prevented in nutrient solution with copper sulfate.

The purpose of this investigation was to examine the effect of copper on the iron uptake of peas in nutrient solutions containing ionizable or complex-bound iron and different nitrogen sources. In order to explain the facts observed on basis of oxidation-reduction conditions, potential measurements were carried out in nutrient solutions during growth.

EXPERIMENTAL

The peas were grown in sterilized nutrient solutions under daylight conditions in the spring and summer of 1948. Erlenmeyer flasks (1000 ml) were used for culture flasks except in the experiment with inoculated peas, where Woulffe flasks (3000 ml) were used. Two sterile seedlings were transferred to each of the culture flasks. In the experiment without nitrogen nutrition the nutrient solution was inoculated with 1 ml of aqueous suspension of H 7 *Rhizobium* strain on transfer of the seedlings. Experiments with nitrate and ammonium nitrogen comprised two parallel series and that with inoculated plants four parallel series.

The nutrient solutions contained per one litre of glass-distilled water the following salts in grams:

	I (with NO_3—N)	II (with NH_4—N)	III (inoculated)
$Ca(NO_3) \cdot 4H_2O$	1.0	—	—
$(NH_4)_2\ SO_4$	—	1.0	—
KH_2PO_4	0.85	0.85	0.85
K_2SO_4	0.4	—	0.4
$MgSO_4 \cdot 7H_2O$	0.3	0.3	0.3
$CaSO_4$	—	0.5	0.5

Copper was removed from concentrated stock solutions with an acid dithitzone treatment. Calcium sulphate was prepared from a copper-free calcium chloride solution by precipitating with a copper-free potassium sulphate solution. Micronutrients were used as a combination A-Z-a according to Hoagland and Broyer but without copper. 10 mg iron were supplied as ferric chloride or ferric citrate to a litre of nutrient solution. Also experiments without iron were carried out in series with nitrate and ammonium nitrogen. Copper was added as copper sulphate. The solutions were adjusted to pH 6.0 with 5 % NaOH.

Iron was estimated colorimetrically with the thiocyanate method according to Scott [5].

Two platinum electrodes were immersed in culture flasks, and daily E_h measurements were made with the Beckman potentiometer. All the E_h values reported are mean values of four electrodes in two culture flasks of parallel experiments. All the E_h values were calculated for pH 6.

RESULTS

The effect of copper addition on the dry matter of sprouts and their ash content in different series appears from the figures in Table 1.

It is seen, that increasing copper content promotes the growth of peas most in ammonium nitrogen solution with ferric citrate as the source of iron. This is in agreement with Arnon [6] who used ferric tartrate as iron source in experiments with barley. Contrarily, with ferric chloride as iron source, copper in ammonium nitrogen solution has a deleterious effect on growth. In all the solutions containing nitrate nitrogen, copper had a beneficial effect on growth. In inoculated nutrient solutions without nitrogen low copper concentrations

Table 1. Dependence of dry weight and ash content of sprouts on copper concentration in nutrient solutions of different composition.

N-source	Iron source	Addition of copper γ/l	Duration of series	Dry weight of sprouts g	Ash content %
NO_3—N	Ferric chloride	0	May 5—June 1	0.398	17.9
»	»	5	»	0.425	19.4
»	»	500	»	0.431	18.0
»	Ferric citrate	0	»	0.842	23.8
»	»	500	»	0.886	24.8
NH_4—N	Ferric chloride	0	June 29—July 20	0.213	33.0
»	»	500	»	0.126	14.5
»	Ferric citrate	0	»	0.195	24.4
»	»	500	»	0.400	22.8
Inoculated	»	0	March 24—May 10	0.640	17.0
»	»	5	»	0.700	17.0
»	»	20	»	0.668	15.9
»	»	200	»	0.608	16.4
»	»	1000	»	0.554	17.7

stimulated the growth of plants, but concentrations above approximately 20 p. p. m. seem to be harmful to plants. Copper seems to increase the ash content of plants in nitrate nutrient solutions, but strongly decreases it in solutions with ammonium nitrogen. With the exception of plants having ferric citrate and copper in nutrient solution, all the plants with ammonium sulphate as nitrogen source grew very poorly. An appreciable acidification of nutrient solution was reached, and in many cases the plants died before flowering.

The iron content of sprouts is graphically presented in Fig. 1.

In nitrate plants copper did not markedly affect iron content when iron was supplied as ferric citrate; with ferric chloride in nutrient solution copper had, however, a very marked antagonistic effect on the iron content of the sprout. In ammonium plants the iron content in ash of sprouts increased markedly on adding copper to cultures with ferric chloride iron as well as with ferric citrate iron. In inoculated plants, small amounts of copper in nutrient solution increased the iron content of sprouts, but the higher copper concentrations had the opposite effect.

The results of the measurements of the oxidation-reduction potentials in nutrient solutions are given in Figs. 2 to 4.

Examination of the curves in Fig. 2 clearly shows that in nitrate nitrogen solutions the addition of copper distinctly prevents the fall in electrode

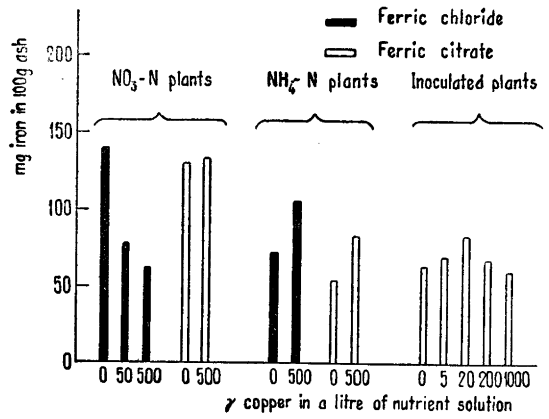

Fig. 1. Effect of copper on iron content of sprouts.

potentials during growth when ferric chlorid is used as the iron source. With ferric citrate as iron source the addition of copper has, however, no marked effect on the potential. In nutrient solutions with ammonium nitrogen (Fig. 3) the addition of copper promotes the fall of the potential in cultures with ferric chloride, later on also in cultures with ferric citrate. The potential curves in inoculated solutions (Fig. 4) are very peculiar. No definite electrode potentials are established in the culture solutions. The wide oscillations of the potential during the initial stage of the growth period dampen later on and become confused. However, a very striking conformity exsists between the potential curves of all the different series, also during the last period of the growth. Therefore, the oscillations can not be accidental only. Higher concentrations of copper have a poising effect on the oscillations of the potential. After a growth period of about three weeks iron in nutrient solution begins to precipitate as ferric hydroxide. This phenomenon is evidently caused by the utilization of citric acid. From this time on the smaller copper concentrations have a reducing effect on the potential, but, on the contrary, the higher concentrations of copper prevent the falling of potential to a low level.

A more detailed study of the reasons for the oscillations of the potentials is beyond the scope of this work. The phenomenon probably is not due to the actual variation of the average potential in the culture solution, but to the variable oxygen concentration in the immediate neighbourhood of the platinum electrode, caused by the varying activity of bacteria.

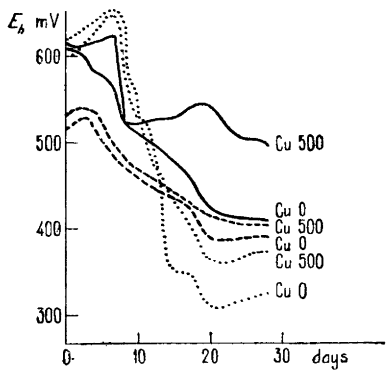

Fig. 2. Effect of copper on electrode potential in nitrate nutrient solution.

..... *Without iron.*
_____ *With ferric chloride.*
– – – *With ferric citrate.*

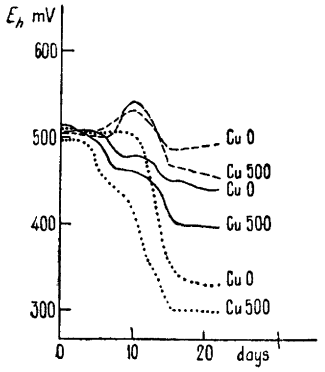

Fig. 3. Effect of copper on electrode potential in ammonium nutrient solution.

..... *Without iron.*
_____ *With ferric chloride.*
– – – *With ferric citrate.*

DISCUSSION

It has often been demonstrated that plants take up iron better in the presence of an organic non-ionized iron complex, such as citrate, tartrate, oxalate, glycerophosphate, or humic acid iron complex [7–11]. On the other hand, plants seem to absorb the ionized iron for the most part as ferrous cation. Ferric iron must be reduced before absorption, *e. g.*, by the reducing substances in soil or by the reducing action of microorganisms or the epidermis of the plant root [9, 12, 13].

In the experiments described above the iron supplied as ferric chloride always precipitated through hydrolysis as ferric hydroxide during the autoclaving of the culture solution. Considering the very little solubility product of $Fe(OH)_3$ (only 10^{-37} at pH 6.0) the actual concentration of ionized ferric iron in culture solution is negligible, and, under these conditions, cannot have any poising effect on the oxidation-reduction potential in the solution. On the contrary, the oxidation-reduction conditions determine the equilibrium $Fe^{+++} + e^- \rightleftharpoons Fe^{++}$ in the solution, and, therefore, decisively influence the ferrous iron uptake of plant.

When iron is supplied as ferric citrate, *i. e.* as a non-ionized organic complex, the oxidation-reduction conditions in nutrient solution cannot exert a decisive effect on the form of occurrence of iron, nor, on the iron uptake as long as there are enough citrate ions in the solution. According to Swenson

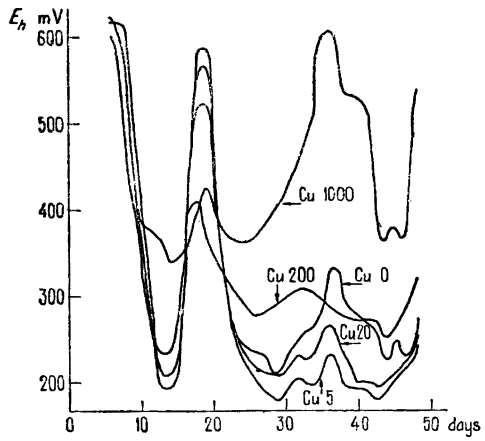

Fig. 4. Effect of copper on electrode potential in inoculated nutrient solution with ferric citrate as the iron source.

et al. [14] of several anions (citrate, mucate, fluoride, gallate, tartrate, gluconate, and salicylate) citrate is the most effective in its ability to coordinate with iron. An increase of the pH value from 3.4 to 6.3 increases the effectiveness of citrate to replace iron from phosphate more than can be attributed to the additional hydroxyl alone.

The results obtained indicate that the correlation between the effect of copper on the iron uptake and on the potential in nutrient solution is very distinct in all the experiments. Thus, the effect of copper on the iron uptake seems to be due to the altering of the oxidation-reduction conditions in the culture solution. In nitrate cultures with ferric chloride where the fall in potential is prevented by the addition of copper, and where copper thus increases the ratio Fe^{+++}/Fe^{++}, copper decreases the iron uptake. In ammonium cultures with ferric chloride as the iron source where copper, on the contrary, promotes the fall in potential, and thus decreases the ratio Fe^{+++}/Fe^{++}, it also promotes the iron uptake. In nitrate cultures with ferric citrate iron, copper has no effect on potential nor on the iron uptake. Even in ammonium cultures with ferric citrate as well as in inoculated cultures the effect of copper on the iron uptake is in correlation with the effect on the potentials. The effect of copper on potential, noticed in older cultures, is possibly caused by the uptake of citric acid from the culture solution.

Lin [15] studied the appearance of iron chlorosis in rice cultures supplying nitrogen as ammonium or nitrate, and never obtained chlorosis in ammonium cultures, but did in nitrate cultures. The experiments described above show that copper may cause iron deficiency in nitrate cultures when organic non-ionizable iron complexes capable of being absorbed are not present. The

fact that Lin was unable to prevent chlorosis by the addition of iron as tartrate can be accounted for by the more rapid decomposition of the iron tartrate complex. In preceding experiments with ferric citrate, iron did not begin to precipitate until about three weeks had elapsed.

Hence we may draw the conclusion that copper has a marked effect on the availability of iron for plants. This effect is very important considering the plant nutrition from the practical point of view.

SUMMARY

The iron uptake of peas was studied in sterile water cultures containing 10 mg Fe in a litre by using different nitrogen sources, and varying the copper concentrations. Moreover, the oxidation-reduction potentials of the culture solutions were observed.

The effect of copper on the iron uptake always was in correlation with the effect on the electrode potential in the nutrient solution.

When iron was supplied as ferric chloride and precipitated by autoclaving, copper (50 and 500 γ Cu in a litre) decreased the iron uptake and also prevented the fall in electrode potential in nitrate culture solution, but in ammonium culture solutions copper increased the iron uptake (calculated on the ash basis) and promoted the fall in potential.

When iron was supplied as ferric citrate, 500 γ of copper had no marked effect on the iron uptake of plant in nitrate culture solution, nor on the potential of the culture solution. In ammonium culture solution with ferric citrate iron, copper increased the iron uptake, and also promoted the fall in potential in nutrient solution after a growth period of about two weeks.

The experiments with inoculated peas in the presence of ferric citrate as the iron source gave wide oscillations of electrode potentials, which in different series were very well synchronized. Also now a negative corralation was observed between the final level of electrode potential and the iron content of plants.

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