EFFECT OF COBALT ON THE GROWTH OF SOYBEANS IN THE ABSENCE OF SUPPLIED NITROGEN

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Cobalt has been demonstrated to be essential for the growth of sheep (Marston and Lee 1952, Anderson and Andrews 1952), certain blue green algae (Holm-Hansen et al 1954) and apparently also is required for certain other algae and microorganisms (Hutner et al 1950, Darken 1953). In general vitamin B_{12} will satisfy the cobalt requirements of these organisms. Investigations of the possible essentiality of cobalt in the nutrition of higher plants have been meager and without appreciable success (Hewitt and Bolle-Jones 1952). Bolle-Jones and Mallikarjuneswara (1957) however have demonstrated significant increases in the growth of rubber (Hevea brasiliensis) and tomato (of unknown origin) plants with a supply of 0.005 ppm. of cobalt in purified sand cultures, but no characteristic foliar deficiency symptoms attributable to cobalt were observed. The present work was undertaken to study the effect of cobalt in small amounts on the growth of inoculated soybean plants (Glycine Max. Merr. var. Roanoke) in the absence of supplied nitrogen. Any responses observed under these conditions would reflect the combined cobalt requirements for the soybean plant and the Rhizobia growing in the symbiotic relationship.

The plants were grown in purified nutrient solutions and the effect of cobalt supplied at 1 ppb. (parts per billion) and 50 ppb. as cobaltous chloride (the 1 ppb. cobalt also was supplied in the form of vitamin B_{12}), was compared with controls in both demineralized and redistilled water to

which no cobalt was supplied. Details of the treatments are given in Table I.

Demineralized water was obtained by passing distilled water through an ion exchange (mixture of Amberlite IRA-400 and IR-120) column 32 inches long. By use of Co^{60} as a tracer it was determined that the exchange resins effectively removed cobalt ions. All the containers in the experiment were washed with 3 N hydrochloric acid and thoroughly rinsed with demineralized water. Demineralized water was used for the purification of salts and the preparation of nutrient solutions. The methods for the purification of the nutrient salts for cobalt was essentially as described by Bolle-Jones and Mallikarjuneswara (1957), excepting those for iron and calcium hydroxide, the details of which will be presented later. Iron was supplied as ferric ethylenediamine di (o-hydroxy phenyl acetic acid) (FeEDDHA). Purified calcium hydroxide was used for adjusting the pH of the solutions. Cobalt added to cultures was prepared from cobalt metal of spectrographic purity by conversion to the chloride form by use of redistilled hydrochloric acid. Vitamin B_{12} was the U.S.P. crystalline compound.

The culture solution contained the following essential macronutrients in milliequivalents and the essential micronutrient elements in ppm: K_2SO_4, 5; $MgSO_4 \cdot 7H_2O$, 4; $CaSO_4 \cdot 2H_2O$, 10; $Ca(H_2PO_4)2H_2O$, 3; and $MnSO_4 \cdot 4H_2O$, 0.15; $CuSO_4 \cdot 5H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.05; H_3BO_3, 0.1; $Na_2MoO_4 \cdot 2H_2O$, 0.02; NaCl, 0.2 and Fe•EDDHA, 1.0 ppm. every week.

The soybean seedlings were germinated between folds of acid-washed filter paper, which was kept moist with 0.1 strength nutrient solution. The germinating seedlings were inoculated with the washed Rhizobia derived from fresh nodules. As soon as the radicals were three inches long, five seedlings were transferred to each of the 10-liter polyethylene pots containing the nutrient solutions. The seedlings were supported with acid-washed Dacron wool and the pots were continuously aerated through Dacron wool filters. In order to curtail the cobalt source from the

seeds, the cotyledons were removed as soon as the primary leaves unfolded. After one week the seedlings in each pot were thinned to four uniform plants. The layout of the experiment was in two growth chambers where the light intensity was maintained at 2000 foot candles, and the temperature at 79 to 81° F. The six culture pots in each replication were randomized and arranged in one row. The two growth chambers carried two replications each. Throughout the experiment the plants were subjected to a 14 hour day. The pH of the nutrients was maintained close to neutrality by adding calcium hydroxide solution from day to day.

The experiment was conducted for 11 weeks, and during the period the tops were cut back to the same height after 5 and 8 weeks respectively, and the tops were allowed to grow again. This procedure was used to minimize the residual cobalt content of the shoots which was derived from the seeds. The fresh and dry weights of the tops from the second cutting and, that of the tops and roots in the last cutting were recorded separately. The samples are being analyzed for the various chemical components and will be included in the detailed paper to follow. The data on the dry weight of the shoots along with the observed growth symptoms for the last cutting is given in Table I.

During the experiment it was noted that the plants grown in the absence of added cobalt were slender, less vigorous and nitrogen deficiency symptoms were apparent. The plants supplied with cobalt were vigorous in growth and dark green in color. The dry weight of the shoots at both the recorded cuttings shows marked differences in weight. The increased dry weight for all the cobalt treatments excepting in the form of vitamin B_{12} was significant at 1% level. The difference between the means of control and vitamin B_{12} cultures were significant at the 5% level. The trends of the second cutting were similar. The data given in Table I is an over-all average for the two growth chambers. It was observed that the growth of plants varied considerably in the two growth chambers. In one the increase in dry weight from the addition of 1 ppb. cobalt was 81%

Table I

The Influence of Cobalt on the Appearance and Dry Weight of Soybean Shoots

Symbols	Treatments	Growth Symptoms	Mean dry wt. (gms/pot)
A & B	0-cobalt, Dem. water	Nitrogen deficiency	16.6*
C	0-cobalt Redistd. water	Nitrogen deficiency	19.6
D	1 ppb. cobalt as Vit. B_{12}	Normal, less vigorous	22.1
E	1 ppb. cobalt as $CoCl_2$	Normal, vigorous	25.3
F	50 ppb. cobalt as $CoCl_2$	Normal, vigorous	24.6

* Treatments A & B were identical and therefore were combined to get a mean of 8 replicates. The other treatment means are for four replicates. L.S.D. between means of A & B vs. any other treatment is 4.8 at 5% and 6.68 at 1%. Over-all L.S.D. between means of any treatment is 5.55 at 5% and 7.64 at 1%.

while in the other growth chamber it was only 30%. It was discovered that in the latter growth chamber the A & B controls showed slightly higher growth which very likely was due to the fact that these were being contaminated from the loose rust that had accumulated on the chamber air ducts. The other growth chamber was free of this defect. Cobalt treatments did not result in any consistent effect on the dry weight of soybean roots. In the comparison of the two types of water used (Treatments A & B vs. C), no consistent differences were noted. On the whole demineralized water was found to be slightly superior to the redistilled H_2O.

Although the details of other findings in this experiment will be published elsewhere, it may be concluded that the soybean plants grown under conditions which forced them to depend on nitrogen from the symbiotic process were distinctly benefitted from small quantities of cobalt. In view of the decreased yields and apparent nitrogen deficiency symptoms of plants grown without cobalt it is suggested that this element is probably involved in some way for the adequate fixation of nitrogen for the

leguminous plants. Detailed investigations are necessary to ascertain the specificity of the response and the quantitative requirements of Rhizobia and of leguminous plants grown with and without the symbiotic bacteria.

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