TECHNICAL PAPERS

Studies of the Molybdenum Nutrition of Plants With Radioactive Molybdenum

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Of the 15 known essential elements for the growth of higher plants, molybdenum is the most striking by reason of the small amounts needed, healthy plants having as little as 10 parts per billion of their green weight as molybdenum.

An association of molybdenum with plant life was first postulated by Ter Meulen (*11*), based on molybdenum assays of organic materials from a wide variety of sources including land and aquatic plants and samples of coal and petroleum collected from different parts of the world. A year earlier, H. Bortels (*3*) found that *Azotobacter chroococcum* required molybdenum where its growth depended upon the fixation of atmospheric nitrogen. Steinberg (*9*) demonstrated that molybdenum was essential for the growth of *Aspergillus niger*.

Direct evidence of the necessity of molybdenum for the growth of higher plants was much delayed, principally because of experimental difficulties in refining nutrient media so that they were sufficiently free from molybdenum to produce deficiency symptoms in plants, and because means of effecting chemical analyses of the small amounts of the element were not available.

An early recognition of the existence of unknown micronutrient elements required for the growth of higher plants was made by Hoagland and Snyder (*5*) in work reported with strawberries grown in culture solution. Their work showed specific responses to copper, boron, manganese, and zinc, and also to an unknown factor within a group of 11 other elements including Rb, Ba, Sr, Cd, Hg, Pb, Bi, Cr, Mo, W, and V.

Arising directly from their observations and intensive work on the zinc nutrition of plants associated with the "little leaf" or rosette disease of fruit trees, a program was undertaken to provide quantities of chemicals, water, and appropriate containers which could be used to grow plants under more rigidly controlled cultural conditions.

Based on Steinberg's (*8*) procedures of purifying nutrient media, large-scale cultural methods satisfactory for consistently producing micronutrient element deficiencies were developed and used to grow crop plants (*10*). Application of such methods established the specific role of molybdenum as an essential element for the growth of higher plants, as reported by Arnon and Stout (*2*).

The small amounts of molybdenum required by the plants, although making chemical investigations difficult, serve as an illustration of "small things that make great differences." For example, Anderson (*1*), reviewing molybdenum investigations in Australia, reports field trials showing a progressive increase in the yield of legumes from 590 to 2,022 lbs dry weight/acre with increasing applications of MoO_3 from 1/64 to 1 oz/acre. Extensive areas in the Mount Lofty Ranges of South Australia having molybdenum-deficient soils of low fertility can be made productive through fertilization provided molybdenum is also applied in amounts of this order of magnitude.

There is much of interest to the student of plant nutrition in the specific role of molybdenum in the physiology of plants. Evidence of an association of molybdenum with nitrogen metabolism exists throughout the work of investigators dealing with this subject since Bortels' original finding that molybdenum was required for the fixation of atmospheric nitrogen by *Azotobacter*. In fact, the remarkable molybdenum response of certain Australian pastures cropped with legumes is thought to be due to an increased efficiency of nitrogen fixation by symbiotic nitrogen-fixing organisms (*1*).

Molybdenum nutrition of higher plants is also apparently associated with the nitrogen cycle. Specifically, Hewitt and Jones (*4*) and Mulder (*6*) have shown that molybdenum-deficient plants do not efficiently reduce nitrate nitrogen after it has been absorbed and translocated to the upper parts of plants. Their findings have been verified in our laboratories. Molybdenum-deficient plants grown with salts further purified with coprecipitation of molybdenum with CuS have shown the effect of supplying minute amounts of molybdenum strikingly reflected in the reduction of nitrate nitrogen in the leaves of the molybdenum-deficient plants. For example, leaves of molybdenum-deficient tomato plants were found to accumulate nitrate to the extent of 12% of their total dry weight. Additions of molybdenum to deficient plants resulted not only in the formation of chorophyll, as evidenced by the chlorotic plants becoming green, but also in the reduction of nitrate from 12% to about 1% on a dry weight basis. Recovery was rapid, 48 hrs being required to bring about these changes.

Radioactive molybdenum isotopes as a means of effecting chemical measurements in submicrogram amounts. Two radioactive isotopes of molybdenum of high specific activity have been prepared for studies of the molybdenum nutrition of plants. They were produced by alpha bombardment of ZrO_2 in the University of California's 60" cyclotron and were isolated in "carrier-free" amounts by ether extraction as indicated under Table 1. The activity has been assigned to formation of Mo^{93} and Mo^{99} through the reactions $Zr^{90}(\alpha n)Mo^{93}$ and $Zr^{96}(\alpha n)Mo^{99}$.

Identity of the radioactive isotopes with molybdenum was made by adding stable molybdenum salts to the radioactive material extracted by ether and then demonstrating a constant ratio of molybdenum to radioactivity

in products of subsequent chemical separations. Two chemical methods were used. First, it was shown that the partition between ether and hydrochloric acid was quantitatively the same for stable molybdenum and the radioisotope (Table 1A); and second, a series of precipitates of $(NH_4)_3PO_4 \cdot xMoO_3$ made from an acid solution of tagged $(NH_4)_2MoO_4$ likewise showed a constant ratio of molybdenum to the radioactivity of each precipitate (Table 1B). Details of yields and methods of preparation of the radioisotopes will be reported elsewhere.

TABLE 1

CHEMICAL IDENTIFICATION OF RADIOACTIVE MOLYBDENUM SEPARATED FROM ZrO_2 IN CARRIER-FREE AMOUNTS[1]

A. Distribution of Mo* (+ carrier Mo) between 6*N* HCl and ether saturated 6*N* HCl

Ratio = Vol. of HCl / Vol. of ether	Mo extracted by ether[2] (μg)	Activity extracted by ether (cpm)	Ratio = Activity extracted / Mo extracted
2.1	1.17	405	346
3.5	0.88	306	348
6.8	0.55	190	346

B. Precipitation of Mo* (+ carrier Mo) as $(NH_4)_3PO_4 \cdot xMoO_3$

PO_4 added (mg)	Wt. of ppt (mg)	Mo in ppt[2] (mg)	Activity in ppt (cpm)	Ratio = Activity / Mo content
0.2	3.8	2.2	527	240
0.4	7.6	4.3	1020	237
0.6	11.5	6.2	1530	247
1.0	18.0	9.6	2359	246

[1] The ZrO_2 was removed from the target and dissolved in the presence of 5 mg of Cb_2O_5 as a "hold-back" carrier for columbian isotopes. Solution was effected by boiling in 12*N* H_2SO_4 down to fuming. NaCl and HCl were added, following cooling and dilution with water, to bring the (H^+) and (Cl^-) to 6*N*. The Mo was extracted with ether saturated with 6*N* HCl.

[2] Spectrophotometric determination of molybdenum by the thiocyanate-stannous chloride method (7).

Absorption of Mo^{93} and Mo^{99} by plants. Short-time absorption experiments with tagged molybdenum have been made using young tomato plants as experimental material. Tomatoes were grown in complete culture solutions. At the time of the experiment single three-week-old tomato plants ranging between 6″ and 10″ in height were transferred to one-quart jars. Oxygen was supplied during the absorption period by passing air through sintered Pyrex aerators into the culture solution.

Some of the experiments were conducted with Mo^{93} (half-life, 6.7 hrs) being the dominant radioisotope, and other experiments have been made using Mo^{99} (half-life, 67 hrs). In each case, 1 μg of molybdenum was tagged with the radioisotope and added to the culture solution as a molybdate salt for adsorption studies with plants.

Particular points of interest resulting from these experiments. (1) A complete accounting of the absorption and subsequent distribution of a single microgram of molybdenum in growing plants has been obtained using an experimental arrangement analogous to that used in absorption studies with the micronutrient elements.

TABLE 2

INFLUENCE OF VARIOUS INORGANIC IONS[1] ON THE ABSORPTION OF SUBMICROGRAM AMOUNTS OF MOLYBDENUM[2] FROM CULTURE SOLUTIONS DURING A 24-HR PERIOD[3]

Cation	NH_4^+		K^+		Ca^{++}	
Anion of salt in solution	Blades	Stems	Blades	Stems	Blades	Stems
NO_3^-	140	15	89	21	173	54
Cl^-	110	22	38	7	57	15
$H_2PO_4^-$	200	68	500	110	230	110
$SO_4^=$	21	1	5	3	26	5

Figures in the table are cpm/gm fresh weight. One μg of $M\overset{*}{o}$ is equivalent to 5,170 cpm.

[1] Salts were used at a concentration of 0.005*N* with respect to the cation.

[2] Mo was added as $\overset{*}{MoO}_4^=$ at a concentration of 1 μg of Mo/liter.

[3] Plants were kept in greenhouse; the day was sunny.

(2) Radioautographs obtained show a distinctly different type of distribution of molybdenum within the plant tissues than for other mineral nutrients, e.g. the macronutrient elements, potassium and phosphorus, or micronutrient elements such as manganese and zinc.

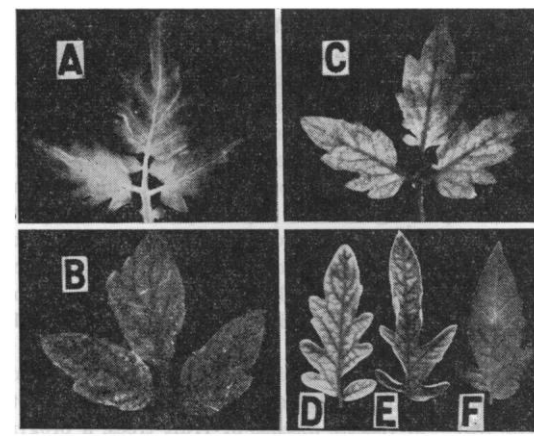

FIG. 1. A, B, C: radioautographs of K^{42}, Mo^{93}, and Mo^{99}, respectively; D, E: photographs of Mo-deficient leaflets; F: photograph of normal leaflet.

(3) Roots accumulate molybdenum rapidly from culture solutions, even though the concentration with respect to molybdenum is initially established at 1 part per billion and is reduced below that value as absorption proceeds.

(4) Translocation of these submicrogram amounts of molybdenum from roots to upper parts of the plant also takes place rapidly, but the amounts translocated are strongly influenced by the concentration of phosphate ion in the culture solution (Table 2). Many other absorption experiments completely verify this phosphate-molybdenum interrelation.

Points 1 and 2 are made evident by the radiographs in Fig. 1. Comparison of the radiograph of potassium in a tomato leaf (Fig. 1A) with the radiographs of molybdenum (B, C) shows that molybdenum is accumulated in interveinal areas in direct contrast with the potassium pattern. It is not accumulated by stem tissue to any great degree—another point of direct contrast with potassium.

It is evident, from the characteristic patterns of molybdenum accumulation, that this element, unlike other mineral nutrient elements of which we have knowledge, is not rapidly accumulated by actively metabolizing plant cells adjacent to the vascular tissue in the upper parts of the plant. Areas within the leaf where the molybdenum does accumulate are also the areas having the greatest number of stomatal openings. Accumulation could therefore result from molybdenum being left at points of greatest water loss.

In the absence of molybdenum, loss of chlorophyll takes place in the same interveinal regions. This may be seen by comparing the photographs of molybdenum-deficient leaflets (D, E) showing the chlorotic areas caused by molybdenum deficiency with the molybdenum radiographs (B, C). Accumulation of molybdenum in the chlorotic areas after it is supplied to the culture solution suggests an alternate possibility of molybdenum being tightly held in these areas by plant compounds directly involved in metabolic processes which are either peculiar to, or more active within, the interveinal areas of the leaf—for example, the reduction of nitrates referred to earlier, which could be considered as one step in the metabolic cycle.

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Molybdenum Deficiency in Serpentine Barren Soils

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The infertility and unique plant distribution patterns of serpentine barren soils recently prompted an investigation of the mineral nutrition of certain native and agricultural species grown on such soils. As a part of this study, tomato plants were grown in pot culture experiments designed to test the effect of variations in the soil calcium level. Marked responses to increased calcium were obtained, similar to those already reported for serpentine soil (*10*), but anomalous symptoms remained. The type of abnormality appeared to be identical with that characterizing molybdenum-deficient plants grown in water cultures in the Division of Plant Nutrition at this University.

The first abnormalities were yellowing and curling of the leaflets of the first or second pair of true leaves. Development of typical molybdenum-deficiency symptoms, as described by Arnon and Stout (*3*) using water cultures and Hewitt and Jones (*7*) in water and sand cultures, followed these initial signs. Pronounced mottling appeared in all true leaves, the veins remaining light green and shading into the chlorotic interveinal areas. Advanced symptoms were puffing of the chlorotic areas and marked upcurling of the leaflet margins. Finally, the tips of the leaflets and areas along the margins began to shrivel and later die. Newly formed leaves were green, but during expansion became mottled and curled.

Molybdenum deficiencies in higher plants grown on soils in Australia, New Zealand, and Central Europe have been reported (*1, 4, 5, 6*), but the writer knows of no previous observation of molybdenum deficiency on soils in the Western Hemisphere. In Australia, legumes grown on certain pasture soils showed marked response to molybdenum in both field and pot tests (*1, 2, 6, 9*), and perennial rye grass responded in pot cultures (*9*). Davies (*5*) observed characteristic molybdenum-deficiency symptoms in cauliflower growing on an acid New Zealand soil and was able to bring about rapid recovery of the plants with molybdate fertilization. Mitchell (*8*), also working in New Zealand, prevented the occurrence of the whiptail disease in cauliflower and broccoli by applications of ammonium molybdate in field plots.

In order to substantiate the symptomatic diagnosis of molybdenum deficiency in the affected tomato plants of this study, chemical analyses were made in the Division of Plant Nutrition to establish their molybdenum content. These showed that the level was less than 0.1 ppm on a dry weight basis (see Table 1), which was lower than any previous assay for molybdenum made in those laboratories on soil-grown plant material.

Molybdenum was then supplied to affected plants in order to check further the indications that they were molybdenum deficient. Direct corroborative evidence was obtained through the recovery of the affected plants after supplying them with molybdenum. Three methods were used: (1) application of small amounts of molybdate salts to the soils, (2) painting of leaflets with a dilute solution of Na_2MoO_4, and (3) direct infiltration of Na_2MoO_4 into cut leaflet tips. Also, a few treatments were made with manganese without success. Specific procedures with results obtained are outlined below.

(1) *Fertilization.* (a) Application of Na_2MoO_4 in solution to the top of the soil at the rate of 0.81 lb of Mo/acre to young mottled plants caused definite greening up in 48 hrs and complete loss of leaf mottling in 4 days. Of 30

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