The Role of Molybdenum in Plant Nutrition

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Molybdenum investigations in New Zealand to date have largely been through field and pot experimentation, the measure of effect being the yield of dry matter. In some cases plants have been analysed, less frequently with prior dissection into their several parts. We have to refer to overseas workers for the more intricate studies of the actual role of molybdenum in plant nutrition. I propose to touch in a superficial way on some of their findings.

ROLES OF MOLYBDENUM:

1. Nitrogen fixation:

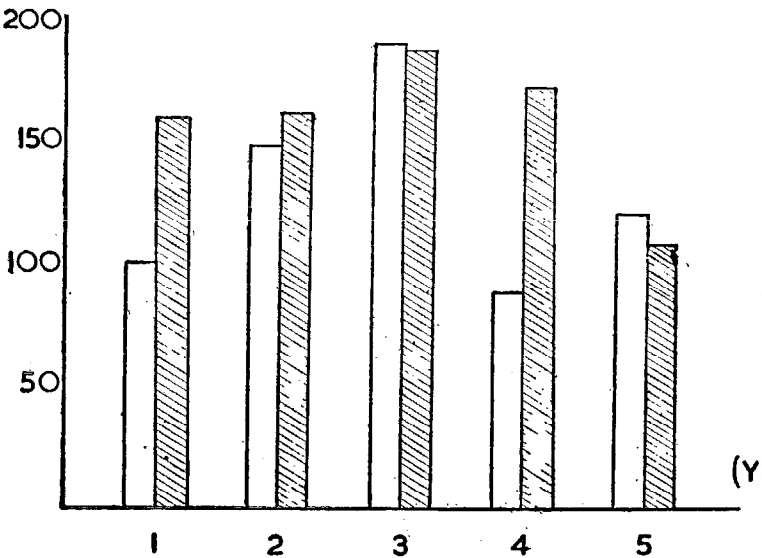
Starting with Bortels' (7) work with **Azotobacter** in 1930 a mass of evidence has been collected demonstrating that molybdenum plays an essential part in nitrogen fixation, both by free living micro-organisms and those existing in a symbiotic relationship. Bortels' results with Azotobacter were rapidly confirmed by a number of continental workers (see Mulder (23) for bibliography). Bortels (8) showed that **Clostridium pasteurianum** and Jensen and Spencer (18) that **Clostridium butyricum** likewise required molybdenum for nitrogen fixation. It has been substantiated that **Azotobacter** can use vanadium in place of molybdenum but Jensen and Spencer were unable to confirm Bortels' observation that the same position held with **Clostridium.** Bortels (10) further showed that molybdenum is necessary for nitrogen fixation by the blue green algae, **Anabaena** and **Nostoc,** including that **Anabaena** which is symbiotic with the water weed **Azolla carolinina. Anabaena azollae** can fix nitrogen only in symbiosis.

It is the part that molybdenum plays in nitrogen fixation by **Rhizobia** in symbiosis with legumes that has particularly attracted the attention of workers in agriculture. Bortels (9) noted the stimulating effect of molybdenum on legumes in the field as did the Russians Obraztzova et alia (27), while Dmitriev (14) observed red clover responses in pot cultures. Anderson (1, 2) obtained the first outstanding field responses with subterranean clover in South Australia, responses of the same order as those reported more recently in New Zealand (12, 19, 20). In conjunction with Thomas, Anderson (4) established that molybdenum was essential for symbiotic nitrogen fixation in leguminous plants and that observed responses were not due to direct molybdenum nutrition or effects through free living soil organisms. Bortels' (9) finding that vanadium also had a stimulating action on legumes has not been the experience of other workers. Anderson and Oertel (3) found no benefit to derive from vanadium sulphate in pot experiments in the presence or absence of molybdenum nor from ammonium vanadate in the field. In Otago, ammonium vanadate, likewise, was found ineffective on a mixed pasture highly responsive to sodium molybdate. Ammonium vanadate has, however, had a definite effect on mixed pastures on certain highly podzolized and molybdenum deficient soils in the far north of New Zealand. The molybdenum and vanadium responses have been additive suggesting that the elements perform different functions. The trials concerned are observational only, but limed and unlimed portions of duplicate plots on Mangonui clay and on Te Kopuru sand are in agreement. Jensen and Betty (17) in a study of the importance of molybdenum in symbiotic nitrogen fixation found a strong concen-

Mo V W TRIAL, OUTRAM

RELATIVE YIELDS GREEN MATTER —

9 CUTS — 18/11/53 — 29/12/54. SUPER = 100



TREATMENTS

1 SUPER 3 CWT, TWICE.

2 1 + 1/80Z Na_2MoO_4

3 1 + 2.5 OZ "

4 1 + 1 LB NH_4VO_3

5 SLAG 3 CWT, TWICE.

ONE BLOCK TREATED
1 LB Na_2WO_4, 3/9/53
(YIELDS SHOWN HATCHED).

tration of molybdenum in the nodules above that in the root substance. (This has been our own experience and that of others). No such concentration of vanadian occurred, suggesting that vanadium was unlikely to be acting in the same way as molybdenum. Only one nodule sample, from **Medicago arabica,** however, was examined for vanadium content. The ability of vanadium to act in place of molybdenum in nitrogen fixation by **Azotobacter** is well established; with **Clostridia** and **Rhizobia** it seems doubtful.

Some workers have reported results from tungstate. Horner et alia (16) demonstrated very clearly that traces of molybdenum occurring as impurity in the tungstate can be responsible for such effects, especially when high concentrations are used. In the molybdenum-vanadium trial of the writer at Outram, already referred to, one block was dressed in September, 1953, with 1lb./acre sodium tungstate. Responses rapidly appeared in the absence of applied molybdenum but not in the presence of 2½ozs./acre sodium molybdate. An exception was in the case of a slag plot which has consistently shown a tungsten depression. These results need confirmation in a replicated trial—the present serves merely as a pointer. Various samples of laboratory sodium tungstate have been examined spectrographically and found to contain traces of molybdenum. Chemical analyses of two samples showed 26 and 66 p.p.m. molybdenum. The sodium tungstate used in the trial contained only 0.04 p.p.m. The matter of possible substitutes for molybdenum has been mentioned as the toxic effect on stock of more than a trace of molybdenum in pasture is well known (Figure 1).

2. Reduction in nitrate:

Steinberg (32) in 1937 showed that the mould **Aspergillus** grew better when supplied with traces of molybdenum when nitrate was the nitrogen source. With ammonium supplied molybdenum made no difference He suggested that molybdenum activated the enzyme nitrate reductase which reduces nitrate as a step towards protein synthesis. Recently Nicholas and Nason (25) have shown that removal of molybdenum by electrodialysis from a cell free extract of **Neurospora crassa** containing nitrate reductase deactivated the enzyme, and that molybdenum salts specifically restored the activity. No other of a number of elements, including vanadium and tungsten, had any effect. On the other hand cell free extracts of **Neurospora** grown in the absence of molybdenum could not be activated by molybdenum addition, implying that molybdenum played a further role in the formation of the protein portion of the enzyme. In the absence of molybdenum, growth of the fungi **Neurospora** and **Aspergillus** in solutions where nitrogen was supplied solely as ammonium, was also reduced to some extent (26). Nitrate reductase was not formed. It seems that a still further function of molybdenum may be involved. The necessity of molybdenum for the activation of nitrate reductase also holds in higher plants for a number of workers have demonstrated accumulation of nitrate in interveinal tissue when molybdenum is deficient. e.g. Wilson and Waring (37). The areas concerned become chlorotic. Molybdenum application leads to a greening of the tissue and a rapid reduction in the amount of nitrate. Spencer and Wood (31) have precisely demonstrated molybdenum to be necessary in the step NO_3 to NO_2. A test of interveinal tissue for nitrate with diphenylamine reagent, where molybdenum deficiency is suspected, is useful in diagnosis. Unnodulated legumes grown with a source of fixed nitrogen require molybdenum for growth (Wilson (38), Meagher et alia (21). In clover the Mo requirement for nitrogen fixation is much greater than that for this other function.

Mulder (24) records that cauliflowers grown in molybdenum deficient soil with nitrogen supplied as NH_4OH, NH_4Cl or NH_4NO_3 developed symptoms typical of molybdenum deficient plants supplied with nitrate. The young leaves were, however, a darker green. Cupping and withering of the leaves are mentioned. In tomato plants grown in the same soil the molybdenum deficiency symptoms observ-

ed in the nitrate and ammonium treated plants respectively were practically the same. Evidently some function of molybdenum other than that of nitrate reduction is involved.

3. Denitrification by Bacteria:

Mulder (23) showed conclusively that molybdenum was essential to denitrification by denitrifying bacteria. When grown anaerobically these depend on nitrate for their oxygen supply.

4. Inhibition of tomato acid phosphatases:

Spencer (30) has demonstrated working with tomatoes that acid phosphatases are inhibited in their action both in vitro and in vivo by molybdate. Possingham (29) studied the change in molybdenum deficient tomato plants over a four day period following addition of sodium molybdate to the culture solution. Growth was increased, the concentration of organic phosphate within the plants greatly increased, and that of inorganic phosphate diminished; results in line with Spencer's finding. It would seem molybdenum is concerned in phosphorus as well as in nitrogen metabolism.

5. Hewitt et al. (15) have shown in water culture that molybdenum deficiency causes a striking reduction in the apparent ascorbic acid content of several crops. They discuss the biological significance of the finding.

This brief review serves to indicate that molybdenum plays not one but many roles in plant nutrition.

The first proof of the essentiality of molybdenum for the growth of higher plants was by Arnon and Stout (6) in 1939, growing tomatoes in water culture. Since that time the need of a great variety of plants for molybdenum has been demonstrated, both in water culture and in soil.

Toxicity.—The only detailed studies of plant injury through molybdenum applications are those of Brenchley (11) growing tomatoes, flax and **Solanum nodiflorum** in pot experiments with various soils. Applications of sodium molybdate were so heavy (the lightest equivalent to approximately 2cwt./acre) that results have little practical interest. Since plants in some soils remained healthy one might conclude they are extraordinarily tolerant to molybdate. The leaves of plants affected by molybdenum toxicity develop a characteristic golden colour shown in an investigation by Warington (35) to be due to globular yellow bodies of a tannin molybdenum complex. Mitchell (22) mentions no adverse effect on cauliflowers of 20lb./acre of ammonium molybdate applied in bands along the line of plants. Mulder (24) in pot experiments on an acid low moor peat rich in iron stone added amounts of sodium molybdate ranging up to the equivalent of 90lb./acre wtihout having any detrimental effect on yield of white clover. The content of molybdenum in the clover rose to 183 p.p.m. In one experiment with potatoes, however, applications above 0.45lb./acre caused a decrease in yield.

Despite the general picture that plants are tolerant to comparatively large applications of molybdenum there have been some unexpected depressions associated with quite low dressings. These need further study.

Interactions.

I do not propose to discuss here the complicated interactions between Fe, Mn, Mo and V studied in water culture by Warington (34, 36) but rather certain relationships perhaps of more immediate practical concern.

(1) Manganese:

Mulder (24) has shown conclusively in experiments with cauliflowers a marked Mn:Mo antagonism. The greater the amount of manganese sulphate applied to a soil, the greater is the amount of

molybdenum required to correct molybdenum deficiency. For example with no manganese addition 0.1 mg. of sodium molybdate per pot was sufficient. With 2 g of manganese sulphate applied, 5 mg. per pot of sodium molybdate became necessary—a fifty fold increase. In acid soils the amount of active manganese present may be great enough to cause manganese toxicity—a condition molybdenum application will not alleviate. Lesser amounts of Mn, not sufficient to cause toxicity may yet induce molybdenum deficiency, and here molybdenum therapy works. In the cauliflowers, Mulder states, manganese toxicity and molybdenum deficiency are somewhat similar, but in the former the margin only of the leaves cup inwards and the leaves stay dark green. With molybdenum deficiency a chlorotic interveinal mottling occurs. It is probably this sensitivity of cauliflowers to manganese that makes such large molybdate applications necessary for control of whiptail.

Mulder was not able to reproduce the manganese sulphate effect on molybdenum uptake in culture solutions. Moreover responses of white clover, spinach and tomato to molybdenum, were practically unaffected by manganese sulphate additions. With cauliflowers he demonstrated that both the manganese and the sulphate of manganese sulphate contributed towards diminishing the effectiveness of applied molybdenum.

(2) **Sulphate.**

Stout et al. (32) followed uptake of molybdenum by tomatoes from culture solutions over a 24 hour period using radio active molybdenum (Mo93 and Mo99). They showed that sulphate checked transfer of molybdenum from the roots to the upper parts of the plants. The depressing effect of sulphate on molybdenum uptake as far as the tops were concerned noted in this delicate laboratory experiment was fully borne out by results of pot experiments on molybdenum uptake of tomatoes and peas as affected by gypsum—a finding out of line with Mulder's experience mentioned above.

In an experiment of the Extension Division of the N.Z. Department of Agriculture, on a marine reclamation at Ahuriri, where pasture molybdenum was approaching a toxic level, sulphur (5 cwt.) and gypsum (1 ton) caused highly significant reductions in molybdenum uptake. Three months after application the gypsum had caused a drop from 10.0 p.p.m. Mo in the herbage to 2.2 p.p.m.

Effects of various treatments on reduction of molybdenum uptake by pasture on a marine reclamation. (Applied 14th June, 1954.)

Treatment	Molybdenum content of herbage (p.p.m.) 6th Sept. 1954 Mo.	Diff. from Control	6th Oct., 1954 Mo.	Diff. from Control
1. Control	10.0	—	5.77	—
2. 1cwt. sulphur	8.67	—1.33	4.77	—1.00
3. 5cwt. sulphur	3.70	—6.30**	1.87	—3.90*
4. 20lb. copper sulphate	7.80	—2.20	5.50	—0.27
5. 3cwt. double super	9.93	—0.07	3.50	—2.27
6. 1cwt. sulphate of ammonia (2 monthly)	8.77	—1.23	3.63	—2.14
7. 3cwt. sulphate of ammonia (2 monthly)	7.27	—2.73*	2.67	—3.10*
8. Gypsum 1 ton	2.17	—7.83**	1.67	—4.10*
Significant differences 5%*	2.55		2.31	
1%**	3.52		N.S.	
S.E. as % M.P.Y.	19.7%		35.3%	

Analyses—J. L. Grigg
Field work—A. J. Coughlan.

3. Phosphate:

In Stout's experiment with radio active molybdenum the ion H_2PO_4 was shown to increase molybdenum uptake. In the same paper he reports very striking increases in molybdenum uptake by subterranean clover caused by phosphate applications, especially in molybdenum treated soil. Molybdate was applied at 1lb. Mo/acre and phosphate as monocalcic phosphate equivalent to approximately 1 ton per acre of superphosphate. Mulder (23) also has shown an effect of phosphate on molybdenum uptake and suggests that in his experiments part of the effect of phosphate in the absence of applied molybdenum has been to make the soil molybdenum more available. Molybdate application, in fact, reduced the amount of phosphate required for maximum growth. In a field at Outram superphosphate gave no response in absence of molybdate, and molybdate no response without applied phosphate. In the Ahuriri lagoon trial already mentioned 3cwt./acre of double superphosphate had no significant effect on molybdenum uptake. Soil phosphate was already high.

(4) Effect of liming:

It has been demonstrated beyond all doubt both in New Zealand and elsewhere that liming increases uptake of molybdenum by plants and that frequently heavy liming produces responses equal to those accruing from applications of molybdenum salts. Liming increases molybdenum uptake particularly when molybdate has been applied. In view of Stout's finding (33) that in water culture Mo uptake was greater in acid solutions (pH about 4) than in solutions near neutrality, it seems that the influence of lime must be indirectly through the soil, for example through reduction of the level of active manganese, or to the $-OH$ ion concentration making molybdate more labile. Piper and Beckwith (28) working with two Australian soils have shown dramatic rises in molybdenum content of **Medicago denticulata** through lifting the soil pH from about 6 to 8.3. Increases in **Erodium cygnorum** and **Hordeum leporinum** were much less pronounced. Lowering the pH through sulphur application decreased molybdenum uptake, no doubt through effect of the $-SO_4$ ion as well as pH.

These interactions are of practical importance. The superiority of basic slag above superphosphate in certain areas may be due not only to the content of molybdenum and probably vanadium but also to a phosphate influence on molybdenum availability not counterbalanced by a sulphate antagonism. The effect of lime on molybdenum availability needs to be watched. As an example molybdenum levels in herbage toxic to stock might result from application of molybdenum to a heavily limed area of a normally molybdenum deficient soil. Further the subsequent liming of soils previously treated over liberally with molybdenum with no ill effect could give rise to similar trouble. The necessity of lime for nodulation of clover can be met by the application of a few cwt. per acre (5). In the absence of manganese or aluminium toxicity, such small applications of lime in conjunction with molybdenum treatment may replace the massive lime applications previously found necessary for a vigorous clover sward. That molybdate must be used with circumspection will be made plain by Dr. Cunningham in the next paper.

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