MOLYBDENUM IN NITROGEN METABOLISM OF LEGUMES AND NON-LEGUMES

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Summary

A study was made of the growth and nitrogen metabolism of plants in relation to molybdenum supply. Four soils low in available molybdenum were used in pot cultures for this work.

Subterranean clover (*Trifolium subterraneum* L.) responded to molybdenum on each of the soils. Flax (*Linum usitatissimum* L.) responded to molybdenum on only one of the soils in the presence of a heavy dressing of manganese sulphate, which reduced the uptake of molybdenum and induced molybdenum deficiency. Oats (*Avena sativa* L.) did not respond to molybdenum on any of the soils, even where manganese sulphate was added.

Deficiency of molybdenum decreased the percentage protein nitrogen in the flax and in the clover, but in different ways. Molybdenum increased the yield of flax only where combined nitrogen, either as nitric acid or ammonium sulphate, was provided. High nitrate accumulation occurred in the flax provided with nitric acid but without molybdenum. The percentage total nitrogen in flax was unaffected by molybdenum, but deficiency of molybdenum decreased the percentage protein nitrogen and increased the percentage non-protein nitrogen. Thus molybdenum was needed by the non-legume for the utilization of absorbed nitrate nitrogen.

Molybdenum markedly increased both the yield and percentage nitrogen of clover where no combined nitrogen was provided, but had little or no effect where nitrogen was supplied as nitric acid or ammonium sulphate. This was despite the fact that each of these nitrogen compounds decreased the uptake of molybdenum by the clover. Deficiency of molybdenum decreased both the percentage protein nitrogen and the percentage non-protein nitrogen in the clover. The evidence with clover shows that molybdenum is directly concerned in symbiotic nitrogen fixation, and that the molybdenum requirement for optimum symbiotic nitrogen fixation is appreciably greater than the requirement for optimum utilization of nitrate nitrogen.

I. INTRODUCTION

In an earlier paper (Anderson and Thomas 1946) it was shown that symbiotic nitrogen fixation in legumes is seriously inhibited by molybdenum deficiency. The legumes were well nodulated but were stunted and contained a low percentage of nitrogen. Molybdenum-deficient clover responded normally to applications of combined nitrogen compounds, and when combined nitrogen was thus supplied there was no response to molybdenum. When ammonium sulphate was used as a source of nitrogen, it reduced the pH of the soil and

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greatly reduced the uptake of molybdenum, but even under these conditions no response to molybdenum occurred. The results indicated that molybdenum is directly concerned in nitrogen fixation.

Nitrogen applied as sodium nitrate increased the growth of the legumes as did molybdenum or ammonium sulphate. However, sodium nitrate increased the pH of the soil and increased the uptake of molybdenum, and it is therefore possible that molybdenum released from the soil contributed in part to the effect of this nitrogen compound.

Evidence obtained with the microorganisms *Aspergillus* and *Azotobacter* indicates that more molybdenum is needed for the utilization of nitrate than of ammonium nitrogen (Steinberg 1937, 1939; Bortels 1936; Mulder 1948). The present experiments were carried out to examine the effect of nitrate nitrogen on the response of plants to molybdenum, and to compare the effects of molybdenum on the nitrogen metabolism of legumes and non-legumes.

II. Experimental

(*a*) *General*

Plants were grown in an unheated glasshouse on soils from the Southern Tablelands of New South Wales, previously shown to be deficient in molybdenum (Anderson 1948). The Bacchus Marsh strain of *Trifolium subterraneum* L. was the legume used. The non-legumes used were flax (*Linum usitatissimum* L.) and Algerian oats (*Avena sativa* L.). The clover seed was inoculated with an effective strain of *Rhizobium* immediately before sowing. Ten plants were grown in each glazed earthenware pot; five plants in each glass pot in Experiment 10; and fifteen plants in each enamel pot in Experiment 11.

All plants were harvested before flowering or when the first few flowers had formed. The plant material was dried rapidly under forced draught at 50°C.

Nutrients other than nitrogen were applied in solution at the beginning of each trial. Nitrogen fertilizers were applied in small successive doses, the nitric acid in solutions containing up to 0.2 per cent. nitric acid. The dressings applied are expressed as cwt. per acre based on the surface area of the pot, and the equivalents in milligrams per pot are given for each treatment. Randomization of treatments was adopted throughout.

Weights of oven-dry soil in each pot are given in the details of particular experiments. The pots were weighed frequently and the soils watered to field capacity. The soils used were all of more acid reaction than pH 5.5. They are identified in the text as:

Soil A.—a grey-brown loam from the Dickson Experiment Station, C.S.I.R.O., Canberra.

Soil B.—a light, yellow-grey, sandy loam of sedimentary origin, from Kingsdale, near Goulburn, N.S.W.

Soil C.—a brown to reddish brown loam of basaltic origin from Roslyn, N.S.W.

Soil D.—a grey to light grey, loamy, coarse sand of granitic origin from Carrick, N.S.W.

The transformation values used in the statistical analysis of the data to secure uniformity of variance within treatments were the square root transformation for dry matter (Tables 8 and 9), log transformations of the form $A + \log (x + B)$ for the data on nodulation, and log transformations of the form $A + \log x$ for the data on chemical analysis. The constant A has been introduced into these transformations primarily to avoid negative values.

(*b*) *Details of Experiments*

Experiment 1.—To test the effect of molybdenum on subterranean clover, at different levels of application of ammonium sulphate and manganese sulphate. Soil *A*. Sown on June 3, 1948, and harvested on October 7. A $2 \times 2 \times 2$ factorial combination of the following fertilizers was used:

Sodium molybdate — nil or 1 lb. per acre ($\equiv$ 1.21 mg. per pot).

Ammonium sulphate — nil or 7 cwt. per acre ($\equiv$ 952 mg. per pot).

Manganese sulphate — nil or 5 cwt. per acre ($\equiv$ 680 mg. per pot).

Replications: 2. Soil: 1.51 Kg. of soil *A* in glazed earthenware pots, with 3 cwt. of monocalcium phosphate per acre on all pots.

Experiment 2.—Details as for Experiment 1. Soil *B*. (1.48 Kg. soil per pot —harvested on September 21).

Experiment 3.—Details as for Experiment 1. Soil *C*. (1.22 Kg. soil per pot).

Experiment 4.—To test the effect of molybdenum on flax, at different levels of application of ammonium sulphate, nitric acid, and manganese sulphate. Soil *B*. Sown on June 3, 1948, and harvested on October 21. A $2 \times 3 \times 2$ factorial combination of the following fertilizers was used:

Sodium molybdate — nil or 1 lb. per acre ($\equiv$ 1.21 mg. per pot).

Nitrogen treatments — nil, 6.25 cwt. ammonium sulphate per acre ($\equiv$ 180 mg. N per pot), or 180 mg. N per pot applied as nitric acid.

Managanse sulphate — nil or 5 cwt. per acre ($\equiv$ 680 mg. per pot).

Replications: 2. Soil: 1.47 Kg. of soil *B* in glazed earthenware pots, with 2 cwt. of monocalcium phosphate per acre on all pots.

Experiment 5.—Details as for Experiment 4, on oats.

Experiment 6.—To test the effect of molybdenum, at different levels of manganese sulphate and potassium sulphate, on oats on soil *A* and on flax on soil *C*. Sown on June 3, 1948, and harvested on October 21. A $2 \times 2 \times 2$ factorial combination of the following fertilizers was used:

Sodium molybdate — nil or 1 lb. per acre ($\equiv$ 1.21 mg. per pot).

Manganese sulphate – nil or 5 cwt. per acre (≡ 680 mg. per pot).

Potassium sulphate – nil or 1 cwt. per acre (≡ 136 mg. per pot) applied 20 days before harvest.

Replications: 1. Soil: 1.51 Kg. of soil *A*, or 1.22 Kg. of soil *C* in glazed earthenware pots, with 2 cwt. of monocalcium phosphate per acre + 180 mg. N per pot applied as nitric acid.

Experiment 7.—Details as for Experiment 6, but with flax on soil *A* and oats on soil *C*, and using 1 cwt. of manganese chloride per acre where potassium was used in Experiment 6.

Experiment 8.—To test the effect of molybdenum on oats, at different levels of application of manganese sulphate. Soil *D*. Sown on June 3, 1948, and harvested on October 21. A 2 × 2 factorial combination of the following fertilizers was used:

Sodium molybdate – nil or 1 lb. per acre (≡ 1.21 mg. per pot).

Manganese sulphate – nil or 5 cwt. per acre (≡ 680 mg. per pot).

Replications: 2. Soil: 1.73 Kg. of soil *D* in glazed earthenware pots, with 2 cwt. of monocalcium phosphate per acre + 2 cwt. of potassium sulphate per acre + 180 mg. N per pot applied as nitric acid.

Experiment 9.—To test the effect of molybdenum on subterranean clover, at different levels of application of nitric acid and manganese sulphate. Soil *D*. Sown on June 3, 1948, and harvested on October 7. A 4 × 2 × 2 factorial combination of the following fertilizers was used:

Sodium molybdate – nil, 1/16 oz. per acre (≡ 4.7 μg. per pot), 1 oz. per acre (≡ 76 μg. per pot), or 1 lb. per acre (≡ 1215 μg. per pot).

Nitric acid – nil or 159 mg. N per pot.

Manganese sulphate – nil or 5 cwt. per acre (≡ 680 mg. per pot).

Replications: 2. Soil: 1.73 Kg. of soil *D* in glazed earthenware pots, with 3 cwt. of monocalcium phosphate per acre + 2 cwt. of potassium sulphate per acre on all pots.

Experiment 10.—To test the effects of molybdenum, ammonium sulphate, and nitric acid on subterranean clover at different stages of growth. Soil *B*. Sown on June 29, 1948. A 2 × 2 factorial combination of the following fertilizers was used for the first harvest on September 1:

Sodium molybdate – nil or 2 oz. per acre (≡ 75 μg. per pot).

Ammonium sulphate – nil or dressings of 0.75 cwt. per acre (≡ 10 mg. N per pot) on July 30 and August 6, then 20 mg. N per pot on August 13 and 24.

The above four fertilizer treatments were also used on pots for the second harvest on September 13 with an additional dressing of 20 mg. N per pot as ammonium sulphate applied on September 3; and on pots for the third harvest

on October 11, with additional dressings of 20 mg. N per pot as ammonium sulphate on September 3 and on October 10.

The design for the third harvest also included a 2 × 2 factorial combination of:

Nitric acid – nil, or 10 mg. N per pot applied on July 30, August 6, 13, 20, and 27, September 3, 9, 16, and 24, and October 1 and 8.

Sodium molybdate – nil, or 2 oz. per acre (≡ 75 μg. per pot).

Replications: 2. Soil: 0.80 Kg. of soil *B* in glass pots, with 2 cwt. of monocalcium phosphate per acre on all pots.

Experiment 11.—To test the effects of molybdenum and nitric acid on subterranean clover and oats. Soils *A* and *B*. Sown on July 2, 1948, and harvested on November 1. A 2 × 2 × 2 × 2 factorial combination as follows was used:

Sodium molybdate – nil or 4 oz. per acre (≡ 696 μg. per pot).

Nitric acid – nil or 330 mg. N per pot).

Two species – subterranean clover in one set of pots; oats in the other.

Two soils – one set using soil *A*; the other using soil *B*.

Replications: 2. Soil: 4.40 Kg. of soils *A* and *B* in enamel pots, with 2 cwt. of monocalcium phosphate per acre on all pots.

Experiment 12.—To test the effect of molybdenum on subterranean clover, at different levels of application of nitric acid and sodium sulphate. Soil *B*. Sown on June 14, 1949, and harvested on October 13. A 2 × 2 × 2 factorial combination of the following fertilizers was used:

Sodium molybdate – nil or 2 oz. per acre (≡ 0.152 mg. per pot).

Two levels of nitric acid – nil or 14.4 mg. N per pot on July 13, 22, and 28, August 5 and 11, and September 1, 8, and 15, then 28.8 mg. N per pot on September 22 and 29.

Sodium sulphate—nil or 1 cwt. per acre (≡ 136 mg. per pot).

Replications: 4. Soil: 1.64 Kg. of soil *B* in glazed earthenware pots, with 2 cwt. of monocalcium phosphate per acre + 2 cwt. of potassium chloride per acre.

(*c*) *Analytical Methods*

Plant tops which were dried rapidly at 50°C. immediately after harvest, were then finely ground, further dried at 101°C., and cooled in a desiccator prior to chemical analysis.

Total nitrogen was determined on 0.2 g. samples by the Kjeldahl procedure using selenium as a catalyst. The final distillation step was carried out in a Parnas-Wagner steam distillation unit using a 2 per cent. boric acid solution to trap the ammonia as described by Ma and Zuazaga (1942).

Protein nitrogen.—The protein was rendered insoluble by treatment with boiling alcohol, and non-protein nitrogen constituents were extracted in alcohol and in water by the following method: Samples of 0.2-0.4 g. are extracted twice with 25 ml. aliquots of redistilled ethyl alcohol and twice with 25 ml. aliquots of water on a boiling water bath. Each extraction is carried on for 30 minutes. The supernatant after each extraction is filtered through a filter paper disc. The plant residue plus the filter paper disc are washed into a Kjeldahl flask and nitrogen estimated as described for total nitrogen.

Non-protein nitrogen was calculated by difference between the total nitrogen and protein nitrogen fractions.

Molybdenum was estimated according to the method of Dick and Bingley (1947) in which a coloured molybdenum thiocyanate complex is developed, extracted into isoamyl alcohol, and the percentage transmission at 465 μ measured in a Coleman Universal spectrophotometer and compared with standard solutions.

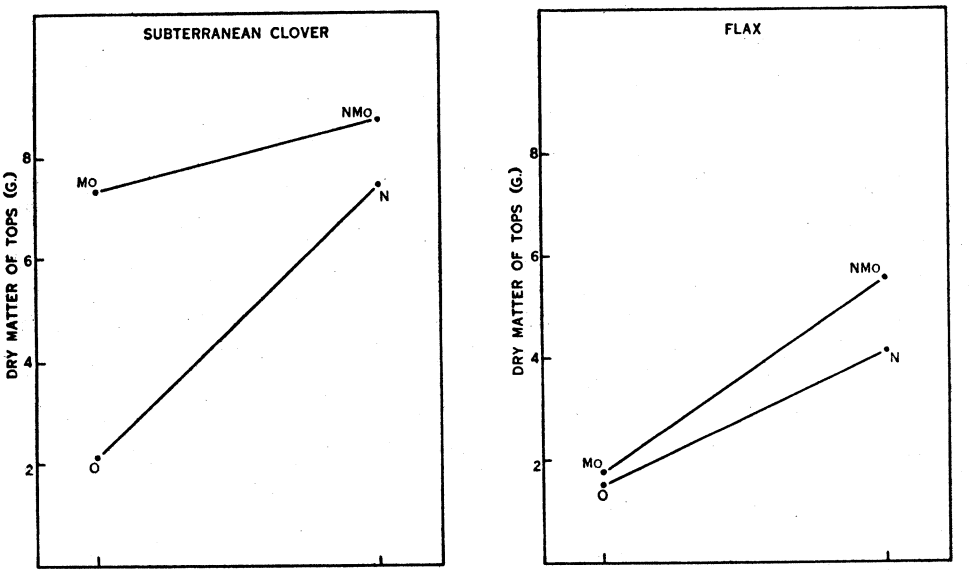

Fig. 1.—Illustrating the contrast in the interaction of nitrogen and molybdenum on a legume (subterranean clover, Experiment 12) and a non-legume (flax, Experiment 4). Soil *B*.

III. Results

(*a*) *Effect of Molybdenum on Symbiotic Nitrogen Fixation*

Molybdenum-deficient subterranean clover was restricted in growth (Tables 1 and 2), and typically nitrogen-deficient in appearance (Plate 1). The percentage nitrogen in the molybdenum-deficient clover was invariably low (Tables 2, 7, and 8, and Fig. 2). Symbiotic nitrogen fixation was therefore inhibited by the low molybdenum supply. The application of molybdenum to the soil improved the colour and increased the percentage nitrogen and growth of these plants.

Non-legumes on the same soils responded markedly to combined nitrogen (Tables 1 and 5, Fig. 1), but the nitrogen-deficient, untreated plants did not respond to molybdenum. This indicates that the increase in the nitrogen content of the clover resulting from the application of molybdenum was due entirely to an increase in symbiotic nitrogen fixation.

TABLE 1

EFFECTS OF NITRIC ACID AND SODIUM MOLYBDATE ON YIELDS OF SUBTERRANEAN CLOVER AND ON OATS; EXPERIMENT 11

(G. dry matter per pot)

	Subterranean Clover				Oats			
	Nil	Na_2MoO_4	HNO_3	HNO_3 + Na_2MoO_4	Nil	Na_2MoO_4	HNO_3	HNO_3 + Na_2MoO_4
Soil *A*	14.50	17.75	20.50	19.50	2.05	1.90	12.75	13.40
Soil *B*	7.15	10.30	13.15	12.80	4.05	3.85	12.50	11.20
Sig. diff. for $P < 0.05$			2.82				1.81	
Sig. diff. for $P < 0.01$			3.86				2.50	

(*b*) *Yield Interactions*

Clover deficient in molybdenum responded in colour and growth to nitrogen applied either as nitric acid or as ammonium sulphate (Tables 1 and 2, Figs. 1 and 2, Plates 1 and 2). Where adequate combined nitrogen was applied the clover plants did not respond to molybdenum and in all cases the response of clover to molybdenum was greatest where no combined nitrogen was added. The interaction was therefore negative.

TABLE 2

EFFECTS OF NITRIC ACID AND SODIUM MOLYBDATE ON THE YIELD AND NITROGEN CONTENT OF SUBTERRANEAN CLOVER; EXPERIMENT 9, SOIL *D* TREATED WITH MANGANESE SULPHATE

(Mean transformation values in italics)

Treatments	Dry Matter per pot (g.)	Total Nitrogen (%)		Total Nitrogen (mg.)	
No N or Mo	2.8	2.00	*0.299*	56	*1.735*
Nitric acid	6.3	2.38	*0.377*	150	*2.176*
Sodium molybdate	7.4	3.21	*0.506*	237	*2.375*
Sodium molybdate + nitric acid	7.8	3.04	*0.482*	237	*2.377*
Sig. diff. for $P < 0.05$	1.7		*0.079*		*0.263*
Sig. diff. for $P < 0.01$	2.3		*0.114*		*0.436*

The nitric acid used contained less than 0.01 p.p.m. of molybdenum. The maximum amount of nitric acid added therefore supplied less than 0.01 μg. molybdenum per pot. The nitric acid treatment decreased the concentration and amount of molybdenum in the plants (Table 3), probably by its influence

TABLE 3

EFFECTS OF NITRIC ACID AND MANGANESE SULPHATE ON THE UPTAKE OF MOLYBDENUM

(Mean transformation values in italics)

	Subterranean Clover Soil *D* Treated with 482 μg. Mo (Experiment 9)		Subterranean Clover Soil Treated with 276 μg. Mo (Experiment 11)		Subterranean Clover Soil *B* (Experiment 12)		Flax Soil *B* Treated with 482 μg. Mo (Experiment 4)	
	Without $MnSO_4$	With $MnSO_4$	Soil *A*	Soil *B*	Without Mo	With 60 μg. Mo	Without $MnSO_4$	With $MnSO_4$
Mo per Kg. (mg.)								
No N	7.52	3.09	7.24	17.79	0.17	1.48		
	1.874	*1.476*	*1.859*	*2.250*	*0.229*	*1.169*		
Nitric acid	1.89	0.73	2.17	9.44	0.11	1.15	4.46	0.72
	1.274	*0.865*	*1.336*	*1.975*	*0.039*	*1.061*	*1.647*	*0.857*
Mo in tops (μg.)								
No N	74	23	128	183	0.38	7.38		
	1.870	*1.340*	*3.107*	*3.261*	*0.579*	*1.864*		
Nitric acid	21	6	42	121	0.56	6.23	15	4
	1.326	*0.738*	*2.626*	*3.081*	*0.749*	*1.794*	*2.179*	*1.601*
Mo per Kg. (mg.)								
Sig. diff. for $P < 0.05$	*0.260*		*0.131*		*0.117*		*0.226*	
Sig. diff. for $P < 0.01$	*0.432*		*0.199*		*0.194*		*0.415*	
Mo in tops (μg.)								
Sig. diff. for $P < 0.05$	*0.321*		*0.199*		*0.158*		*0.394*	
Sig. diff. for $P < 0.01$	*0.533*		*0.301*		*0.262*		*0.723*	

on soil pH and molybdenum availability. The pH of the soil in the pots at harvest was not significantly influenced by the nitric acid (Table 4), but increase in acidity would be expected to have occurred, particularly prior to the absorption of the nitrate by the plants.

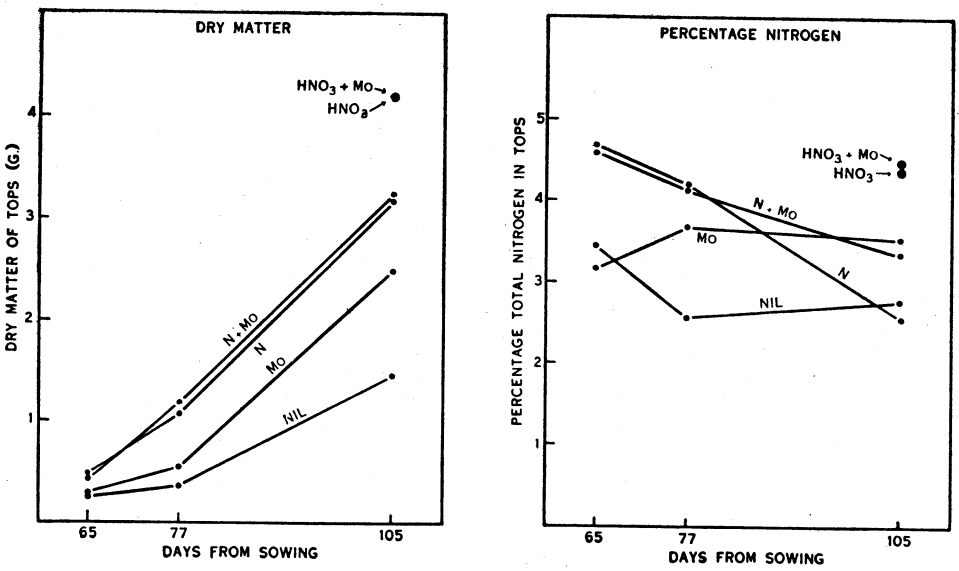

Fig. 2.—Effects and interaction of nitrogen and molybdenum on the yield and percentage nitrogen of subterranean clover at three harvests (Experiment 10). The nitrogen was applied as sulphate of ammonia in the main trial. The values obtained where nitric acid was used are also given.

The results obtained with nitric acid show that, on the soils studied, clover was deficient in molybdenum for symbiotic nitrogen fixation, but not for the

TABLE 4

EFFECTS OF MANGANESE SULPHATE AND NITRIC ACID ON MOLYBDENUM-DEFICIENT SUBTERRANEAN CLOVER; EXPERIMENT 9, SOIL *D*

	Nil	Nitric Acid	Manganese Sulphate	Manganese Sulphate + Nitric Acid	Manganese Sulphate + Sodium Molybdate	Manganese Sulphate + + Sodium Molybdate + + Nitric Acid	Sig. Diff. for $P < 0.05$	Sig. Diff. for $P < 0.01$
Dry matter (g.)	5.8	7.7	2.7	6.5	7.1	7.9	1.20	1.66
Final pH of the soil	5.32	5.34	5.21	5.28	5.21	5.27	0.31	0.43
Relative estimated weight of nodules	48	47	47	45	47	34	7	10

utilization of the added nitrate nitrogen. Even a substantial reduction in molybdenum uptake did not inhibit nitrate utilization.

Manganese sulphate reduced the uptake of molybdenum by subterranean clover (Table 3), and accentuated nitrogen deficiency (Table 4 and Plate 1, Figs. 1 and 2). It also reduced the uptake of molybdenum by flax (Table 3), and in one of the trials with soil *B* induced molybdenum deficiency in flax provided with ammonium sulphate (Table 5). In the same trial, where nitric acid was used, manganese sulphate also provided the additional sulphur needed for the normal growth of flax, and induced molybdenum deficiency (Tables 5 and 6). In the presence of manganese sulphate on this soil, flax responded to molybdenum only where nitrogen was provided. The interaction was positive and significant (Fig. 1).

TABLE 5

EFFECT OF MANGANESE SULPHATE ON THE RESPONSE OF FLAX AND OATS TO SODIUM MOLYBDATE WHEN GROWN WITHOUT NITROGEN AND WITH AMMONIUM SULPHATE AND NITRIC ACID; EXPERIMENTS 4 AND 5, SOIL *B*

Treatments	Dry Matter of Flax (g.)		Dry Matter of Oats (g.)	
	Without Manganese Sulphate	With Manganese Sulphate	Without Manganese Sulphate	With Manganese Sulphate
No Mo, S, or N	1.55	1.50	2.00	2.95
Sodium molybdate	1.45	1.75	2.05	3.00
Ammonium sulphate	4.75	3.70	9.80	9.45
Ammonium sulphate + sodium molybdate	4.85	5.00	9.50	9.75
Nitric acid	3.05	4.10	6.20	10.40
Nitric acid + sodium molybdate	3.40	5.55	5.50	10.65
Sig. diff. for $P < 0.05$	0.83		0.92	
Sig. diff. for $P < 0.01$	1.17		1.29	

The results with flax therefore show a striking contrast to those obtained with clover. Flax required treatment with both nitrogen and molybdenum for normal growth, while either molybdenum or nitrogen promoted normal growth of clover.

(*c*) *Effect of Molybdenum on the Nitrogen Fractions in Plants*

Molybdenum increased both the percentage and total amount of nitrogen in the clover plants (Tables 2, 7, and 8), and increased the percentage protein nitrogen (Table 8). The percentage of non-protein nitrogen was also increased by the molybdenum (Tables 7 and 8). There was therefore no evidence that the added molybdenum was needed by the clover for the conversion of the non-protein to protein nitrogen. The results are consistent only with the hypothesis that molybdenum increased the nitrogen supply by increasing symbiotic nitrogen fixation.

On pots treated with nitrate nitrogen (Table 8), the addition of molybdenum increased the percentage protein nitrogen in flax (main effect significant at $P < 0.01$). Molybdenum did not increase the percentage total nitrogen. The yield and total amount of protein nitrogen was increased, but the

total amount of non-protein nitrogen was unaffected by molybdenum. Tests with diphenylamine showed high nitrate accumulation in the molybdenum-deficient flax treated with manganese sulphate and nitric acid, but not in the molybdenum-treated plants. This confirmed the recorded decrease in percentage non-protein nitrogen (Table 8). In flax, therefore, molybdenum was needed for the efficient conversion of non-protein to protein nitrogen.

TABLE 6

EFFECT OF MOLYBDENUM ON THE YIELD OF FLAX, OATS, AND SUBTERRANEAN CLOVER, ON DIFFERENT SOILS TREATED WITH 5 CWT. MANGANESE SULPHATE PER ACRE (NON-LEGUMES PROVIDED ALSO WITH NITRIC ACID; EXPERIMENTS 1-9)

Species	Soil *A*		Soil *B*		Soil *C*		Soil *D*	
	Nil	Na_2MoO_4	Nil	Na_2MoO_4	Nil	Na_2MoO_4	Nil	Na_2MoO_4
Subterranean clover (g.)	7.7	14.1*	2.1	4.0*	11.9	14.8*	2.8	7.4*
Flax (g.)	6.6	6.8	4.1	5.5*	10.5	9.7		
Oats (g).	12.3	12.7	10.4	10.6	12.8	12.9	11.2	11.4

* Increase due to molybdenum significant at $P < 0.01$.

(*d*) *Progressive Effects of Molybdenum and Nitrogen on Legumes*

Treatment of clover seedlings with combined nitrogen increased the percentage nitrogen and improved the colour and growth before any active symbiotic nitrogen fixation occurred. Molybdenum was without effect at this early stage (Fig. 2).

TABLE 7

EFFECTS OF SODIUM MOLYBDATE AND NITRIC ACID ON THE YIELD, PROTEIN NITROGEN, AND TOTAL NITROGEN OF SUBTERRANEAN CLOVER; EXPERIMENT 10, SOIL *B*, HARVEST 3

Treatments	Dry Matter (g.)	Total Nitrogen (%)	Total Nitrogen (mg.)	Protein Nitrogen (%)	Protein Nitrogen (mg.)	Non-Protein Nitrogen (%)	Non-Protein Nitrogen (mg.)	Number of Nodules
No Mo or N	1.54	2.50	38.5	1.92	29.6	0.58	8.9	364
Sodium molybdate	2.40	3.07	73.6	2.01	48.2	1.05	25.4	83
Nitric acid	4.18	3.73	154.8	2.53	105.8	1.19	49.0	1
Nitric acid + sodium molybdate	4.19	3.92	164.3	2.62	109.7	1.30	54.6	1
Sig. diff. for $P < 0.05$	0.42	0.68	14.1	0.25	9.4	0.52	16.1	
Sig. diff. for $P < 0.01$	0.58	1.03	21.4	0.38	14.2	0.78	24.4	

Later, molybdenum increased the yield and percentage nitrogen. In the case cited (Fig. 2), where ammonium sulphate was applied the percentage nitrogen fell as the plant developed. Hence, while only combined nitrogen

increased the percentage nitrogen at the first harvest, only the molybdenum increased the percentage nitrogen at the last harvest. In the same experiment, the amount of nitrogen applied as nitric acid was sufficient to maintain a high percentage nitrogen, and molybdenum had no effect on either the yield or percentage nitrogen of these nitrogen-treated plants. The results show further the dependence of symbiotic nitrogen fixation on molybdenum.

(*e*) *Nodulation*

Molybdenum decreased the number of nodules on subterranean clover, as did combined nitrogen. Abnormally large numbers of nodules occurred on plants deficient in molybdenum and nitrogen (Tables 7 and 9), indicating that under conditions of inactive symbiotic nitrogen fixation and low nitrogen supply the susceptibility of the plant to infection is greatly increased.

TABLE 8

EFFECT OF SODIUM MOLYBDATE ON YIELD, PROTEIN, AND TOTAL NITROGEN OF SUBTERRANEAN CLOVER (EXPERIMENT 1, SOIL *A*) AND FLAX (EXPERIMENT 4, SOIL *B*), TREATED WITH 5 CWT. MANGANESE SULPHATE PER ACRE (FLAX PROVIDED ALSO WITH NITROGEN AS NITRIC ACID)

	Dry Matter (g.)	Total Nitrogen (%)	Total Nitrogen (mg.)	Protein Nitrogen (%)	Protein Nitrogen (mg.)	Non-Protein Nitrogen (%)	Non-Protein Nitrogen (mg.)
Subterranean clover–							
No molybdate	7.70	1.95	150	1.61	124	0.33	26
Sodium molybdate	14.15†	2.97†	420†	2.46†	347†	0.51†	73†
Flax–							
No molybdate	4.10	2.48	102	1.43	59	1.05‡	43
Sodium molybdate	5.55†	2.48	138	1.70	95*	0.77	43

* Effect due to molybdenum significant at $P < 0.05$.
† Effect due to molybdenum significant at $P < 0.01$
‡ High nitrate in this treatment shown by diphenylamine test.

The effect of molybdenum on nodule size was markedly influenced by the sulphur status of the plant (Table 9). Under conditions of moderate sulphur deficiency, molybdenum more than doubled the yield of the clover but did not affect the number of nodules attaining a length of over 2 mm. Mean size was not determined, but may well have been increased by molybdenum as a result of the reduction in the proportion of small, late-formed nodules. Where sulphur was present, providing optimum conditions for symbiotic nitrogen fixation, molybdenum greatly increased the number of large nodules. This occurred even though molybdenum decreased the total number of nodules, indicating that molybdenum had a positive effect on nodule size.

Treatment with combined nitrogen, which depresses symbiotic nitrogen fixation, decreased the number of large nodules, particularly under conditions of sulphur deficiency. (For full data on sulphur in these studies see Anderson and Spencer 1950.)

TABLE 9

EFFECTS OF NITRIC ACID AND SODIUM MOLYBDATE AT TWO LEVELS OF SODIUM SULPHATE ON THE YIELD AND NODULATION OF SUBTERRANEAN CLOVER; EXPERIMENT 12, SOIL *B*

(Mean transformation values in italics)

Treatments	Dry Matter (g.)	Number of Nodules per 10 cm. of Tap Root	Number of Nodules per 10 cm. of First Order Branch Roots	Number of Nodules per 10 cm. of Second Order Branch Roots	Number of Nodules > 2 mm. in Length	Relative Estimated Weight of Nodules
Without sulphate						
No Mo or N	2.20	1.77	3.43	2.00	9.7	63
	1.48	*0.55*	*1.60*	*1.36*	*1.14*	*1.76*
Sodium molybdate	4.77	1.10	0.97	0.40	10.7	8
	2.18	*0.42*	*1.19*	*0.83*	*1.16*	*0.90*
Nitric acid	5.00	0.43	0.50	0.27	2.0	4
	2.24	*0.20*	*1.00*	*0.74*	*0.78*	*0.57*
Nitric acid + sodium molybdate	5.25	1.33	0.07	0.13	2.3	4
	2.29	*0.48*	*0.83*	*0.62*	*0.80*	*0.57*
With sulphate						
No Mo or N	2.13	0.87	3.27	4.63	9.3	80
	1.46	*0.36*	*1.58*	*1.65*	*1.06*	*1.90*
Sodium molybdate	7.27	1.10	1.13	0.87	19.0	30
	2.68	*0.42*	*0.83*	*1.03*	*1.36*	*1.48*
Nitric acid	7.42	1.10	0.40	0.23	6.3	7
	2.72	*0.42*	*0.99*	*0.69*	*1.00*	*0.80*
Nitric acid + sodium molybdate	8.70	1.10	0.13	0.10	14.7	8
	2.95	*0.42*	*0.87*	*0.59*	*1.26*	*0.90*
Sig. diff. for $P < 0.05$	*0.23*	*0.20*	*0.23*	*0.29*	*0.24*	*0.28*
Sig. diff. for $P < 0.01$	*0.30*	*0.28*	*0.32*	*0.40*	*0.33*	*0.39*

IV. DISCUSSION

Molybdenum-deficient clover showed typical symptoms of nitrogen deficiency, with pale green to yellow leaves and red-brown coloration of the stems. When provided with combined nitrogen the clover did not show these symptoms.

In flax, molybdenum deficiency induced by added manganese sulphate was not sufficiently acute to produce visual symptoms. Chlorosis is known to be a symptom of acute molybdenum deficiency in non-legumes as well as legumes.

In addition, deficiency of molybdenum in plant metabolism also induces characteristic lesions in several plant species (Arnon and Stout 1939; Piper 1940; Hewitt and Jones 1947; Mulder 1948; Dunne and Jones 1948; Millikan 1948; Vanselow and Datta 1949).

Deficiency of protein results from molybdenum deficiency both in clover and flax. The correlation between leaf colour and protein content is well known (Walkley 1940; Hanson 1941). However, the comparison of effects of molybdenum on a legume and a non-legume grown in a deficient soil show that the reasons for the increase in protein content is not the same in both cases.

Yield interactions between molybdenum and combined nitrogen on legumes were negative, response to molybdenum being much greater where the plants were not provided with combined nitrogen but depended on symbiotic nitrogen fixation for their nitrogen supply. Both combined nitrogen and molybdenum increased the yield and protein content of clover by increasing the nitrogen supply – molybdenum doing so by increasing symbiotic nitrogen fixation.

Yield interactions between molybdenum and combined nitrogen on flax were positive, the need for molybdenum being greater where combined nitrogen was provided. The molybdenum was needed by flax for the utilization of the combined nitrogen. The nitrogen-molybdenum interaction on yield obtained here with flax is of the same type as the nitrogen-phosphorus interaction (Anderson and Thomas 1946) and the nitrogen-sulphur interaction (Anderson and Spencer 1949, 1950) on non-legumes and on legumes with insufficient molybdenum for optimum symbiotic nitrogen fixation.

The difference in effects of molybdenum on clover and flax is also shown by the nitrogen fractions in the plant tops. Molybdenum increased the percentage non-protein as well as the percentage protein nitrogen in the clover, thus increasing the percentage total nitrogen. In flax, molybdenum increased the percentage protein nitrogen and decreased the percentage non-protein nitrogen. The non-protein nitrogen fraction includes nitrate which accumulated in the flax provided with nitrate nitrogen but without molybdenum. By contrast, no nitrate was detected in the pale green molybdenum-deficient clover. The results show that whereas the added molybdenum was needed by the clover for symbiotic nitrogen fixation, it was needed by the non-legume for protein formation from the absorbed combined nitrogen.

The soils used, though deficient in available molybdenum for symbiotic nitrogen fixation, were not absolutely deficient in this element. The need for molybdenum in oats is established (Piper 1940), yet oats did not respond to molybdenum in any of the present trials, and presumably obtained sufficient molybdenum from the soil for their requirements. Clover did not respond at all to molybdenum in some of the experiments where nitrate nitrogen was provided, and some molybdenum was detected in these plants. It is quite probable that molybdenum is needed for metabolism in the host legume, as in non-legumes. However, the concentration of molybdenum in clover plants deficient in molybdenum for symbiotic nitrogen fixation was higher than in

the normal plants provided only with combined nitrogen. Thus the molybdenum level adequate for the utilization of nitrate nitrogen was insufficient for symbiotic nitrogen fixation.

With inadequate molybdenum and inadequate nitrogen the nodulated clover plants were stunted by nitrogen deficiency, even though nodules contain a much higher concentration of molybdenum than other plant organs (Bertrand 1940; Jensen and Betty 1943), and notwithstanding the fact that the concentration of molybdenum in the stunted plants was more than was needed for the utilization of combined nitrogen. This suggests that much higher concentrations of molybdenum are needed in the nodules for symbiotic nitrogen fixation than in the plant for the utilization of combined nitrogen.

Some of the apparent anomalies in the literature can be explained on the basis of a differential need for molybdenum by plants, depending upon the nitrogen source. In media with a sufficiently low content of available molybdenum, responses to molybdenum would probably occur irrespective of the nitrogen source. Vanselow and Datta (1949) have reported responses of citrus plants in water culture to molybdenum when provided either with ammonium or nitrate nitrogen. In media of limited purity with respect to molybdenum, whether soils or artificial culture, additional molybdenum would be needed. only for nitrogen fixation. The nature of effects of molybdenum on plants in the field would then depend upon the degree of deficiency and on the nitrogen supply.

The results obtained here with flax grown in soil provided with nitrate nitrogen are in agreement with the results obtained by Mulder (1948) with tomato plants grown in water culture. Mulder found that molybdenum increased the protein content and decreased the nitrate content of tomato plants where nitrate nitrogen was used. Hewitt, Jones, and Williams (1949) found that deficiency of molybdenum in cauliflower plants resulted in a considerable reduction in the concentration of most amino acids in the plant. Accumulation of nitrate in molybdenum-deficient plants has frequently been recorded (Hewitt and Jones 1947; Wilson and Waring 1948; Mulder 1948; Stout and Meagher 1948).

Strict comparison of the need for molybdenum in the plant for the utilization of nitrate and ammonium nitrogen is not possible in the present experiments where the nitrogen compounds were applied to the soil, as nitrification of the ammonia might be expected. However, it is interesting to note that comparable responses were obtained with flax where either nitric acid or ammonium sulphate was used, and that no nitrate accumulation was detected with diphenylamine in the molybdenum-deficient flax where ammonium sulphate was used. Mulder (1948) obtained evidence suggesting that plants provided with ammonium nitrogen do not require molybdenum, although Vanselow and Datta (1949) obtained similar response of citrus plants to molybdenum in water cultures supplying either ammonium or nitrate nitrogen.

Increase in symbiotic nitrogen fixation with molybdenum was associated with a decrease in the number of nodules. However, plants provided with

molybdenum were sufficiently well nodulated to provide for their nitrogen requirements, suggesting that reduction in nodulation was not due to a direct inhibiting effect of molybdenum on *Rhizobium*, but was brought about as a result of the increase in nitrogen fixation. Combined nitrogen also frequently reduces nodulation. It checks the deformation of the root hairs by *Rhizobium*, thus preventing infection, and checks the growth of nodules already formed (Thornton 1935). The mechanism by which these effects are brought about is not understood, but both carbohydrate and nitrogen in the plant may be involved (Wilson 1940; Thornton 1947). It is interesting to note that, in the present experiments, the decrease in the number of nodules formed where molybdenum was applied was associated with an increase in the percentage of both non-protein and protein nitrogen.

There is strong additional evidence that decreased nodulation with molybdenum was not due to a direct inhibiting effect of molybdenum on *Rhizobium*. The greatest decrease in the number of nodules on lucerne (Anderson and Oertel 1946) occurred with the two lowest levels of molybdenum, and little further decrease resulted from a more than 200-fold increase in concentration. In the absence of lime, where the plants were too poorly nodulated for their nitrogen requirements, molybdenum caused no decrease at all in the number of nodules.

In the present experiments molybdenum decreased the number of smaller nodules on the later-formed root branches. This decrease in the number of smaller nodules would have tended to increase the mean size of nodules. However, there was evidence that molybdenum had a positive effect on nodule size, as the molybdenum-treated plants had more nodules over 2 mm. in length than did the molybdenum-deficient plants with their greater number of nodules. This occurred particularly where sulphur was adequate for optimum nitrogen fixation, and might be expected to result from the increase in nitrogen fixation in the nodules.

Burk (1934) reported responses of *Azotobacter* to either molybdenum or combined nitrogen, and absence of response to molybdenum where combined nitrogen was provided. This result indicated that molybdenum is particularly needed by *Azotobacter* for nitrogen fixation. Bortels (1936) and others later obtained responses of *Azotobacter* to molybdenum where nitrate nitrogen, asparagine, or ammonium nitrogen was used. The results of Burema and Wieringa (1942) and Mulder (1948), however, have established the particular need of *Azotobacter* for molybdenum when dependent on elemental nitrogen.

The complete absence of response of *Azotobacter* to molybdenum in the early experiments where combined nitrogen was provided was not necessarily due to any impurity of molybdenum in the nitrogen compounds used. It can be explained on the basis of the differential need for molybdenum depending upon the nitrogen source, molybdenum impurity in the basal medium being sufficient for the utilization of combined nitrogen, but not for nitrogen fixation.

The results here obtained with legumes are therefore in agreement with those obtained by others with *Azotobacter*. They indicate that molybdenum

is needed by legumes in the symbiotic nitrogen fixation process, either in the initial reaction with gaseous nitrogen or in a later reaction which is not an essential step in either nitrate or ammonium utilization.

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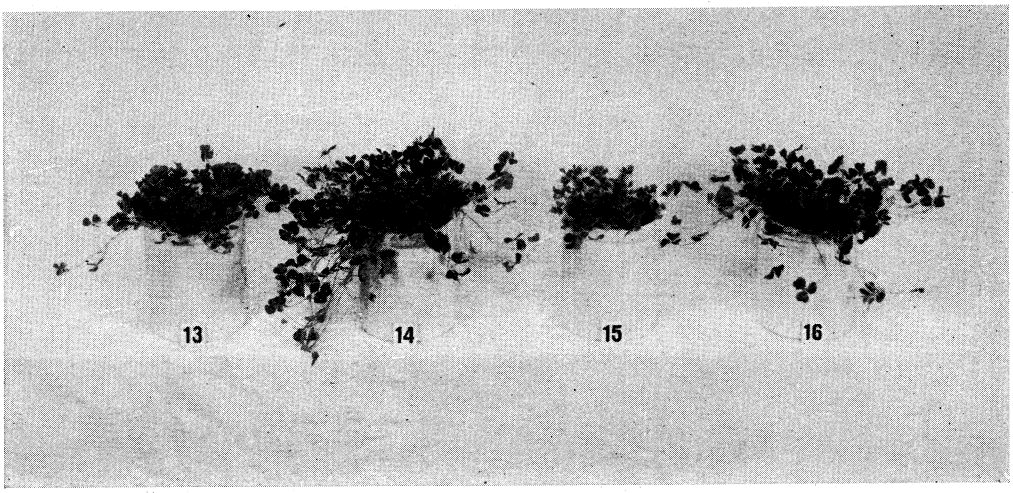

Fig. 1.—Molybdenum deficiency in subterranean clover accentuated by manganese sulphate (Experiment 9).

(13) No molybdenum.
(14) 1 lb. Sodium molybdate per acre.
(15) 5 cwt. Manganese sulphate per acre.
(16) 1 lb. Sodium molybdate + 5 cwt. manganese sulphate per acre.

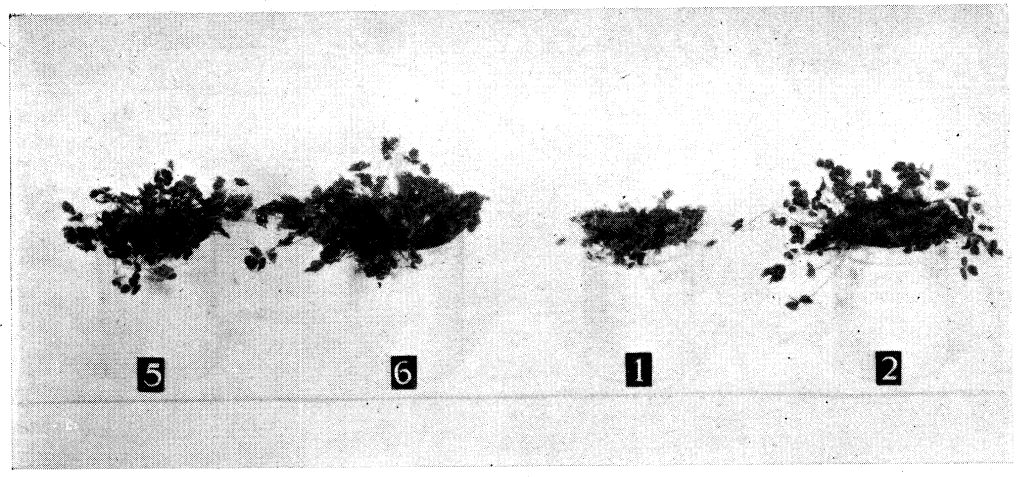

Fig. 2.—Nitrogen deficiency in subterranean clover accentuated by decreasing molybdenum uptake with manganese sulphate (Experiment 9).

(5) No molybdenum.
(6) Nitric acid (100 mg. N per pot).
(1) 5 cwt. Manganese sulphate per acre.
(2) Nitric acid (100 mg. N per pot) t. manganese sulphate per acre.

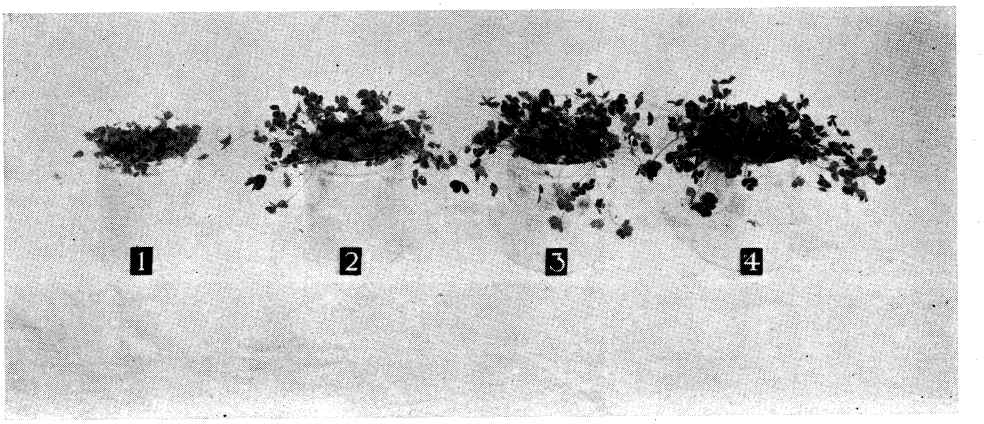

Fig. 1.—Molybdenum-nitrogen interaction on subterranean clover in the presence of manganese sulphate (Experiment 9).

(1) No molybdenum.
(2) Nitric acid (100 mg. N per pot).
(3) 1 lb. Sodium molybdate per acre.
(4) Nitric acid (100 mg. N per pot) + 1 lb. sodium molybdate per acre.

Fig. 2.—Molybdenum-nitrogen interaction on subterranean clover without manganese sulphate on a soil more deficient in molybdenum (Experiment 12).

(21) No molybdenum.
(22) 2 oz. Sodium molybdate per acre.
(23) Nitric acid (115 mg. N per pot).
(24) 2 oz. Sodium molybdate per acre + nitric acid (115 mg. N per pot).