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Effect of molybdenum, manganese and magnesium on the reduction of nitrate in spinach and oat

Virkning af molybdæn, mangan og magnesium på reduktion af nitrat i spinat og havre

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Summary

In experiments with supply of molybdenum, manganese and magnesium to oat and spinach in pot culture, deficiency of Mo caused increased concentration of nitrate and decreased concentration of reduced nitrogen and reduced *in vivo* nitrate reductase activity (NRA) in spinach leaves, while the effects in oat were much less pronounced.

Deficiency of manganese caused an increased concentration of nitrate, but NRA and the content of reduced N were also increased. The apparent influence of Mn is proposed to be due to a dilution effect caused by a yield-increasing effect of Mn.

Only small and non-significant effects of Mg on the content of nitrate and reduced nitrogen and NRA in oat and spinach were found.

No effect of Mo and Mn, above the amount giving maximum yield, on content of nitrate and on NRA was observed.

Key words: Nitrate, nitrate reductase activity, molybdenum, manganese, magnesium, spinach, oat.

Resumé

Forsøg med molybdæn, mangan og magnesium til havre og spinat i karforsøg viste, at mangel på molybdæn medførte en forøget koncentration af nitrat og en nedsat nitratreduktaseaktivitet (NRA) og koncentration af reduceret kvælstof i spinat, medens effekten på havre var betydeligt mindre.

Manganmangel i havre medførte ligeledes et forøget indhold af nitrat, men tillige en forøgelse i indhold af reduceret N og forøgelse i NRA. Den tilsyneladende virkning af Mn formodes at være forårsaget af en fortyndingseffekt på grund af mangans udbyttefremmende virkning.

Der blev kun fundet små og usystematiske virkninger af Mg på indholdet af nitrat og reduceret kvælstof og på NRA i havre og spinat.

Der blev ikke fundet nogen virkning af Mo og Mn på nitratindhold og NRA over den mængde, der gav det maksimale udbytte.

Nøgleord: Nitrat, nitrat-reduktaseaktivitet, molybdæn, mangan, magnesium, spinat, havre.

Introduction

The plant uptake of nitrogen from the soil occurs mainly as nitrate, which must be reduced to ammonium before incorporation in amino acids and proteins.

The assimilation and the content of nitrate in plants are dependent on various internal and external factors among which also some of the inorganic nutrients besides nitrogen are supposed to be of importance (6, 10, 11, 28).

The most well-known example is molybdenum, which is a component of the enzyme nitrate reductase, catalysing the reduction of nitrate to nitrite (28). Deficiency of Mo in plants is often followed by an increased concentration of nitrate (4, 7, 13, 19), and a decreased nitrate reductase activity (NRA) is observed (14, 26, 32).

Manganese has also been found to be of significance for the assimilation of nitrate (1, 3).

In oat (23) and cauliflower (13) higher nitrate concentration was found in plants deficient in manganese than in healthy plants and *Vielemeyer et al.* (38) and *Liebenow* (24) found that addition of manganese to deficient plants decreased the content of nitrate.

The specific effect of manganese in nitrate assimilation is still uncertain. However, from experiments with spinach, *Maldonado et al.* (25) have proposed a possible role of Mn in reactivation of inactivated nitrate reductase mediated by photochemical reactions.

The importance of Mn for the nitrate assimilation may also be due to an effect on the reduction of nitrite. *Amberger* (1) found a decrease in the content of nitrite in oat and maize, especially in roots, with an increasing supply of Mn.

Although no direct influence of magnesium in the nitrate assimilation process is known, some apparent effect has been observed. *Nowakowski et al.* (29) found an increase of nitrate in Italian ryegrass deficient in Mg. In wheat seedlings *Harper* and *Paulsen* (9) observed a decreased nitrate reductase activity with Mg-deficiency, but the concentration of nitrate was also decreased, which was supposed as a reason for the low NRA.

An indirect effect of Mg possibly can be expected from its influence on other metabolic processes in the plants, such as the photosynthesis, where Mg is significant for the structure and function of the chlorophyll molecule and for activation of the enzyme ribulose biphosphate carboxylase. Light stimulates the reduction of nitrate, but the exact mechanism is unknown. One explanation is that during photosynthesis some metabolites are produced essential for reduction of NAD to NADH, which donates electrons for reduction of nitrate (33).

Manganese is also essential for the photosynthetic activity (16) and thus an indirect influence on the nitrate reduction might be expected too. In a number of enzymic processes Mn and Mg can substitute each other (5).

The purpose of the experiments was to study the influence of Mo, Mn and Mg, on the content of nitrate and activity of nitrate reductase in connection with the influence of the nutrients on the yield. Also if there was any effect of the nutrients at levels above the ones necessary for obtaining maximum yield.

Spinach and oat which are known to accumulate nitrate (6) were selected as test plants.

Material and methods

Pot cultures

The spinach and oat plants (cultivar Matador and Selma respectively) were grown in either large pots (d. 25 cm; h. 39 cm) or small pots (d. 14 cm; h. 28 cm). The growing medium and the basic fertilization were selected according to the experimental nutrient as follows:

A) *Molybdenum experiments:* Large pots with 15 kg of a mixture of silica sand and peat (6:1 w/w), amended with 15 g of $CaCO_3$, which gave a pH of 4.5 in the medium. The basic fertilizers consisted of 5.0 g NO_3-N, 5.0 g K, 2.0 g P, 1.0 g Mg and 0.2 g Mn/pot.

The supply of Mo was: Exp. A I: 0-5-10-20 mg Mo/pot (spinach and oat), Exp. A II: 0-1-2-5-10-20 mg Mo/pot (spinach).

B) *Manganese experiments:* Exp. B I: Large pots with 24 kg of a mixture of silica sand and a loamy sand (4:1 w/w). Basic fertilization as for Mo, except the manganese, but with addition of 0.01 g Mo/pot. The supply of Mn was: 0-100-200-400 mg Mn/pot (spinach and oat). Exp. B II: Small pots with 3.7 kg of a mixture (1:1 w/w) of silica sand and a humus-rich manganese deficient soil. Basic fertilization 1.0 g NO_3-N, 1.0 g K, 0.4 g P, 0.15 g Mg, 0.03 g Cu/pot. Supply of Mn: 0-12.5-25-50-100-200-500 mg Mn/pot (oat).

C) *Magnesium experiments:* Exp. C I: Large pots with growing medium and basic fertilization as for manganese in Exp. B I, except the amount of Mg, but with 0.2 g Mn. Mg supply 0-0.5-1.0-2.0 g Mg/pot (spinach and oat). Exp. C II: Small pots with 5 kg of silica sand. Basic fertilization 1.0 g NO_3-N, 1.0 g K, 0.4 g P, 10 mg Mn, 20 mg Fe, 1 mg Cu, 1 mg Zn, 5 mg B, 0.5 mg Mo/pot. Mg supply 0-12.5-25-50-100-500 mg/pot (spinach and oat).

The plants were supplied with demineralized water as required.

For the analyses plants were sampled at intervals during the growth as indicated in the results. In most instances young expanded leaves were used for the analyses.

Analyses

Total nitrogen, nitrate-nitrogen, molybdenum, manganese and magnesium were determined on dry matter and nitrate reductase activity (NRA) on fresh matter.

The Kjeldahl method was used for determination of total nitrogen after reduction of nitrate with salicylic acid. NO_3-N was determined with the xylenol-method (36). Reduced nitrogen was taken as the difference between total nitrogen and nitrate-nitrogen.

For determination of molybdenum the plant material was wet digested with perchloric-sulfuric acid followed by complexation of Mo with dithiol and extraction with isoamylacetate and spectrophotometric determination at 680 nm (2).

For manganese a modification of the method of *Piper* (31) was used. After digestion of the plant material with nitric-perchloric-sulfuric acid the manganese was oxidized to permanganate by periodate and determined spectrophotometrically at 530 nm.

Magnesium was determined by atomic absorption spectophotometry after dry ashing of the plant material.

Nitrate reductase activity (NRA) was determined by an in vivo method which was modified from methods described by *Jaworski* (18) and *Streeter* and *Bosler* (35).

From the plants harvested in the morning (8.00–9.00 h) young expanded leaves were selected. The leaves were cut to 1–2 cm pieces with a pair of scissors and after mixing a sample of 2–3 g fresh matter was weighed for incubation. The pieces were vacuum-infiltrated repeatedly until settled in 100 ml of the incubation medium composed of 0.1 M K-phosphat, pH 7.5; 0.1 M KNO_3; 0.1% Triton-X-100 and 10 mg chloramphenicol per litre.

At intervals aliquots (0.5–2.0 ml) were taken from the medium and treated with 0.5 ml 1% sulfanilamide in 3 M HCl and 0.5 ml 0.02% N-(1-naphthyl)ethylenediamine dihydrochloride and the absorption measured at 540 nm. As there was an initial delay in the generation of nitrite the NRA was calculated from the difference between two measurements taken with an interval of 30-45 min. in a period where the formation of nitrite was linear.

Results

Molybdenum

In the first experiment (A I) the yield of spinach was increased by molybdenum, but not significantly above 5 mg Mo per pot (Table 1). The concentration of nitrate was strongly decreased and reduced nitrogen increased, but again no effect above 5 mg Mo was found. The concentration of nitrate was much higher in the stalks than in the leaves, but the effect of Mo was small.

For both harvests the effect of Mo was similar, except for the concentration of Mo, which in-

Table 1. Effect of Mo on yield of spinach leaves and concentration of nitrate, reduced nitrogen and molybdenum in dry matter at two harvests. Mean of 2 samplings for each harvest. Experiment A I, spinach.

Indflydelse af Mo på udbyttet af spinatblade samt koncentration af nitrat, reduceret kvælstof og molybdæn i tørstof ved to høsttidspunkter. Gennemsnit af 2 prøveudtagninger ved hver høst.

	Har-[1]) vest	Addition of Mo mg/pot 0	5	10	20	LSD[2]) (P = 0.05)
Leaf						
dry matter yield	1	0.24	0.68	0.69	0.63	0.12
g/plant	2	0.89	4.65	4.79	4.64	1.07
NO_3-N in leaves	1	1.38	0.38	0.40	0.42	0.46
% of DM	2	1.64	0.52	0.44	0.47	0.08
NO_3-N in stalks	1	2.00	1.77	1.66	1.90	–
% of DM	2	1.99	1.70	1.63	1.60	–
Reduced N in	1	3.92	4.54	4.65	4.64	0.31
leaves, % of DM	2	3.58	4.51	4.56	4.60	0.56
Molybdenum	1	0.1	0.3	0.4	0.7	0.4
µg/g DM	2	0.4	0.3	0.2	0.3	0.3

[1]) Harvest 1 and 2 at 33 and 46 days after emergence respectively.

[2]) LSD: Least significant difference.

Table 2. Effect of Mo on yield and concentration of nitrate, reduced nitrogen and molybdenum in dry matter of shoots of green oat at two harvests and the yield of grain and straw at maturity. Mean of 2 samplings at each harvest. Experiment A I, oat.

Indflydelse af Mo på udbytte og koncentration af nitrat, reduceret kvælstof og molybdæn i tørstof i grøn havre ved to høsttidspunkter samt udbyttet af kerne og strå ved modenhed. Gennemsnit af 2 prøveudtagninger ved hver høst.

	Har-[1]) vest	Addition of Mo mg/pot 0	5	10	20	LSD[2]) (P = 0.05)
Dry matter yield	1	0.78	0.90	0.87	0.82	0.21
g/plant	2	2.15	2.54	2.26	2.37	0.70
NO_3-N	1	1.46	1.16	1.13	1.15	0.18
% of DM	2	1.46	1.30	1.37	1.34	0.19
Reduced N	1	4.14	4.16	4.20	4.17	0.27
% of DM	2	3.31	3.34	3.38	3.44	0.31
Molybdenum	1	0.3	1.0	2.0	3.1	0.7
µg/g DM	2	0.3	1.6	2.6	3.8	0.7
Grain, gDM/pot	Maturity	145	150	151	141	11
Straw, gDM/pot	Maturity	109	113	112	109	5

[1]) Harvest 1 and 2 at 44 and 53 days after emergence respectively.

creased at the first harvest, but had no consistent course at the second harvest.

For oat (Table 2) the effect of Mo on yield and concentration of nitrate was much smaller than for spinach, but the concentration of Mo was raised much more.

In the second experiment (A II) with spinach Mo was added in smaller increaments than in the first experiment, to obtain a more detailed information about the effect of small amounts of Mo. The results appear in Fig. 1.

The largest relative change was found for nitrate and NRA, which vary in an opposite manner with increasing supply of Mo. The relative change in reduced nitrogen is small. The greatest effect of Mo was found at the first small increaments and with the exception of the concentration of Mo no changes in any of the qualities were significant above the 10 mg Mo/pot.

The points in Fig. 1 represent the mean of more samplings during the growth period. Fig. 2 shows the relation between the increase in yield during growth and the content of nitrate, the nitrate reductase activity and the content of reduced nitrogen respectively for different amounts of Mo. If no Mo is given the content of nitrate rises steeply as the yield increases, while the nitrate reductase activity decreases steeply indicating an increased deficiency of Mo with the growth of the plants. There is first an increase and next a decrease in the content of reduced nitrogen. The curves are flattened as the amount of Mo is raised.

Manganese

For experiment B I the growing medium was not known to be especially deficient in manganes and the purpose was to see if Mn had any effect on internal qualities such as content of nitrate or re-

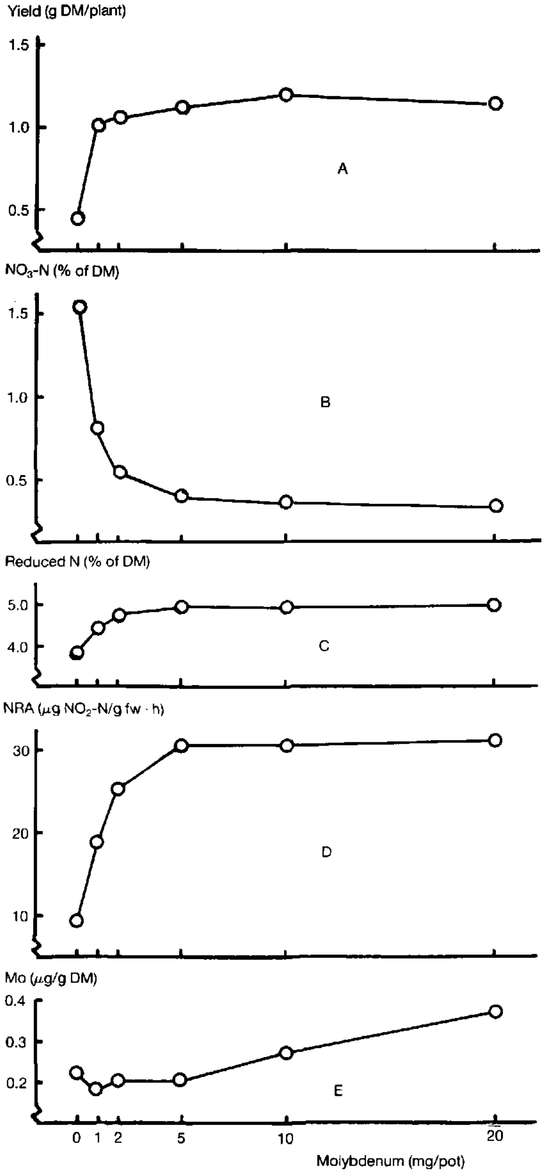

Fig. 1. Influence of Mo on dry matter yield of leaves (A), concentration of nitrate (B), reduced N (C), nitrate reductase activity (D) and concentration of Mo in spinach leaf-samples (E). Each point is the mean of 6 samplings in the period 21–31 days after emergence. (Experiment A II).

Indflydelse af Mo på udbyttet af bladtørstof (A), koncentration af nitrat (B), reduceret N (C), nitratreduktaseaktivitet (D) og koncentration af Mo (E) i spinatblade. Hvert punkt repræsenterer gennemsnittet af 6 prøveudtagninger i perioden 21–31 dage efter fremspiring.

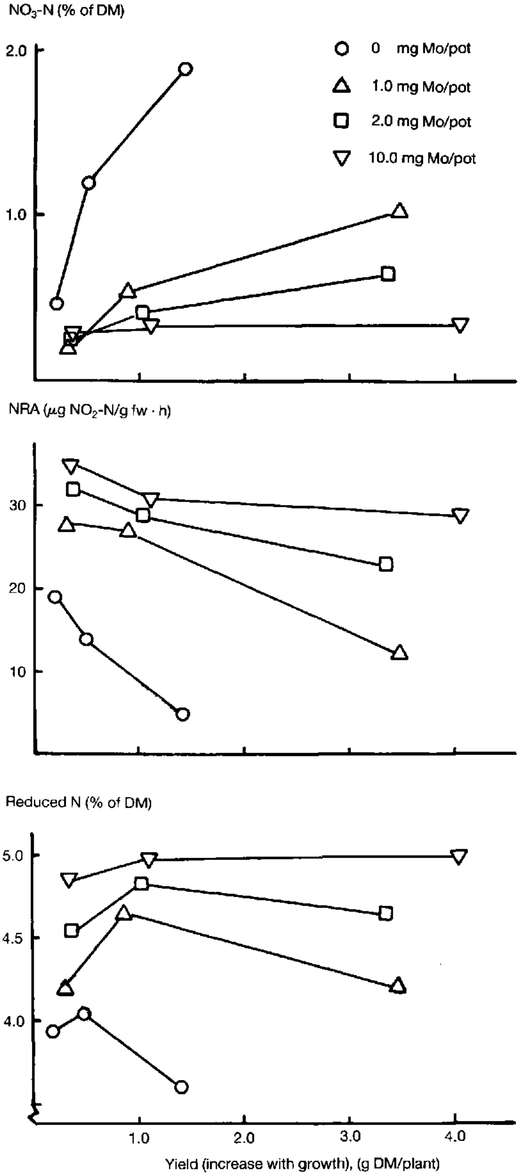

Fig. 2. Nitrate concentration, nitrate reductase acitivity and concentration of reduced nitrogen in spinach leaves in relation to the yield during the growth at different levels of molybdenum.

Growth period 14–31 days after emergence. Each point mean of 3 samplings- (Experiment A II).

Nitratkoncentration, nitratreduktaseaktivitet og koncentration af reduceret kvælstof i spinatblade i relation til udbyttet under væksten ved forskellige molybdæniveauer. Vækstperiode 14–31 dage efter fremspiring. Hvert punkt er gennemsnit af 3 prøveudtagninger.

Table 3. Yield of dry matter and content[1]) of nitrate, reduced nitrogen and manganese in spinach and oat at different levels of Mn. Mean of 2 samplings at each harvest. Experiment B I.

Udbytte af tørstof og indhold af nitrat og reduceret kvælstof og mangan i spinat og havre ved forskellige mangantilførsler. Gennemsnit af 2 prøveudtagninger ved hver høst.

	Har-[2]) vest	Spinach				Oat			
		g Mn/pot			LSD	g Mn/pot			LSD
		0	0.2	0.4	P = 0.05	0	0.2	0.4	P = 0.05
Dry matter yield	1	0.94	1.01	1.06	0.25	0.87	0.86	0.90	0.13
g/plant	2	6.99	6.57	6.79	1.00	2.68	2.71	2.51	0.45
NO_3-N	1	0.37	0.37	0.44	0.12	1.09	1.09	1.13	0.16
% of DM	2	0.50	0.43	0.48	0.11	1.31	1.26	1.30	0.21
Reduced N	1	4.60	4.53	4.69	0.19	4.41	4.38	4.44	0.52
% of DM	2	4.82	4.83	4.92	0.34	3.45	3.47	3.40	0.24
Manganese	1	29	31	53	3	27	38	60	10
µ/g DM	2	12	19	24	17	20	36	51	6
Grain (oat) g DM/pot	Maturity					147	149	150	7

[1]) For spinach the content is in the leaves, for oat in the shoots.
[2]) Harvest 1 and 2 were for spinach at 33 and 46 days and for oat at 44 and 53 days after emergence respectively.

duced nitrogen above normal levels of Mn. From Table 3 it can be seen that manganese had no significant effect on either yield or the nitrogen fractions measured, but there was an increase in the content of Mn as was expected.

The results from experiment B II with a Mn-deficient soil is shown in Fig. 3. The yield clearly increased with the supply of manganese, at least up to 200 mg Mn/pot. The concentration of Mn naturally was also increased. A small decrease was found with increasing supply of manganese in the content of nitrate and reduced nitrogen and the nitrate reductase activity. However, the effect of Mn on these qualities is apparently not a direct one, but is due to its effect on the yield. From Fig. 4 it appears that the content of nitrate in the leaves decreases as the yield increases with the growth of the plants.

The course of the change in the nitrate content is nearly identical for all the levels of Mn, and indeed the curves for the lower levels tend to be the lowest situated.

The nitrate reductase activity showed an increase from the first to the second sampling followed by a decrease to the third sampling. Often a rather high day to day variation was found in the NRA.

The content of reduced nitrogen tends to be highest at the lowest addition of Mn.

Magnesium

Experiment C I with increasing amounts of Mg to spinach and oat on a non-deficient medium (the same as used for the non-deficiency experiment, B I, with Mn) gave similar results (not shown) as obtained with Mn (Table 3), i.e. no effect of Mg on the yield, and content of nitrate and reduced nitrogen. The concentration of Mg was increased, for spinach from 0.1 to 0.7% Mg and for oat from 0.1 to 0.3% Mg of dry matter, when the amount of Mg was increased from 0 to 2.0 g Mg/pot.

An experiment (C II) with Mg to spinach and oat grown in pure silica sand showed an effect of Mg on the yield (Table 4), but was only significant for the first amount of Mg.

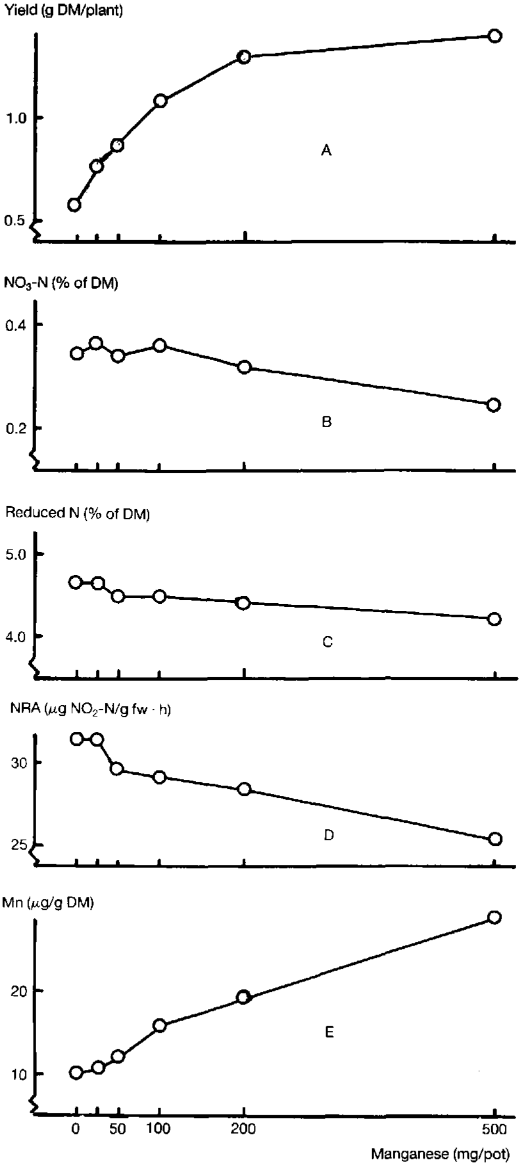

Fig. 3. Influence of Mn on dry matter yield of shoots of oat plants (A), concentration of nitrate (B), reduced N (C), nitrate reductase antivity (D) and concentration of Mn in leaf-samples (E). Each point mean of 4 samplings in the period 36–42 days after emergence. (Experiment B II).

Indflydelse af Mn på tørstofudbyttet af grøn havre (A), koncentration af nitrat (B), reduceret N (C), nitratreduktaseaktivitet (D) og koncentration af Mn (E) i bladprøver. Hvert punkt er gennemsnit af 4 prøveudtagninger i perioden 36–42 dage efter fremspiring.

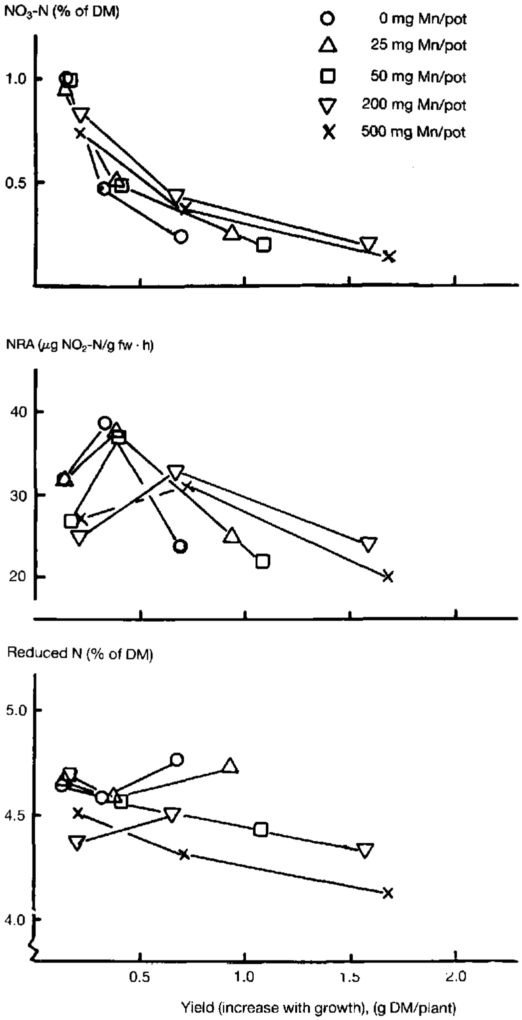

Fig. 4. Nitrate concentration, nitrate reductase activity and concentration of reduced nitrogen in oat leaf samples in relation to the yield of the shoots during growth at different levels of manganese. Growth period 27–42 days after emergence. Each point mean of 1 (1. harvest) or 2 (2. and 3. harvest) samplings. (Experiment B II).

Nitratkoncentration, nitratreduktaseaktivitet og koncentration af reduceret kvælstof i havreblade i relation til udbyttet af hele toppen under væksten ved forskellige manganniveauer. Vækstperiode 27–42 dage efter fremspiring. Hvert punkt er gennemsnit af 1 (1. høst) eller 2 (2. og 3. høst) prøveudtagninger.

Table 4. Effects of Mg on yield of shoots and on concentrations of nitrate, reduced nitrogen and magnesium and on nitrate reductase activity in leaves of spinach and oat. Mean of 4 samplings in the period 26–36 days after emergence. Experiment C II.

Indflydelsen af Mg på udbytte af top og på koncentrationen af nitrat, reduceret kvælstof og magnesium og på nitratreduktaseaktiviteten i blade af spinat og havre. Gennemsnit af 4 prøveudtagninger i perioden 26–36 dage efter fremspiring.

	Species	Addition of Mg mg/pot					LSD
		0	12.5	25	100	500	(P = 0.05)
Yield	Spinach	0.35	0.71	0.80	0.67	0.77	0.17
g DM/plant	Oat	0.47	0.76	0.76	0.81	0.86	0.18
NO_3-N in leaves	Spinach	0.50	0.42	0.40	0.47	0.48	0.08
% of DM	Oat	1.10	1.09	1.11	1.10	1.09	0.06
Reduced N in leaves	Spinach	5.34	5.29	5.22	5.26	5.29	0.12
% of DM	Oat	4.81	4.89	4.92	4.84	4.86	0.08
NRA in leaves	Spinach	63	57	55	60	60	8
μg NO_2-N/g fw · h	Oat	44	39	41	41	38	4
Magnesium in leaves	Spinach	0.12	0.16	0.25	0.62	1.44	0.11
% of DM	Oat	0.08	0.11	0.13	0.20	0.39	0.02

The concentration of Mg was increased to an even higher value than in experiment C I, a high level was reached especially in spinach.

The concentration of nitrate and reduced nitrogen and the nitrate reductase activity were not consistently or significantly affected by Mg.

The concentration of nitrate was somewhat higher in oat than in spinach, while the NRA and the concentration of reduced nitrogen were smaller.

Discussion

Of the three nutrients investigated only Mo had an evident influence on the concentration of nitrate and reduced nitrogen and on nitrate reductase activity.

This effect of Mo is known from various experiments (4, 7, 13, 14, 19, 26, 32) and is in accordance with its known function as an indispensable component of the enzyme nitrate reductase, necessary for the activity of the enzyme (28).

The exact role or function of Mo is unknown but it is proposed that Mo participate in the transfer of electrons to nitrate at its reduction to nitrite and that Mo changes its oxidation state in the process (15, 21).

For plants without symbiotic nitrogen fixation the only established role of Mo is in the reduction of nitrate (14). Thus the influence of Mo on the yield of the plants must be through its influence on the nitrate reduction, and thereby indirectly also the rate of synthesis of enzymes, the keys in the plant growth processes.

Spinach was found to be much more sensitive than oat to deficiency of Mo, in agreement with *Johnson et al.* (19), who found spinach to be the most Mo-demanding among various plant species, while oat together with other cereals required only small amounts of Mo. On special soils such as peat soils with high iron contents deficiency of Mo has been demonstrated in oat (34).

The stronger increase in content of Mo in oat than in spinach at increasing addition of Mo indicates a greater ability of oat to take up Mo from the growing medium.

The concentration of Mo in spinach at the zero level does not confirm the deficiency of Mo indicated by the low yield at this level. Normally deficiency occur at concentration lower than the 0.2–0.3 µg Mo/g DM found here (8, 19). The reasons for the discrepancies may be inaccurate analyses in the present experiment, but unfortunately only small amounts of substances were available and a re-examination of the results was not possible.

The apparent decreasing effect of Mn on the concentration of nitrate in oat leaves in the deficiency experiment (Fig. 3) cannot with certainty by ascribed to a direct influence on the nitrate reduction processes in the plants, but can also be due to a dilution effect (17) in consequence of the yield-increasing effect of Mn. As the oat plants developed the content of nitrate decreased in nearly the same manner for all the treatments (Fig. 4).

Some of the instances referred to in the literature, of increased concentration of nitrate at deficiency of manganese may possibly also be a dilution effect as the yield has often been reduced (30, 38).

The maximum yield is not reached with certainty at the highest amount of Mn and the curves for nitrate, reduced nitrogen and NRA still seem to be decreasing (Fig. 3). Though, the concentration of Mn reached about the same level as for the lowest level of Mn in the first experiment (B I, Table 3) and is above the Mn-concentration normally considered as the limiting value for Mn-deficiency (27).

From the present results an influence of Mn on the later steps in the nitrate assimilation, e.g. reduction of nitrite, is not excluded. However, the concentration of reduced nitrogen is not higher at the high levels of Mn (Fig. 3 and 4), but instead tends to be lower.

One explanation could be that some of the reduced nitrogen at the lower levels of Mn is non-protein nitrogen due to a decreased synthesis of proteins (enzymes) at deficiency of manganese and thereby the production of non-nitrogenous substances is retarded.

For magnesium no consistent effect on nitrate, reduced nitrogen or NRA was found. Only at the zero-level the plants were strongly deficient according to the yield. The concentration of Mg indicate a possible deficiency also at the first addition of Mg (12, 22, 39).

Thus the decrease in yield at deficiency of Mg is probably not due to an influence of Mg on the reduction of nitrate, but is probably caused by an effect on the photosynthetic activity which is found to be decreased at deficiency (37).

Apparently supply of the three nutrients at levels above the ones necessary to obtain maximum yield had no effect on the content of nitrate or nitrate reductase activity. Whether even higher, e.g. toxic amounts, could have an influence can not be decided from the present experiments. *Jones* and *Menary* (20) found that high, yield-decreasing levels of Mn caused a decreased NRA and an increased concentration of nitrate in lettuce.

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