

On Interactions of Anion Uptake in Alfalfa and Orchardgrass by Pot Culture and Water Culture

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Numerous investigators have demonstrated¹⁻³⁾ that the availability of nitrogen (N) limits plant growth on most humid grassland, under intensive alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) production. The level of available plant nutrients in the soil frequently falls below a critical level because of heavy removal of nutrients in the hay^{4,5)}.

Therefore application of fertilizer, especially nitrogen (N) have been tried. But excessive application of those nitrogen (N) fertilizer from manure or urine result in over absorption of nitrate (NO_3) legume and grass, and those nitrate is caused for nitrite (NO_2) poisoning of dairy cattle and other animals.

This study was conducted to interact anions (NO_3^- , Cl^- , H_2PO_4^- , SO_4^{2-} , SiO_3^{2-}) uptaken by alfalfa and orchardgrass in water and pot culture.

Material and Method

In the pot culture method, alfalfa (Du Puits) and orchard grass (Kitami-dori) were sowed into polyethylene pots (12 cm by 12 cm deep with 4 small drainage holes in the bottom) containing soil (4:1 mixture of Nopporo clay loam soil and sand). The soil mixture contained 12.4 mg/100 g dry soil of available P_2O_5 , 40 mg/100 g dry soil of exchangeable K_2O and had pH 6.5 with H_2O .

Nitrate-nitrogen ($\text{NO}_3\text{-N}$) levels with KNO_3 were 50, 150, and 450 mg/pot, and silicate (SiO_3) levels with silica gel of 100 mesh for chromatography were 0.4, 0.8 and 1.6 g/pot. Each pot sowed was placed in a growth chamber set at 27°C during the day and 21°C at night, and all pots were watered with demineralized water as needed.

While, in the water culture method, seedlings of alfalfa and orchardgrass were grown in trays with the previously described 4:1 soil mixture for 30 days in a green house. The seedlings were planted in a pot with the water

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culture solution. Nitrate-nitrogen ($\text{NO}_3\text{-N}$) levels of the solution with KNO_3 were 3, 9, and 15 meq./l, and Cl , H_2PO_4 and SO_4 were 1, 3 and 9, meq./l, respectively.

These plants were harvested, and the anions (NO_3^- , Cl^- , H_2PO_4^- , SO_4^{2-} , SiO_3^{2-}) analyzed. Four replications of each treatment were prepared, and the plant sample were made after tissues had been ground to 40 mesh size.

Results

Response of silicate and nitrate. Percentage of silicate in the leaves of orchardgrass increased with each increment of applied silicagel from 0.87 per cent in the control to 1.20 per cent when 1.6 g/pot, but in alfalfa, it did not increase with all application rates. On the other hand, in the water

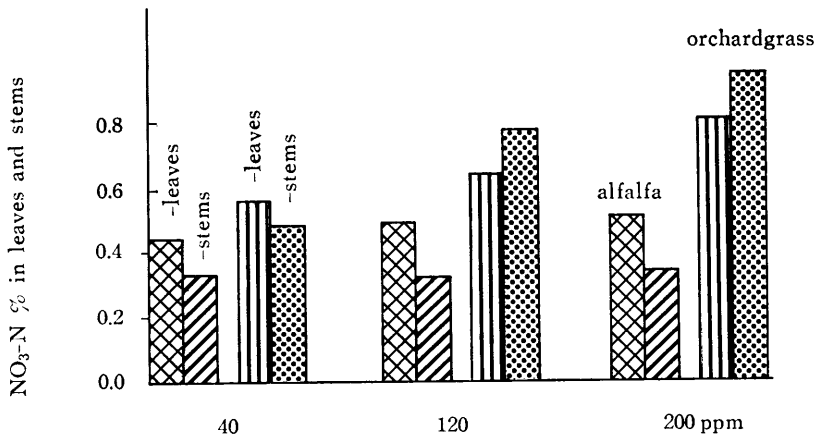


Fig. 1. $\text{NO}_3\text{-N}$ level in water culture solution.

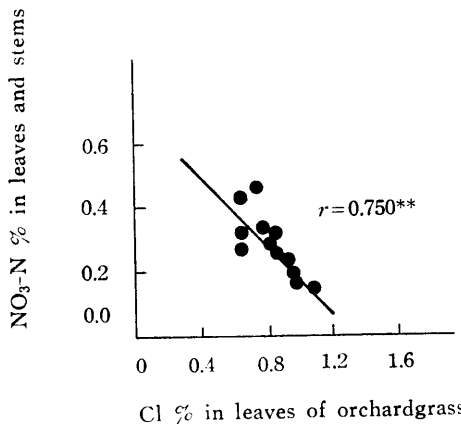


Fig. 2. Relationship between NO_3^- and Cl^- in leaves of orchardgrass.

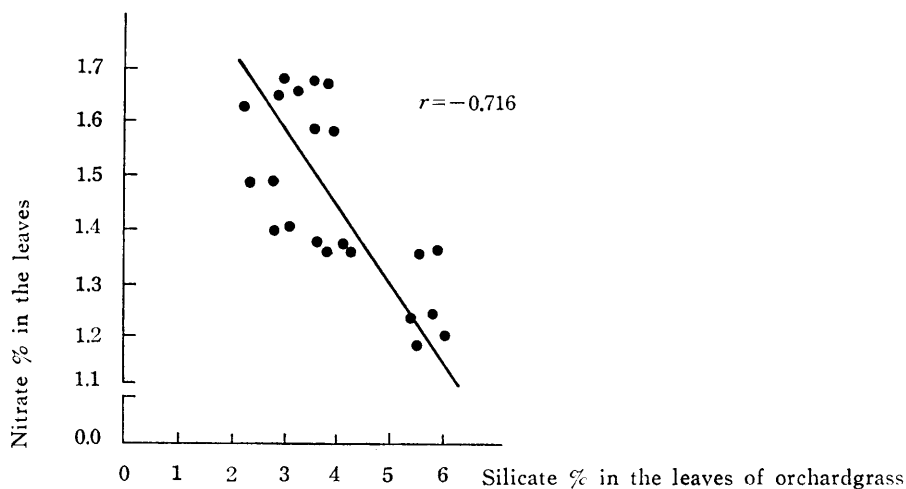


Fig. 3. Relationship between nitrate and silicate in leaves of orchardgrass.

culture method, when alfalfa and orchardgrass were compared at the same rate of applied the nitrate nitrogen, leaves and stems of each plant responded to increasing nitrate were different (Fig.1), namely concentration of nitrate increased with the increment of nitrate applied, for leaves and stems of orchardgrass.

Relationship between NO_3^- and other anions. The relationship between NO_3^- and Cl^- concentration in leaves of orchardgrass was shown Fig. 2. The increase of the Cl^- concentration in the tissue was decreased in NO_3^- uptake. Also, the relationship between NO_3^- and H_2PO_4^- concentration in the leaves was similar to the pattern of Cl^- and NO_3^- .

In other experiments with pots the relationship between nitrate and silicate concentration in leaves of orchardgrass was shown Fig. 3, namely, the increase of the silicate concentration was decreased in nitrate content, and also interaction of silicate and phosphorous was found in the leaves. The correlation coefficient r was -0.716 and -0.647 respectively. But no statistical significance was found for the interaction of NO_3^- and SO_4^{2-} .

Discussion

The high accumulation of nitrate and silicate in the leaves and stems of orchardgrass were shown from the results with alfalfa and orchardgrass by water culture. Also, these accumulations of silicate and nitrate caused competition between anions: NO_3^- and Cl^- or NO_3^- and H_2PO_4^- . These accumulations and competition were not found in the alfalfa.

Nitrate concentration in plants increased with the rising of nitrate level of pot and water, and was greater in orchardgrass than in alfalfa tissue.

The competition of the nitrate and chlorine was seen in leaves of both orchardgrass in water or pot culture; the chlorine increase in leaves was decreased the uptake of nitrate. Also the competition of nitrate and phosphorus was similar to nitrate and chlorine, but no statistical significance was found between nitrate and sulphur. Nitrate and silicate or nitrate and phosphorus interaction was found in leaves of orchardgrass grown in pot culture. The correlation coefficient r was -0.716 and -0.647 respectively.

The relationships of anions indicated that absorption of nitrate in plants was controlled by the other anions, except sulphur. Therefore, the poisoning of dairy cattle by excess absorption of nitrate by plants was controlled phosphate, chlorine, silicate, and other anions.

Summary and Conclusion

Excessive absorption of nitrate in legumes and grass was caused nitrate poisoning in dairy cattle. This study was conducted to observe the interacting of anions (NO_3^- , Cl^- , H_2PO_4^- , SO_4^{2-} , SiO_3^{2-}) uptaken by alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.)

In the pot culture method, alfalfa (Du Puits) and orchardgrass (Kitamidori) were sowed into polyethylene pot (12 cm by 12 cm deep with 4 small drainage holes in the bottom) containing soil (4:1 mixture of Nopporo clay loam soil and sand).

The soil mixture contained 12.4 mg/100 g dry soil of available P_2O_5 , 40 mg/100 g dry soil of exchangeable K_2O and had pH 6.5 with H_2O .

Nitrate-nitrogen ($\text{NO}_3\text{-N}$) levels with KNO_3 were 50, 150 and 450 mg/pot, and silicate (SiO_3) levels with silicagel of 100 mesh for chromatography were 0.4, 0.8 and 1.6 g/pot. Each pot sowed was placed in a growth chamber set at 27°C during the day and 21°C at night, and all pots were watered with demineralized water as needed.

In the water culture method, seedlings of alfalfa and orchardgrass were grown in trays with the previously 4:1 soil mixture for 30 days in a green house. The seedlings were planted in pots with a water culture solution. Nitrate-nitrogen ($\text{NO}_3\text{-N}$) levels of the solution with KNO_3 were 3, 9 and 15 meq./l, and Cl^- , H_2PO_4^- , SO_4^{2-} were 1, 3 and 9 meq./l, respectively.

Those plants were harvested, and the anions (NO_3^- , Cl^- , PO_4^{3-} , SO_4^{2-} , SiO_3^{2-}) analyzed. Four replications of tissues from each treatment were ground 40 mesh size.

Nitrate concentration was increased with the rising of nitrate level and was greater in orchardgrass than in alfalfa tissues. The $\text{NO}_3^- \times \text{Cl}^-$ competition was found in leaves of both orchardgrass in water or pot culture; the Cl^- increase in the leaves of orchardgrass decreased with NO_3^- uptake.

Also the $\text{NO}_3^- \times \text{PO}_4^{3-}$ competition was similar to $\text{NO}_3^- \times \text{Cl}^-$, however no

statistical significance was found for the $\text{NO}_3^- \times \text{SO}_4^{2-}$ interaction. $\text{NO}_3^- \times \text{SiO}_3^{2-}$ and $\text{NO}_3^- \times \text{PO}_4^{3-}$ interaction were found in the leaves of pot cultures orchardgrass grown. The correlation coefficient r was -0.716 , and -0.647 , respectively.

In conclusion, anion interaction was observed in grass: nitrate, chlorine, phosphorous, and silicate, except for sulphate, uptaken up in the leaves of orchardgrass were shown to compete reciprocally.

Key words: Interaction of anions, Alfalfa (*Medicago sativa* L.) Orchard-grass (*Dactylis glomerata* L.) Water culture, Pot culture, Nitrate.

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要 約

アルファルファ (*Medicago sativa* L.) とオーチャードグラス (*Dactylis glomerata* L.) の硝酸イオン吸収を、他の塩素、燐酸、硫酸および硅酸イオンの共存下において、土耕法と水耕法によって検討した結果、はつぎのようであった。

アルファルファよりオーチャードグラスの方が、その体内の硝酸イオン濃度を増大する傾向にあった。土耕法でも、水耕法においても、オーチャードグラスの葉部では、 NO_3^- と Cl^- の競合が認められた。すなわち Cl^- 吸収量の増加は NO_3^- 吸収量を低下させた。また NO_3^- と PO_4^{3-} の競合も NO_3^- と Cl^- に類似していた。しかし NO_3^- と SO_4^{2-} には、その関係は見えなかった。オーチャードグラスの土耕法における NO_3^- と SiO_3^{2-} および NO_3^- と PO_4^{3-} の競合はそれぞれ $r = -0.716$, および $r = -0.647$ であった。

結論として、オーチャードグラスにおいては SO_4^{2-} を除いて、 NO_3^- , Cl^- , PO_4^{3-} , および SiO_3^{2-} の吸収において、相互に競合することが確認された。