AMMONIUM AND NITRATE UPTAKE BY BARLEY (*HORDEUM VULGARE L.*) AS INFLUENCED BY THE PRESENCE OR ABSENCE OF KAOLIN IN NUTRIENT SOLUTIONS

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ABSTRACT

In an effort to simulate field conditions a new clay-containing slurry technique of growing plants in nutrient solution was attempted.

Barley seedlings (Hordeum vulgare L. cv. Mazurka) were grown in nutrient solutions containing 2mM NO_3^- ; 2mM NH_4^+ ; and 1mM NO_3^- + 1mM NH_4^+ , all with and without 5% (w/v) kaolin content.

Rate of uptake of nitrogen, its translocation rate and the composition of the xylem bleeding sap were monitored in plants grown in all solutions.

Results obtained showed no significant differences between kaolin-containing and non-kaolin-containing solutions, although kaolin did appear to have some effect on immobilizing NH_4^+ ions (between 9 and 17% of ions in solution). This effect was insufficient for the purpose of using kaolin in the proposed slurry technique.

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CHAPTER 1 - INTRODUCTION

Dry plant material generally contains between 2 and 4% nitrogen, which is a relatively small amount when compared to the carbon content of most plants, which can often range as high as 40% of the dry material. But, because it is such an indispensible elementary constituent of numerous organic compounds of general importance, such as proteins, nucleic acids, amino acids and co-enzymes, among others, nitrogen is generally of extreme importance to all plants.

Plants generally derive the nitrogen which they require for metabolism from the soil solution. The majority of soil nitrogen is derived predominantly by biological activities associated with the nitrogen cycle. The soil nitrogen status is a function of the process of nitrogen fixation, microbial decomposition, nitrification and denitrification. Beevers (1976), states that the soil nitrogen can be divided into organic and inorganic fractions, with the majority being in the organic form. The inorganic component can be subdivided into fixed ammonia, exchangeable ammonia, nitrate (NO_3^-) and nitrite (NO_2^-).

With the exception of certain highly specialized plants such as insectivores and those engaging in symbiosis with mycorrhizal or nitrogen-fixing organisms, plants depend almost entirely on the dissolved forms of inorganic nitrogen in the soil for supplies of this essential element (Pate, 1973). The main nitrogen sources under natural conditions are the ions, ammonium and nitrate. With regard to agriculture, the forms of nitrogen most used are also the ammonium and nitrate ions (DeKock and Kirkby, 1969).

In most terrestrial ecosystems, organic materials are decomposed through microbial action with the release of ammonium in what is termed ammonification.

Ammonium made available in this way is then microbially converted first to nitrite and then to nitrate, in the nitrification process. It is principally in this highly oxidized form (NO_3^-) that nitrogen is made available to higher plants (Hewitt, et al, 1976). Both ammonium and nitrate can be assimilated by microflora and higher plants however.

The supply of mineral nitrogen in most plants is generally dependent on the rate of mineralization (i.e. ammonification and nitrification) carried out by micro-organisms, except where symbiotic or non-symbiotic nitrogen fixation is occurring to any great extent. In most soils, inorganic nitrogen is continuously being formed from organic nitrogen by mineralization. In turn, some of the mineral nitrogen is being transferred into organically bound nitrogen by soil microbes and thus effectively immobilized. The quantity of mineral nitrogen present in the soil and available to the plant is thus dependent on both processes, mineralization and immobilization.

Schrader, et al (1972), state that most plant roots are thought to absorb nitrate more readil y than ammonium, while Haynes and Goh (1978), state that in some soils plants may show more growth and greater nitrogen absorption when nitrate rather than ammonium is the source of nitrogen. This is partly because these soils bind added ammonium, thus rendering it unavailable to plants. This fixation of ammonium occurs when the cations calcium, potassium, magnesium and sodium in the expanded lattice of certain clay minerals are replaced by ammonium ions. Black and Waring (1972), have shown that soils which contain large quantities of micaceous clay minerals, such as vermiculite, illite and montmorrilonite, are able to fix added ammonium nitrogen. Clays consist of layers of hydrated silica and alumina (Hopkins, 1965). The two most important forms of clay occurring in soils belong to the kaolin

and montmorrilinoid groups. Clays of the kaolin group consist of alternate layers of silica and alumina (Si-Al) held firmly together, while clays of the montmorrilinoid group consist of two layers of silica to one of alumina (Si-Al-Si), held less firmly together. This factor makes it easier for NH_4^+ ions to exchange with cations in the lattice of the negatively charged clay of the montmorrilinoid group than with those of the kaolin group.

The evidence on the availability of recently fixed ammonium to plants suggest that it is low, while native fixed ammonium in many soils which have not been previously fertilized with ammonium, is generally unavailable to plants (Haynes and Goh, 1978).

Results obtained have shown considerable reductions in plant growth and nitrogen uptake caused by the fixation of applied ammonium. The availability of nitrogen to plants appears to be inversely related to ammonium fixation (Haynes and Goh, 1978).

The level of ammonium-nitrogen available to plants in the soil is further decreased when micro-organisms immobilize the applied nitrogen. Jansson (1971), showed that micro-organisms prefer ammonium- to nitrate-nitrogen when a choice is possible. Research has also found lower recoveries of applied nitrogen by plants from ammonium fertilizers as compared with nitrate fertilizers because of a corresponding increase in percentage of ammonium-nitrogen immobilized, both by the soil and by micro-organisms (Haynes and Goh, 1978).

In general, the reactions of nitrate in soils are not very important as far as plant nutrition is concerned. Nitrate is not immobilized by clay containing soils because its negative charge acts to repel it from these soils. Nitrate may be lost from the soil solution via denitrification back to the

atmosphere through the action of denitrifying bacteria.

The other important mechanism whereby nitrogen is lost to the plant is through the action of leaching. Because most temperate soils possess an overall negative charge on their colloids which repels the nitrate ion, the latter may move downwards through the soil faster than the drainage water. In tropical soils however, losses of nitrate by leaching are less than from most temperate soils (Haynes and Goh, 1978). In spite of these losses however, nitrate remains the major source of nitrogen for most plants.

When ammonium fertilizers are applied to non-acidic soils, the NH_4^+ ion is rapidly converted to nitrate-nitrogen by soil bacteria . Similarly, urea is also rapidly nitrified after hydrolysis, so that in agricultural practice there is little difference to be expected from the use of either nitrate or ammonium fertilizers (DeKock, 1972). Differences in effect have however, become apparent between these forms of nitrogen with the introduction of nutrient culture techniques.

In the field, most plant roots are thought to absorb nitrate more readilly than ammonium, generally because ammonium is more prone to immobilization by the soil and micro-organisms than is nitrate (Schrader, et al, 1972).

Diffe ring results have been obtained when growing plants in nutrient solutions however. DeKock and Kirkby (1969), state that conclusive evidence now exists to show that plants grow differently when supplied with the two forms of nitrogen in culture media.

Fried, et al (1965), showed that ammonium-nitrogen was taken up more rapidly by young rice seedlings and wheat than nitrate nitrogen when each was the

only nitrogen source. When both NH_4^+ and NO_3^- were present in the same nutrient media at the same concentration however, NH_4^+ was taken up at a faster rate and was thought to inhibit NO_3^- uptake (Fried, et al, 1965).

Schrader, et al (1972), quoting Minotti, Williams and Jackson (1969), reported that the rate of ammonium uptake from nutrient solutions of the same concentrations by wheat seedlings was considerably faster than nitrate uptake, when each was the only nitrogen source. Schrader, et al (1972), demonstrated that when corn (Zea mays L.) was grown in nutrient solutions of the same concentration under greenhouse conditions, the rates of nutrient uptake of both nitrate-nitrogen and ammonium nitrogen were similar, with neither being retarded in the presence of the other. These results were confirmed in work performed by Warncke and Barber (1973).

More recently it has been demonstrated by Lewis and Chadwick (in press) that barley seedlings (Hordeum vulgare L.) growing in nutrient solutions containing ammonium-nitrogen as the nitrogen source, had a faster rate of nutrient uptake than similar plants grown in nitrate supplied solutions.

From the above brief survey of the work so far performed, it can be seen that seedlings, when grown in nutrient solutions, seem to absorb the ammonium ion more rapidly and in preference to the nitrate ion. This is probably because NO_3^- absorption is an active process, reliant on energy dependent permeases, whereas NH_4^+ absorption is largely a simple diffusion process.

But, in the field, most plants use nitrate rather than ammonium because of the immobilization of the latter. Thus it can be seen that studies of the rates of inorganic nitrogen uptake, its incorporation into organic nitrogenous

compounds, and the translocation of both inorganic and organic nitrogen through the plant, with plants grown in liquid nutrient solutions, do not provide a true simulation of conditions pertaining to the soil. Because of this, and also because of the difficulties involved in monitoring the rate of nutrient uptake by seedlings grown in potted soil, it was decided to try a new technique involving the use of a clay-containing slurry in an effort to simulate field conditions.

Barley seedlings (Hordeum vulgare L.) were grown in nutrient solutions containing, as nitrogen sources, ammonium only, nitrate only and a 1:1 mix of ammonium and nitrate. Seedlings were also grown in the same solutions just mentioned, but with the addition of 5% (w/v) kaolin.

The rate of uptake of nitrogen, its translocation rate and the composition of the xylem bleeding sap were monitored in plants grown in all solutions. Results were then compared in an effort to establish whether the addition of kaolin to the nutrient solution affected these variables in any way, and thus to establish whether the slurry technique might provide an adequate simulation of soil conditions.

CHAPTER 2 - MATERIALS AND METHODS

Barley plants (Hordeum vulgare L. cv. Mazurka) were grown in different nutrient solutions over a period of 21 days.

Eight 3.0 litre plastic pots were prepared by filling with distilled water and fixing fine nylon mesh over their openings. Between 200 and 300 seeds were placed on top of this mesh. The seeds were then allowed to germinate over a period of three days in complete darkness, after which they were uncovered. On day 4, the distilled water was replaced with nitrogen-free Long Ashton nutrient medium. This solution was topped up daily until day 7. At this point the following nitrogen supplements were made up and added to fresh Long Ashton solution, which then replaced the old nutrient solution present in the pots:

a) 2mM NO_3^- —— added to 2 pots.

b) 2mM NH_4^+ —— added to 2 pots.

c) 1mM NO_3^- + 1mM NH_4^+ —— added to 2 pots.

d) 2 pots were used as controls and had no nutrients added to them.

The contents of the pots were changed every 48 hours until day 14, at which point they were changed every 24 hours until day 20.

To the 2mM NH_4^+ solutions were added 2g $CaCO_3$ per litre and to the 1mM NH_4^+ solutions, 1g $CaCO_3$ per litre. This was to prevent NH_4^+ toxicity (Hewitt, 1966; Maynard and Barker, 1969), keeping the pH of the solutions between 5.8 and 6.2. pH values were checked daily.

Nutrient solutions were aerated using small Whisper 700 air pumps connected to polythene manifolds which were placed at the bottom of the

pots. This was done to prevent the buildup of bacterial nitrate reductase observed in barley root systems when grown in poorly aerated solutions (Blevins, Lowe and Staples, 1976, quoted by Lewis, et al, 1982). It also prevented the excretion of NO_2^- by young barley roots in oxygen deficient conditions, as observed by Lewis, et al (1982).

The plants were grown to 20 days old in Conviron Controlled Environment Growth Cabinets with a 20°C (max.) day temperature and an 18°C (min.) night temperature. Day length was fixed at 14 hours. Light intensity was kept constant at 295 microeinsteins m^{-2} s^{-1}, and was measured at periodic intervals at the plant surface, using a Crump Quantum Radiometer-Photometer. Humidity was kept constant at 70% and was measured at regular intervals.

A second set of plants was prepared as described, but 150g of kaolin (obtained from Serina Kaolin, Fish Hoek, Cape Town) were added to each pot, giving an effective 5% (w/v) kaolin solution. The kaolin was kept in solution via aeration from the air pumps. This was only partially successful as kaolin tended to settle to the bottom of the pots over a period of time.

Nitrate and Ammonium Assays of Nutrient Media.

1ml of nutrient solution was removed from each pot at hourly intervals, beginning at 8am and terminating at 4pm (i.e. over a period of 8 hours). A Gilson Pipetman dispensor was used to extract the sample. Prior to removal of the sample, the old nutrient solution in each pot was replaced with a freshly made-up solution. The 1ml removed was replaced by adding 1ml of nutrient-free Long Ashton solution to each pot. Samples, which were placed into test tubes, were then deep-frozen prior to assaying. Assays were

carried out in order to determine how much NO_3^- and NH_4^+ were taken up by the plants over the eight hour period. Mean hourly rates per plant could then be calculated and the results compared.

Nitrate was determined by using the Salicylic Acid method as outlined by Cataldo, et al (1975).

0.2ml aliquots of each sample were pipetted into boiling tubes and mixed thoroughly with 0.8ml of 5% (w/v) salicylic acid in concentrated H_2SO_4. After 20 minutes at room temperature, 19ml of 2N NaOH were slowly added with a repipette to raise the pH above 12. Samples were cooled to room temperature and absorbance at 410nm was determined in a Beckman Model 42 Spectrophotometer. Two replicates per sample per pot were analysed. A standard calibration curve was determined in the same way as outlined above, using a range of from 0 to 150 μg NO_3^- -nitrogen per ml (see Fig.1). Standards were also incorporated into each set of samples. Blanks, consisting of 0.2ml distilled water, 0.8ml of concentrated H_2SO_4 (without salicylic acid) and 19ml of 2N NaOH were also assayed. The salicylic acid-H_2SO_4 reagent was made fresh for each assay while the nitrate standards were stored at 0^oC. Nitrate-nitrogen concentrations were expressed as μg NO_3^- -N ml^{-1}.

Ammonium was determined by using the Manual Indo-Phenol Blue method as outlined by Stock (1983).

2mM NH_4^+ solutions were diluted 10 times, while 1mM NH_4^+ + 1mM NO_3^- solutions were diluted 5 times. To 2ml of the diluted sample , or standard, the following reagents were sequentially added with Gilson Pipetman dispensors:

a)1.6ml 10% (w/v) sodium potassium tartarate solution.

b)0.2ml 0.16% (w/v) sodium nitroprusside solution.

c)0.4ml sodium phenate reagent prepared fresh each day by dissolving 12.5g phenol in 70ml 40% NaOH and diluting to 100ml.

d)0.2ml sodium hypochlorite with 5% available Cl.

The reagents were mixed and the solution made up to 10ml. After 20 minutes incubation in a waterbath at 40°C the solutions were cooled for 2 minutes in an ice water bath and the absorbance was read within 10 minutes at 625nm on a Beckman Model 42 Spectrophotometer.

Batches of 15 samples, 3blanks and 5 standards were run simultaneously. 2 replicates per sample per pot were analysed. Standards were prepared in the range 0.1 to 3.0 μg N ml^{-1}.

In an effort to determine how much NH_4^+ was being held by the kaolin, Kjeldahl analyses of the samples were performed. Inconclusive results were obtained and it was decided to try a different method. Standard solutions were prepared (i.e. 2mM NH_4^+ in Long Ashton medium and 1mM NH_4^+ + 1mM NO_3^- in Long Ashton medium) to which were added 5% (w/v) kaolin. Prior to addition of the kaolin, samples were taken from the solutions and deep-frozen. Kaolin was then added and the solutions were allowed to stand for 24 hours. Air was bubbled through the solutions as previously described. At the end of the 24 hour period further samples were extracted. These and the first set of samples were then analysed for ammonium content by the Manual Indo-Phenol Blue method. The amount of ammonium held by the kaolin could then be calculated.

Transpiration Rate

The flow of water through the plant, induced by transpiration, provides a transport system for minerals from the surrounding solution. The constant removal of solution thus has the effect of mobilizing nutrients and carrying them to the roots and thus to the rest of the plant. By measuring the amount of water lost via transpiration it is possible to assess the rate of delivery of inorganic nitrogen from the root to the leaf via the xylem stream. The transpiration rates of the plants grown under the above conditions were measured as follows:

At day 20 the pots were weighed every hour (from 8am to 4pm - i.e. a period of 9 hours) prior to removal of the 1ml sample. A Mettler PC 8000 balance was used (see Fig. 2). Growth addition due to photosynthesis was assumed to be negligible during the 8 hours. After the experiment the rate of water loss (ml^{-1} $plant^{-1}$ $hour^{-1}$) was calculated.

Collection and Analysis of Xylem Sap.

Xylem sap was collected on the day following the sampling and transpiration experiments (i.e. day 21). Shoots of about half the plants were severed just below the point of insertion of the first leaf (at 8am). The stumps were washed with demineralized water and blotted. Xylem sap expelled from the stumps was then collected by Pasteur pipette during the next hour (see Fig. 3). The procedure was repeated at 12 noon (i.e. 4 hours later) with the remaining plants. Freeze dried sap was used for amino acid and ammonium analyses. This was carried out on a Beckman 120C analyser using a lithium buffer method.

Fresh sap was analysed for NO_3^- content using the salacylic acid method as previously described.

Results were statistically tested for significance using Lord's Range Test for small samples.

CHAPTER 3 - RESULTS

Transpiration Rates.

Figures 4 to 7 indicate transpiration rates over the 8 hour period under consideration. Table 1 summarises the mean transpiration rates of seedlings grown under the 4 nutrient regimes.

Referring to plants grown in solutions without the addition of 5% kaolin it can be seen that the highest transpiration rate was recorded for those grown with the 1:1 mix of NH_4^+ and NO_3^-, followed by those grown with the 2mM NO_3^-, 2mM NH_4^+ and the control (Table 1).

With reference to plants grown in solutions containing 5% kaolin, it can be seen that the highest mean transpiration rate was recorded for those grown with the 1:1 mix, followed by 2mM NH_4^+, 2mM NO_3^- and the control.

Comparing transpiration rates between plants grown with nutrient solutions containing and lacking 5% kaolin, it can be seen, by referring to Figures 4 to 7, and Table 1, that:

i) 1:1 mix -- the transpiration rates of plants grown in kaolin- and non-kaolin-containing solutions were similar.

ii) 2mM NH^+_4 -- a higher transpiration rate was evident with the addition of kaolin.

iii) 2mM NO_3^- -- transpiration rate of plants grown in solutions lacking kaolin was higher than those grown in solutions containing kaolin.

iv) Control -- transpiration rates of plants grown in kaolin-and non-kaolin-containing solutions were similar.

Table 2 indicates the significance of differences between transpiration rates resulting from growth with different nitrogen sources.

In plants grown in solutions containing kaolin, it can be seen that transpiration rates of those grown with 2mM NO_3^-, 2mM NH_4^+ and the 1:1 mix were significantly higher (at 5% level) than the transpiration rates of the control plants. No significant differences were apparent when comparing transpiration rates of plants grown on NO_3^- and NH_4^+, NO_3^- and the 1:1 mix, or NH_4^+ and the 1:1 mix.

The same situation was apparent when looking at plants grown with nutrient solutions lacking the 5% kaolin.
Table 3 shows that there were no significant differences in transpiration rates between plants grown in kaolin-containing as opposed to kaolin-free solutions.

Nitrogen transport in xylem sap.

Nitrate-nitrogen.

In solutions containing 5% kaolin, at the 2 hour analysis, it was apparent that roots were transferring nitrate in large quantities to the shoot (17 μmol ml^{-1}, i.e. 55% of the total nitrogen - see Table 4 and Fig. 8). This was also the case in plants grown without kaolin (30% of total nitrogen - Table 4; Fig. 8), although the amount of nitrate nitrogen present in the sap was lower than that of plants grown in kaolin-containing solutions.

At the 6 hour analysis, the amount of nitrate transported in the xylem sap of kaolin-grown plants had dropped to 25% of the total nitrogen, while that of non-kaolin plants had increased to 41% of the total nitrogen (Table 4;

Fig. 8). At this stage the actual amount of nitrate transported by the differently treated plants was very similar.

In plants grown with the 1:1 mix of NH_4^+ and NO_3^-, containing kaolin, the amount of NO_3^- transported at the 2 hour mark was 20% of the total nitrogen (Table 4; Fig. 8). In the case of plants grown in the non-kaolin containing solutions, only traces of nitrate were present. The situation was the same at the 6 hour collection, except that the amount of nitrate carried in the xylem sap of kaolin-grown plants had dropped to 11% of the total nitrogen (Table 4; Fig. 8).

In the case of plants grown with NH_4^+ as a nitrogen source, nitrate was absent from the xylem sap (Table 4; Fig. 8).

The plants displaying the highest quantity of nitrate in the xylem sap were generally those grown with the 2mM NO_3^- solution as a nitrogen source, followed by those grown on the 1:1 mix, with zero nitrate in those grown in 2mM NH_4^+ as a nutrient source.

Ammonium-nitrogen.

In solutions of 2mM NO_3^-, both containing and lacking 5% kaolin , the level of ammonium-nitrogen was generally low in all cases (2-3% of total nitrogen) and did not fluctuate markedly between treatments or with time (Table 4; Fig. 9).

In the case of plants grown with a 1:1 mix of NH_4^+ and NO_3^-, the amount of ammonium present at all times and in both treatments was again low (0.5%-2% of total nitrogen).

At the 2 hour period, the amount of NH_4^+ - nitrogen was similar for these plants, but at the 6 hour point, this had, in the case of solutions containing kaolin, dropped by more than half. In the case of plants grown in solutions lacking kaolin, the amount had doubled (Table 4; Fig. 9).

In solutions of 2mM NH_4^+ it can be seen, by reffering to Fig. 9, and Table 4, that at no time did NH_4^+ - nitrogen represent a great percentage of total nitrogen transported through the sap (from 0.5% - 1% of total nitrogen). There was also not much difference at the 2 hour or the 6 hour mark between plants treated with kaolin and those not treated with kaolin. In plants grown with kaolin however, the amount of NH_4^+ present in the xylem sap at the 6 hour mark was twice that of the 2 hour mark. This was also the case for plants grown without kaolin (Table 4; Fig. 9).

Looking at the overall picture however, it can be seen that the NH_4^+ - nitrogen content of the bleeding sap did not differ greatly between nutrient solutions and that the actual amount of NH_4^+ - nitrogen present in the bleeding sap was low compared to the 2mM NO_3^- and 2mM NH_4^+ nutrient solutions (Table 4; Fig. 9).

Organic nitrogen.

At the 2 hour point, the organic nitrogen content of the xylem sap of plants grown in 2mM NO_3^- plus kaolin was 19% of the total nitrogen content, compared to 31% for non-kaolin treated plants (Table 4; Fig. 10). After 6 hours the kaolin treated plants had an organic nitrogen content comprising 36% of the total nitrogen, compared to 24% for plants grown without kaolin.

In the case of the 1:1 mix, organic nitrogen levels did not fluctuate greatly.

These ranged from 39% - 44% (after 2 and 6 hours respectively) in the kaolin treated plants, to 46% - 48% (at 2 and 6 hours respectively) in the case of plants grown in the solution lacking kaolin (Table 4; Fig. 10).

In the case of plants grown in the 2mM NH_4^+ solutions, the levels of organic nitrogen ranged between 49% - 50% of total nitrogen content at all times (Table 4; Fig. 10) and for both treatments.

Overall, the xylem sap of plants grown on NH_4^+ - containing solutions had the highest organic nitrogen content, followed by the 1:1 mix, and then the 2mM NO_3^- treatment.

In all treatments the main form of organic nitrogen was in the form of glutamine, ranging from 30% of the total nitrogen content (2mM NH_4^+), to 21% (1:1 mix) to 12% (2mM NO_3^-). This was followed, in all cases, by asparagine, alanine, serine and arganine (Table 4).

Uptake of nutrients from solution.

Figures 11 to 15 indicate the amount of nitrogen, in the form of NO_3^-, NH_4^+ and both, removed from solution over the 8 hour period under consideration. The curves shown are generally of a power curve nature. In the case of absorption of the NO_3^- ion from the 2mM NO_3^- solution treated with kaolin, reference to Figure 13 shows some fluctuation after approximately 5 hours.

Reference to Table 5 shows, in the case of the solutions containing 5% kaolin, that the overall rates of uptake were similar in the 1:1 mix and the 2mM NO_3^- solution. The rate of uptake for the 2mM NH_4^+ solution was approximately half that of the other two. In the 1:1 mix, NO_3^- was taken up at a faster rate

than NH_4^+.

In the case of solutions lacking the kaolin component, the rates of uptake were similar in all cases. In the 1:1 mix , uptake of NO_3^- was similar to that of NH_4^+.

When comparing kaolin-treated to non kaolin-treated solutions, it can be seen (Table 5) that in every case the rate of nutrient uptake was faster for the plants using the kaolin treated solutions.

Statistical tests performed on these results did not show any significant differences in rates of uptake.

There were, in certain cases, high standard errors of the mean resulting from the fact that only two pots were used to calculate mean rates of nutrient uptake in every case. These values have consequently not been shown in Figures 11 to 15. There was however, generally good agreement between replicates taken from individual pots.

Immobilization of NH_4^+ ion by kaolin.

Reference to Table 6 shows that 16% of the NH_4^+ ion present in a 2mM NH_4^+ solution was immobilized. The figure for the 1:1 mix was 9%.

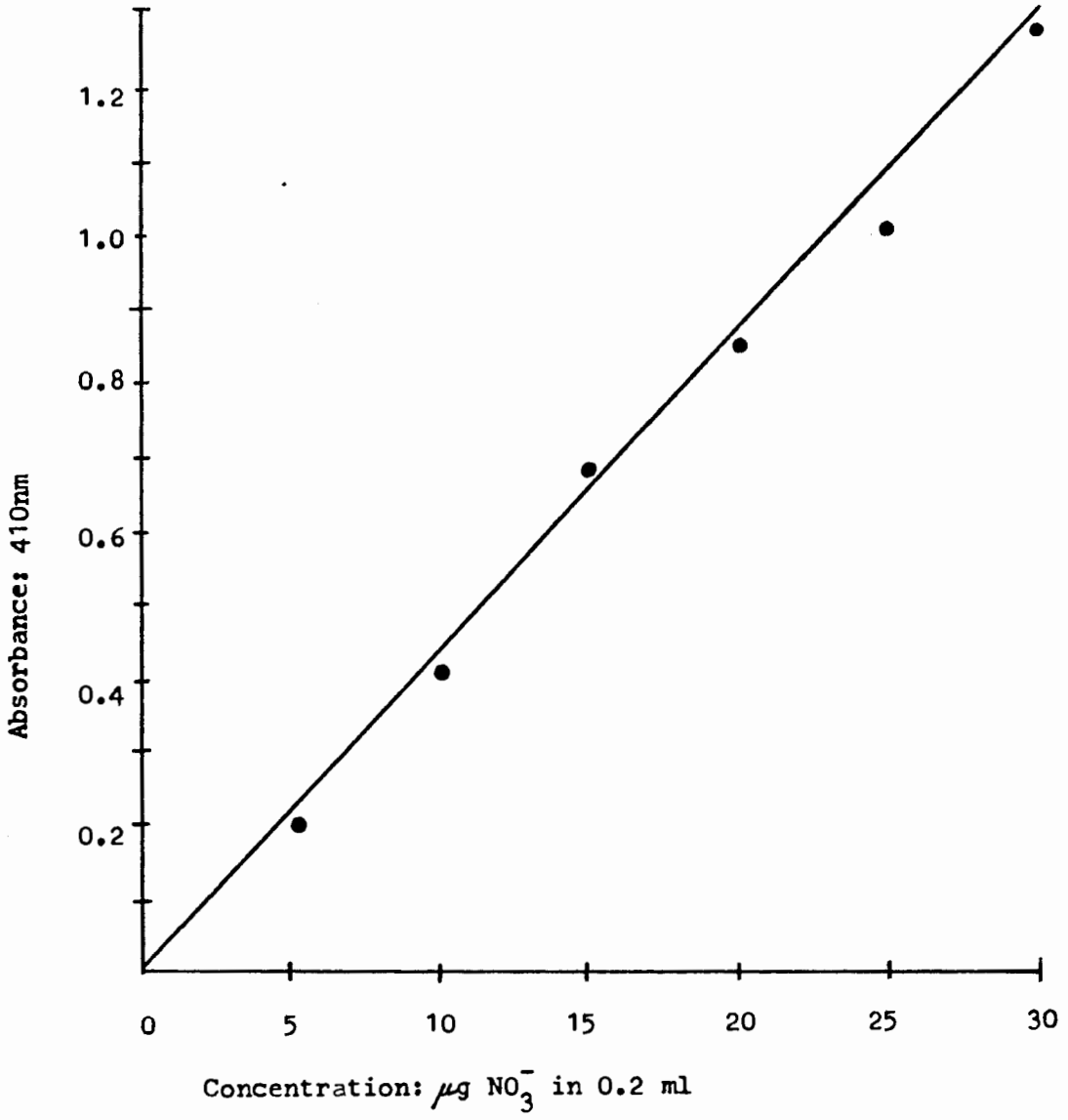

Figure 1: Standard curve showing the linear response between absorbance and NO_3^- - N, using the method of Cataldo, et al (1975). Absorbance was determined directly in 0.2 ml samples. Results were means of 3 replicates. Regression equation: y= 0. 0071 + 0.043x; r = 0.998.

Figure 2: Weighing of pots for determination of transpiration rates.

Figure 3: Collection of xylem bleeding sap.

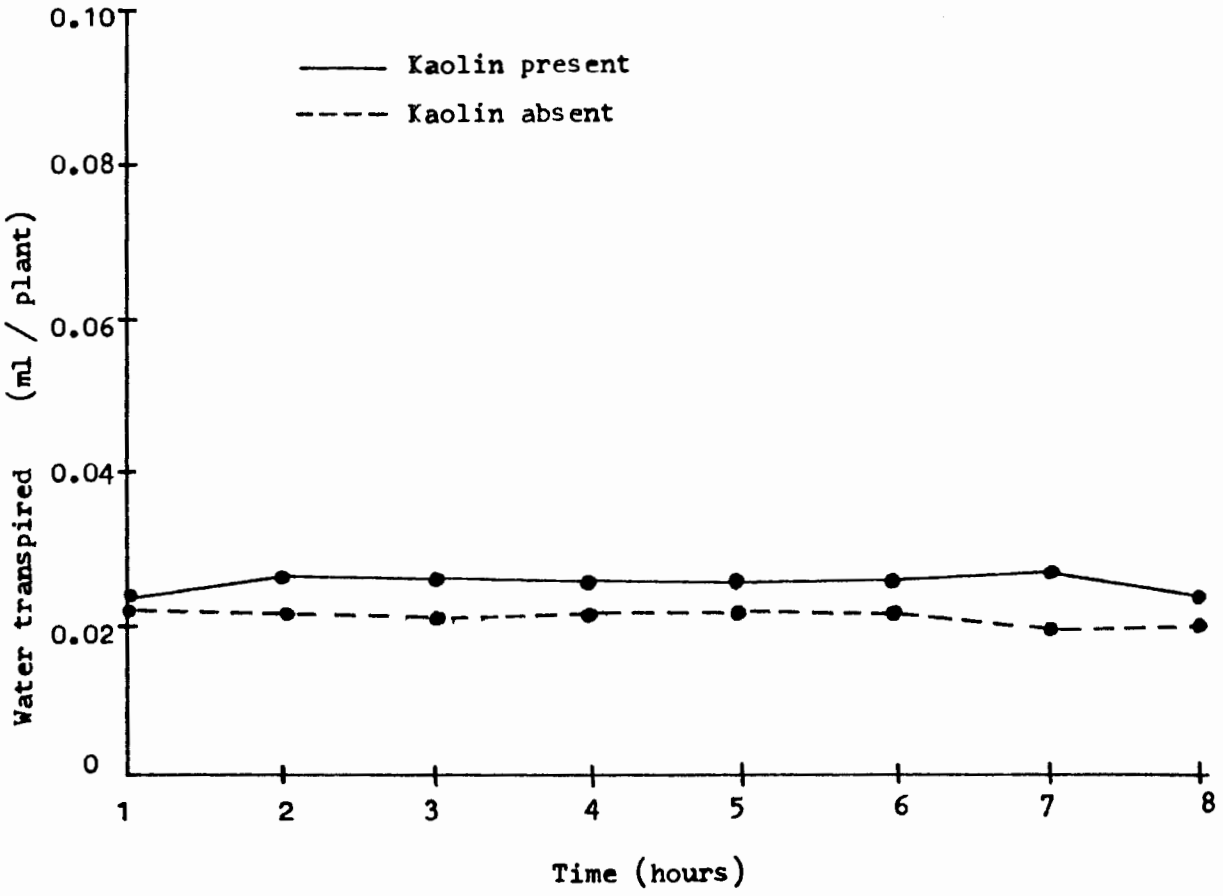

Figure 4: Transpiration rate of 20 day old barley seedlings grown in nitrogen-free solutions, with and without 5% (w/v) kaolin, measured at hourly intervals over 8 hour period. Points represent mean values of 2 pots.

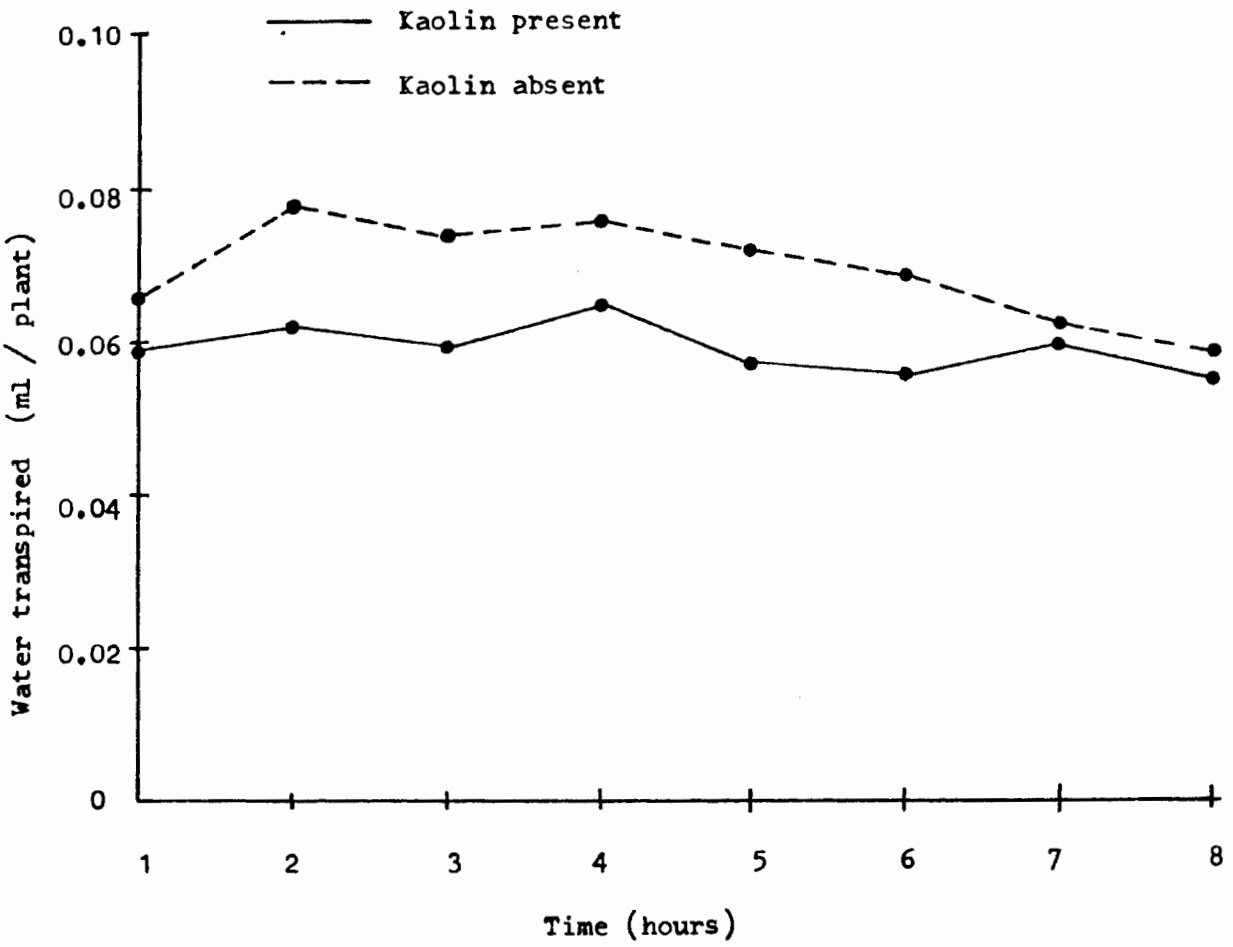

Figure 5: Transpiration rate of 20 day old barley seedlings, grown in nutrient solutions containing 2mM NO_3^- - N, with and without 5%(w/v) kaolin, measured at hourly intervals over 8 hour period. Points represent mean values of 2 pots.

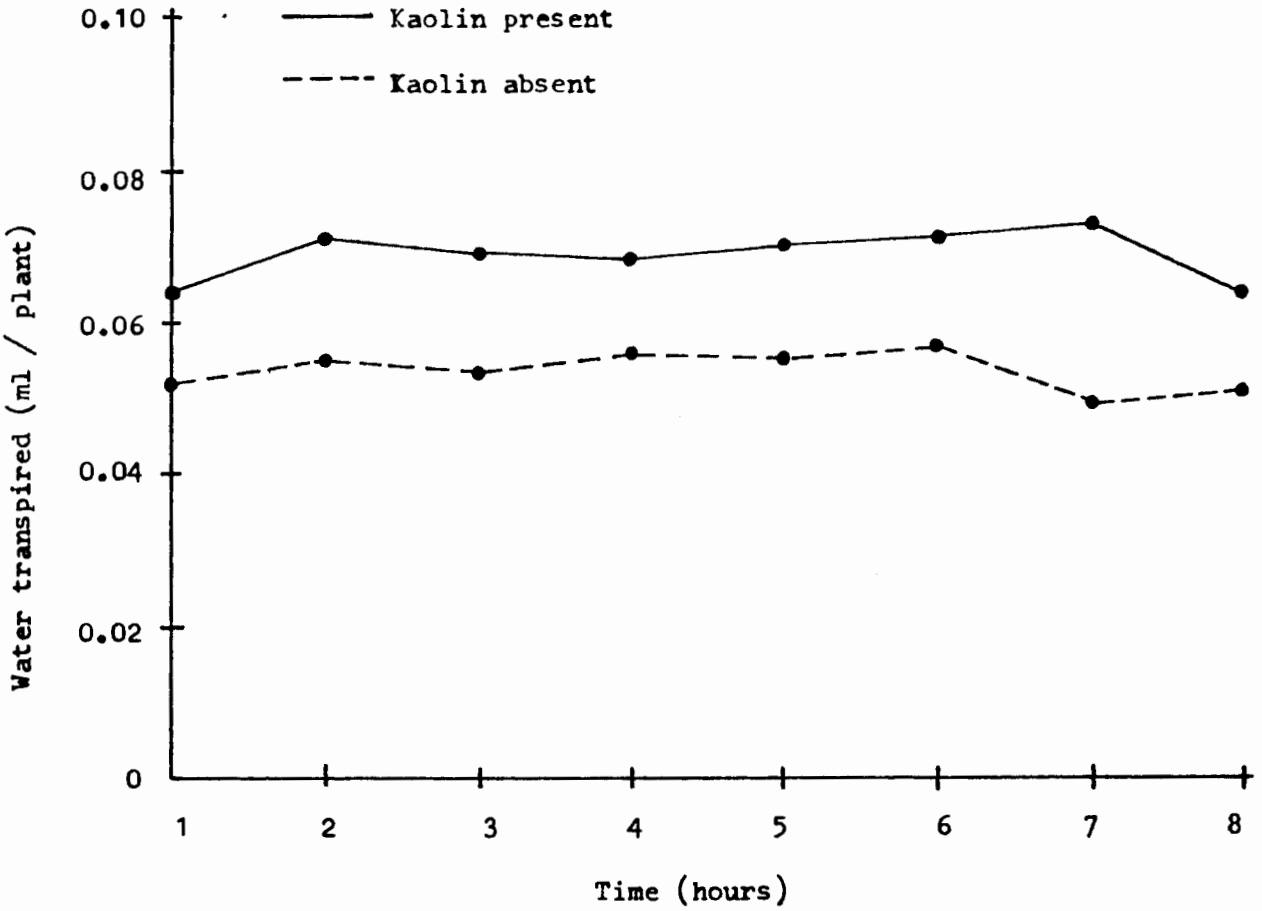

Figure 6: Transpiration rate of 20 day old barley seedlings grown in nutrient solutions containing 2mM NH_4^+ - N with and without 5% (w/v) kaolin, measured at hourly intervals over 8 hour period. Points represent means of 2 pots.

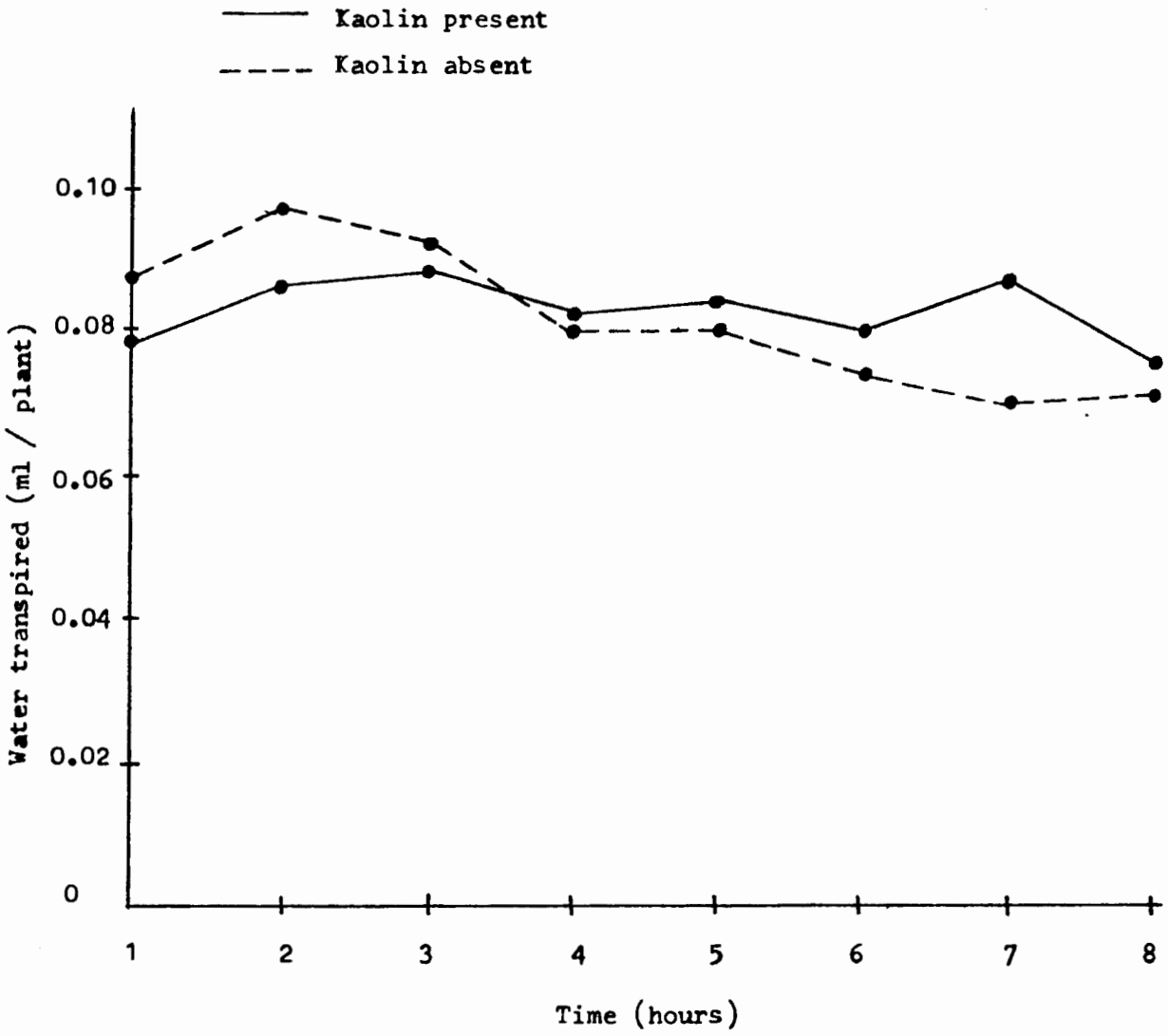

Figure 7: Transpiration rate of 20 day old barley seedlings, grown in nutrient solutions containing 1mM NO_3^- + 1mM NH_4^+ with and without 5% (w/v) kaolim, measured at hourly intervals over 8 hour period. Points represent means of 2 pots.

Table 1: Mean transpiration rate (ml $plant^{-1}$ hr^{-1}) of 20 day old barley seedlings for 4 nutrient regimes (N-free; 2mM NO_3^- ; 2mM NH_4^+ ; 1mM NO_3^- + 1mM NH_4^+) with and without 5% (w/v) kaolin content, measured over 8 hour period at 20°C. Results indicate means of 2 pots and standard errors of the means.

Nutrient Solution	With Kaolin	Without Kaolin
	ml hr^{-1} $plant^{-1}$	ml hr^{-1} $plant^{-1}$
N - Free (control)	0.025 ± 0.0007	0.021 ± 0.0006
2mM NO_3^-	0.059 ± 0.009	0.070 ± 0.009
2mM NH_4^+	0.069 ± 0.007	0.053 ± 0.008
1mM NO_3^- + 1mM NH_4^+	0.083 ± 0.0007	0.081 ± 0.011

Table 2: Results of Lord's Range Test for Small Samples performed on data expressed in Table 1. Tests performed to ascertain significance of differences between transpiration rates of 20 day old barley seedlings resulting from growth in 4 different nutrient solutions. (+ = significant at 5% level; 0 = not significant).

Tested for significance between	With Kaolin	Without Kaolin
Control and NO_3^-	+	+
Control and NH_4^+	+	+
Control and 1:1 mix	+	+
NO_3^- and NH_4^+	0	0
NO_3^- and 1:1 mix	0	0
NH_4^+ and 1:1 mix	0	0

Table 3: Results of Lord's Range Test for Small Samples performed on data expressed in Table 1. Tests performed to ascertain significance of differences between transpiration rates of 20 day old barley seedlings grown in nutrient solutions with and without 5% (w/v) kaolin. (+ = significant at 5% level; 0 = not significant; +k = 5% kaolin present; -k = kaolin absent).

TESTED FOR SIGNIFICANCE BETWEEN	SIGNIFICANCE
N - free (+k) & N - free (-k) (control)	0
NO_3^- (+k) & NO_3^- (-k)	0
NH_4^+ (+k) & NH_4^+ (-k)	0
1:1 mix (+k) & 1:1 mix (-k)	0

Table 4: Nitrate, ammonium and amino compound composition (μmol N ml^{-1}) of xylem sap of 21 day old barley seedlings at 2 hours and 6 hours after commencement of feeding. Plants were grown on nitrate, 1:1 nitrate + ammonium or ammonium at nitrogen concentrations of 2mM. (+K = Kaolin present; -K = Kaolin absent).

Compound	NO_3^-				1:1 NO_3^- + NH_4^+				NH_4^+			
	2 hrs		6 hrs		2 hrs		6 hrs		2 hrs		6 hrs	
	+K	-K	+K	-K	+K	-K	+K	-K	+K	-K	+K	-K
ASP	0.183	0.173	0.233	0.137	0.183	0.083	0.276	0.092	0.160	0.149	0.135	0.113
THR	0.219	0.265	0.465	0.219	0.423	0.159	0.634	0.286	0.318	0.184	0.313	0.369
SER	0.432	0.512	1.104	0.449	1.430	0.395	2.124	0.821	0.762	0.512	0.668	0.898
ASN	1.598	1.369	2.322	1.258	3.910	2.543	2.169	4.297	16.107	4.755	13.779	8.695
GLU	0.092	0.107	0.147	0.125	0.094	0.044	0.172	0	0	0.046	0.052	0
GLN	1.742	3.594	8.971	3.570	9.740	6.822	6.776	23.093	27.011	6.720	19.708	32.153
PRO	0	0	0	0	0	0	0	0	0	0	0	0
GLY	0.012	0.012	0.067	0.019	0.082	0.012	0.151	0.041	0.046	0.018	0.009	0.030
ALA	0.149	0.186	0.801	0.198	5.512	0.248	8.333	0.732	0.392	0.269	0.239	0.717
VAL	0.255	0.263	0.391	0.209	0.279	0.065	0.506	0.230	0.319	0.345	0.238	0.180
CYS	0	0	0	0.014	0.032	0	0.007	0	0.028	0	0	0
MET	0	0	0.043	0	0	0.009	0	0.039	0	0.006	0.009	0.027
ILE	0.112	0.143	0.145	0.055	0.124	0.048	0.029	0.052	0.072	0.083	0.074	0.059
LEU	0.142	0.206	0.159	0.173	0.170	0.057	0.238	0.087	0.091	0.086	0.093	0.059
TYR	0.029	0.045	0.073	0.025	0.063	0.011	0.094	0.043	0.031	0.027	0.037	0.016
PAL	0.071	0.100	0.099	0.050	0.100	0.022	0.139	0.061	0.050	0.102	0.043	0.040
LYS	0.338	0.489	0.765	0.447	0.461	0.317	0.524	0.518	0.481	0.336	0.612	0.562
HIS	0.186	0.257	0.401	0.275	0.169	0.071	0.345	0.060	0.083	0.081	0.156	0.167
ARG	0.488	0.732	1.204	0.932	0.550	0.456	1.240	0.524	0.564	0.264	0.862	0.844
DAB	0.123	0.102	0.418	0.155	1.216	0.081	3.594	0.176	0.216	0.125	0.173	0.117
TOTAL ORGANIC N	6.18	8.55	18.74	8.31	24.54	11.44	27.35	31.15	46.72	14.10	37.14	45.04
AMMONIUM - N	1.08	1.03	0.93	1.08	0.75	0.56	0.31	1.27	0.48	0.34	0.78	0.62
TOTAL ORGANIC + AMMONIUM - N	7.26	9.58	19.67	10.11	25.29	12.00	27.66	32.42	47.20	14.44	37.92	45.66
NITRATE - N	17.62	8.20	13.44	14.52	12.90	TRACE	6.95	TRACE	TRACE	0	0	0
TOTAL N	32.14	27.36	52.78	34.74	63.48	24.00	62.27	64.84	94.00	28.88	75.84	91.32

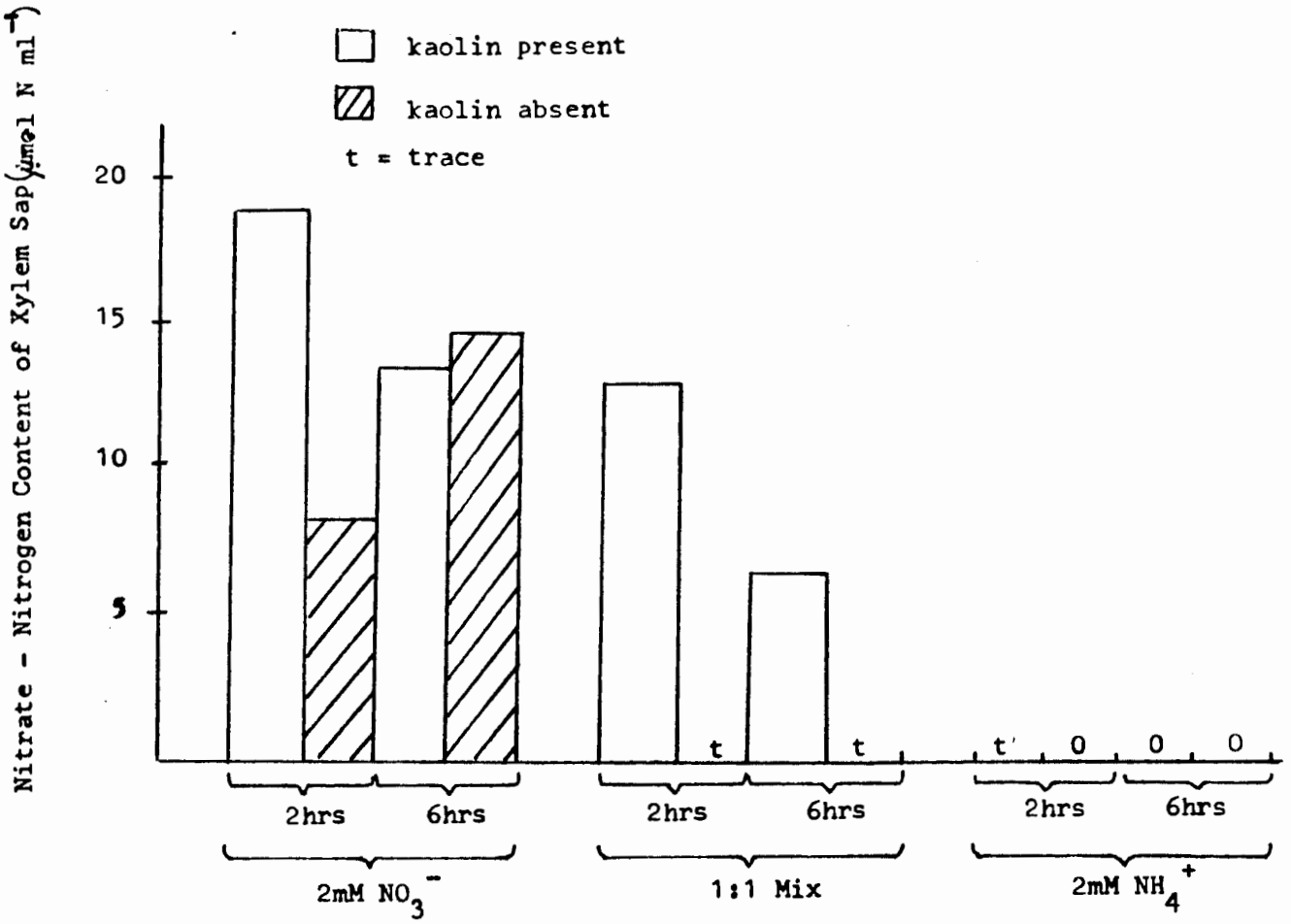

Figure 8: Total Nitrate - Nitrogen Content of Xylem Sap of 21day old barley plants measured 2 hours and 6 hours after commencement of feeding. Results represent mean values of 2 pots.

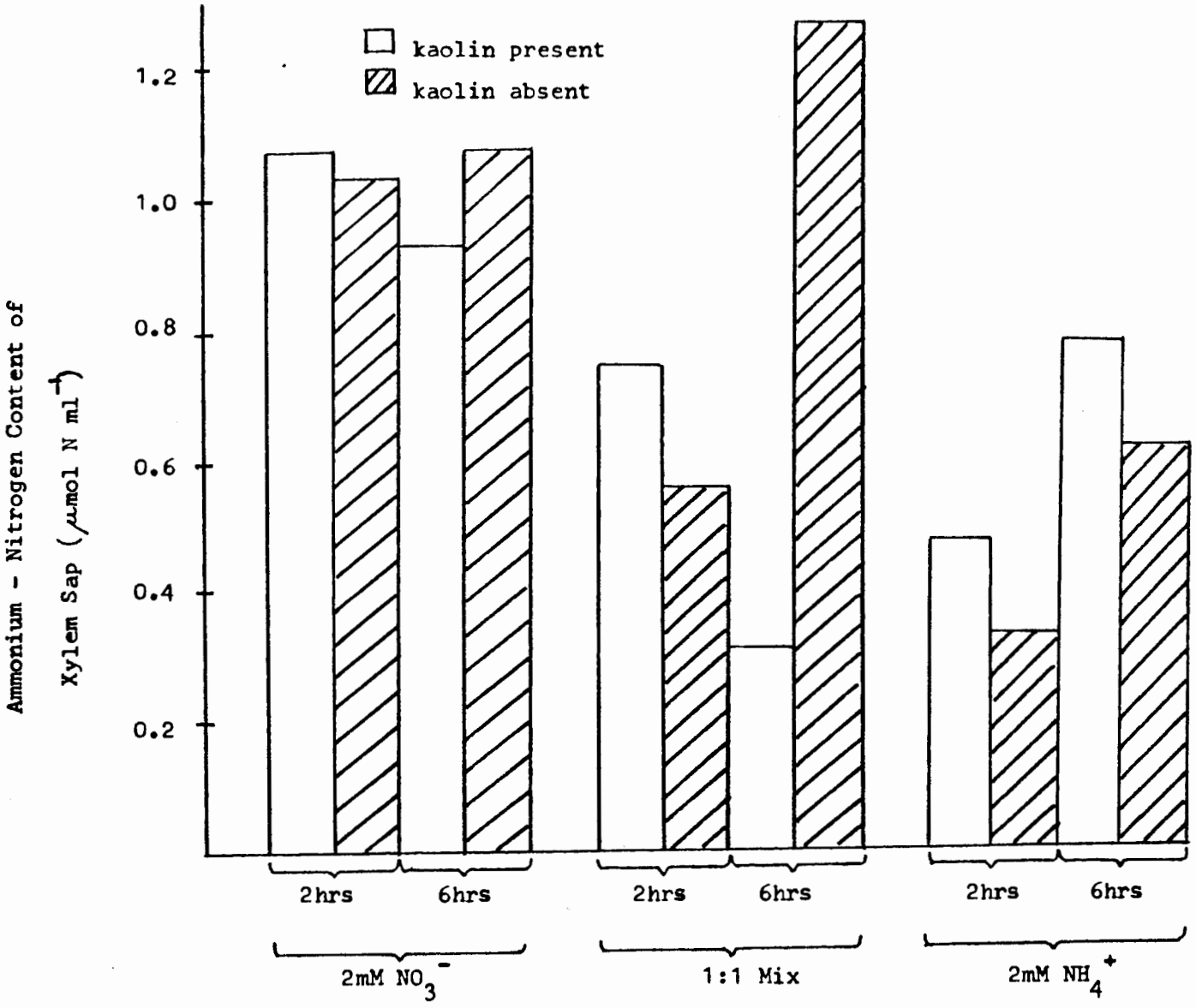

Figure 9: Total Ammonium - Nitrogen Content of Xylem Sap of 21 day old barley plants measured 2 hours and 6 hours after commencement of feeding. Results represent the mean values of two pots.

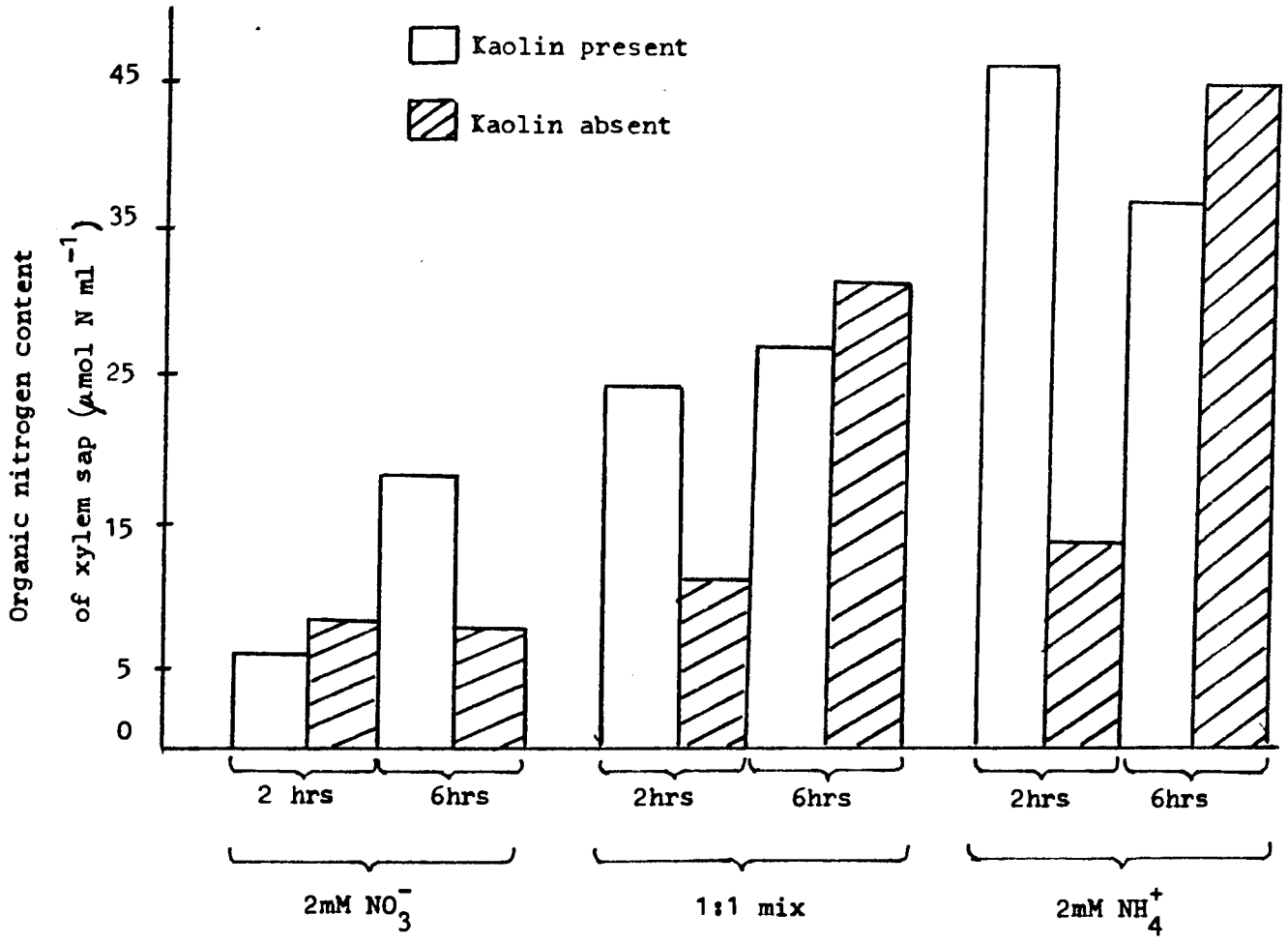

Figure 10: Total organic nitrogen content of xylem sap of 21 day old barley plants measured 2 and 6 hours after commencment of feeding. Results represent mean values of 2 pots.

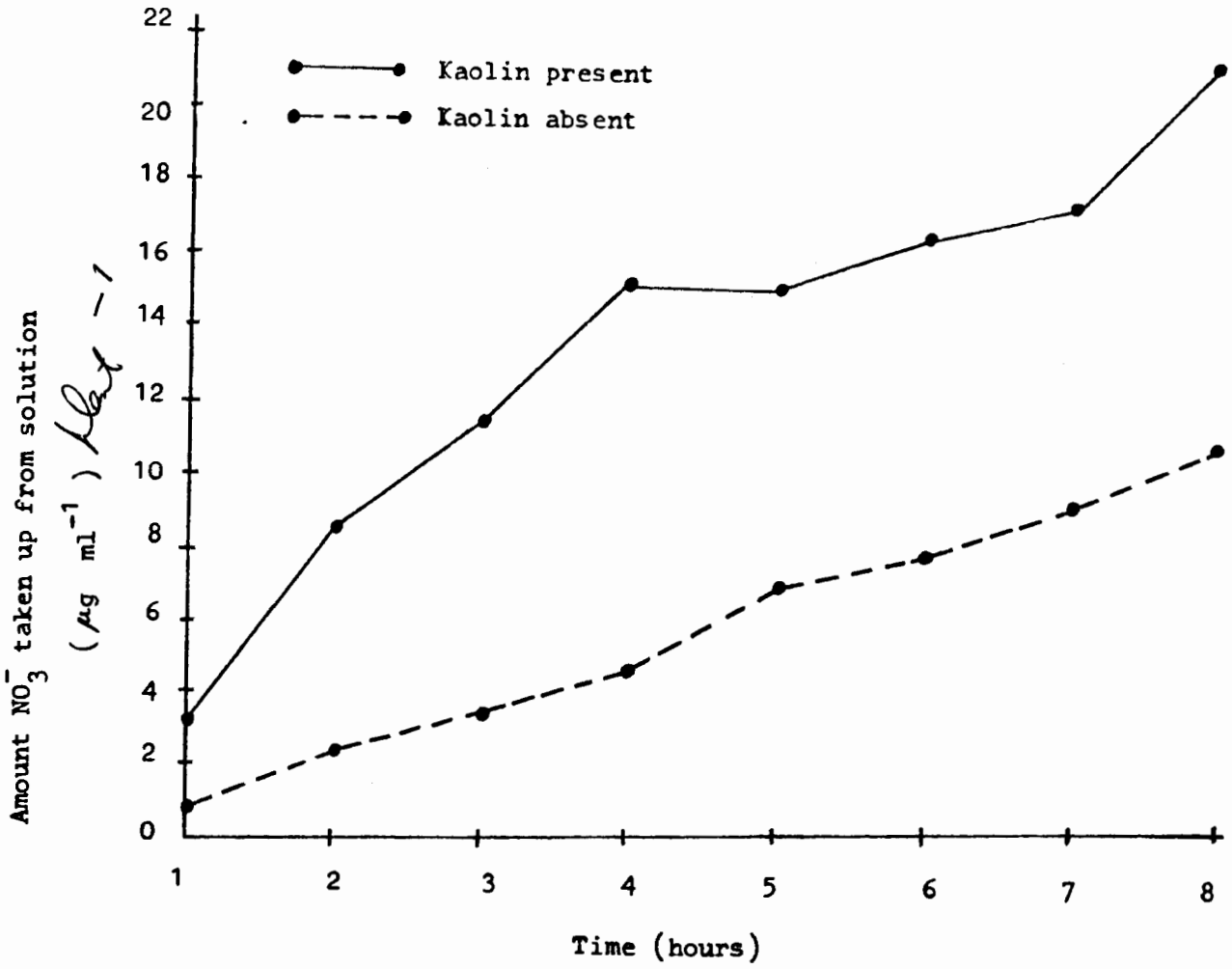

Figure 11: Amount of NO_3^- ion removed from 2mM NO_3^- nutrient solution by 20 day old barley plants, measured over an 8 hour period. Points represent mean values of 2 pots, 2 replicates per sample per pot.

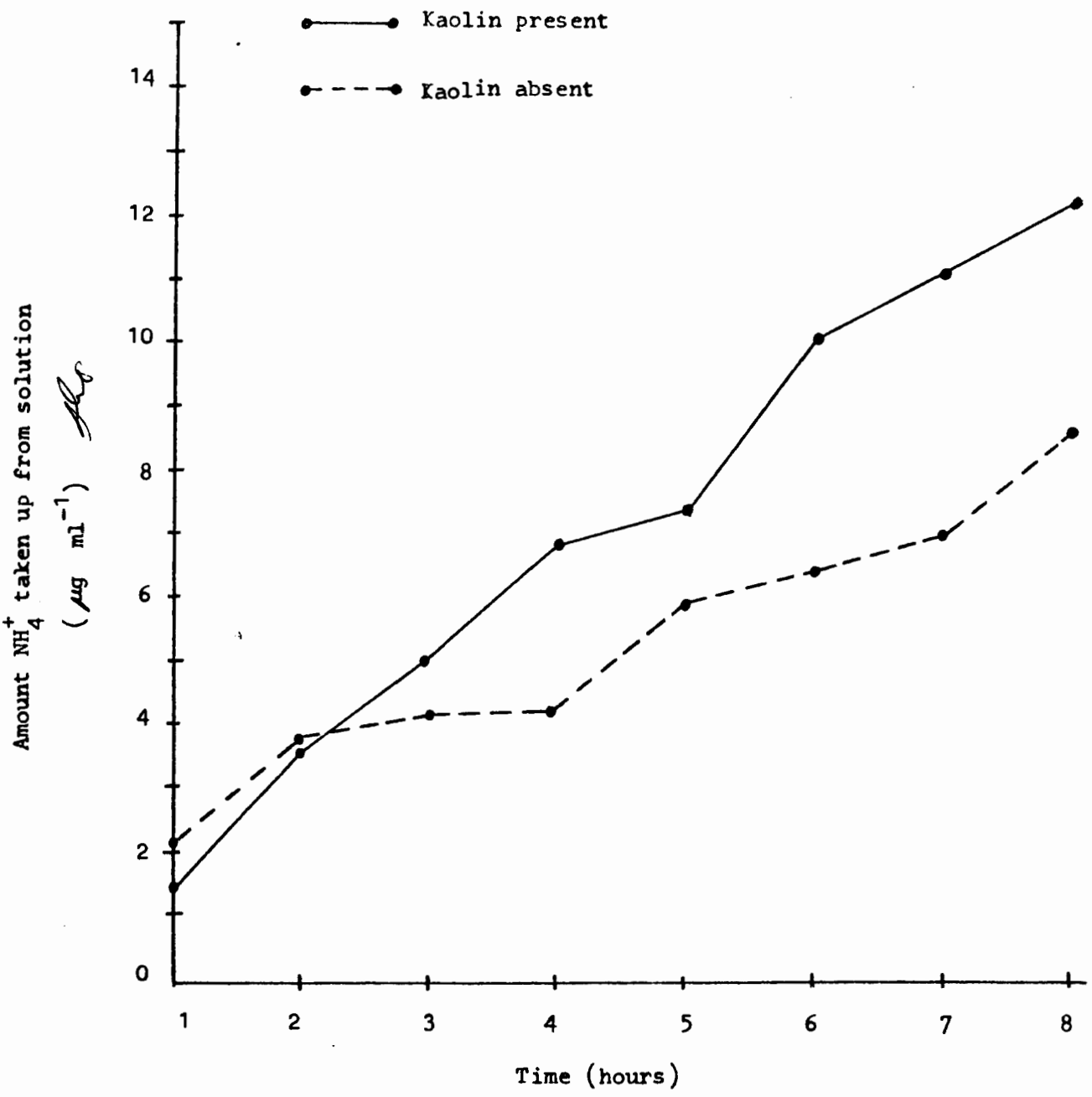

Figure 12: Amount of NH_4^+ ion removed from 2mM NH_4^+ nutrient solution by 20 day old barley plants, measured over an 8 hour period. Points represent mean values of 2 pots, 2 replicates per sample per pot.

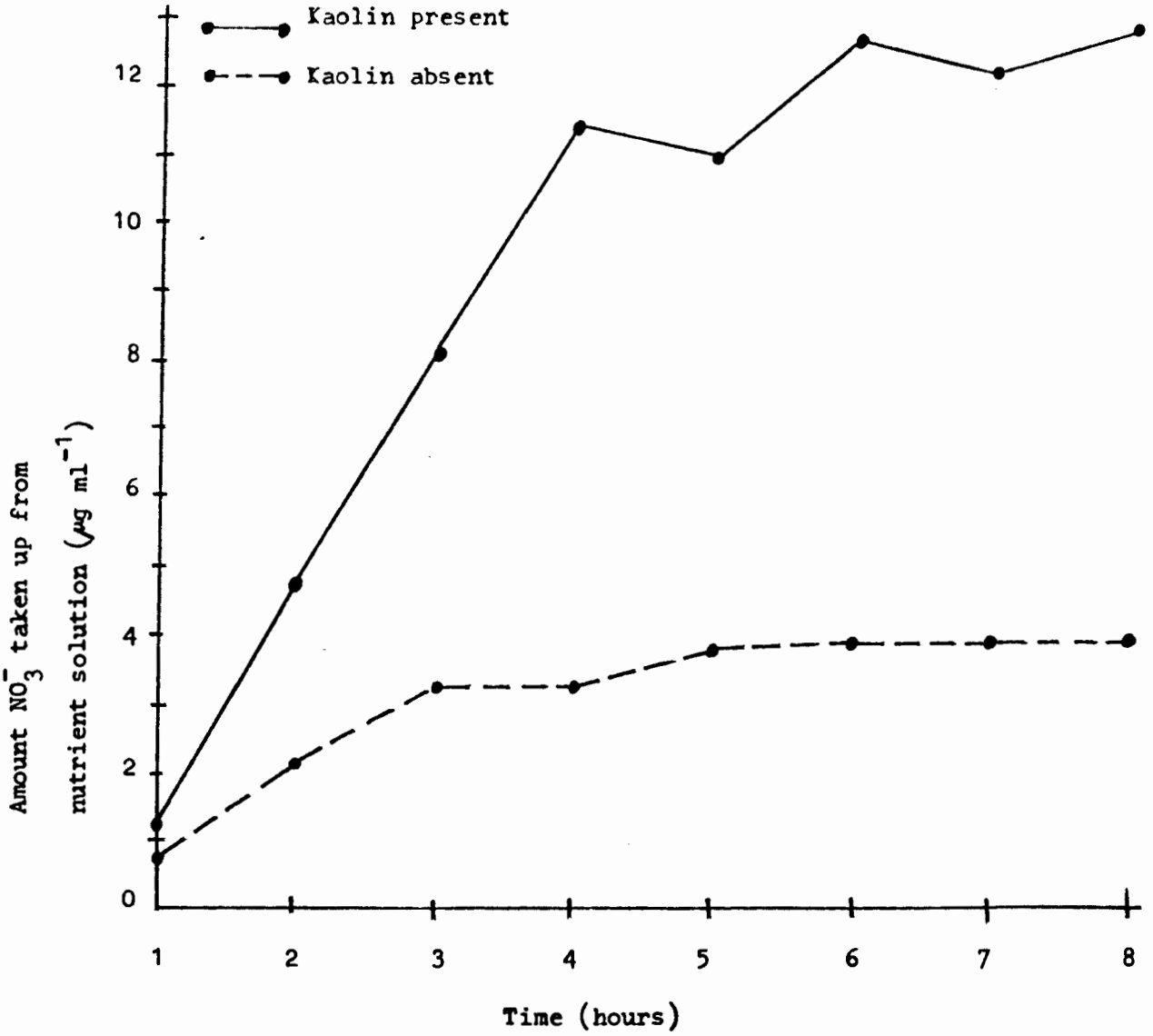

Figure 13: Amount of NO_3^- ion removed from 1mM NO_3^- + 1mM NH_4^+ nutrient solution by 20 day old barley plants, measured over an 8 hour period. Points represent mean values of 2 pots, 2 replicates per sample per pot.

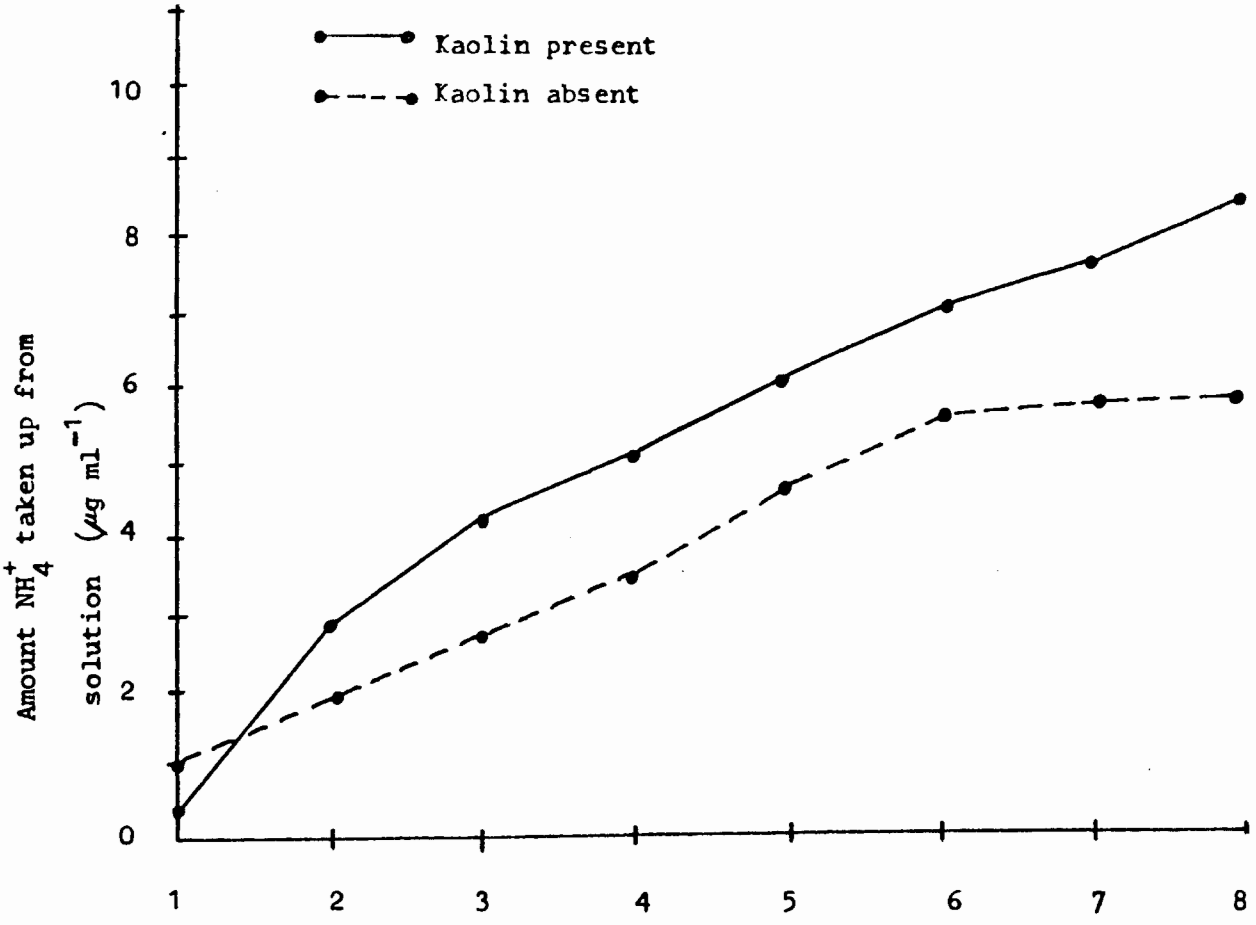

Figure 14: Amount of NH_4^+ ion removed from 1mM NO_3^- + 1mM NH_4^+ nutrient solution by 20 day old barley plants, measured over an 8 hour period. Points represent mean values of 2 pots, 2 replicates per sample per pot.

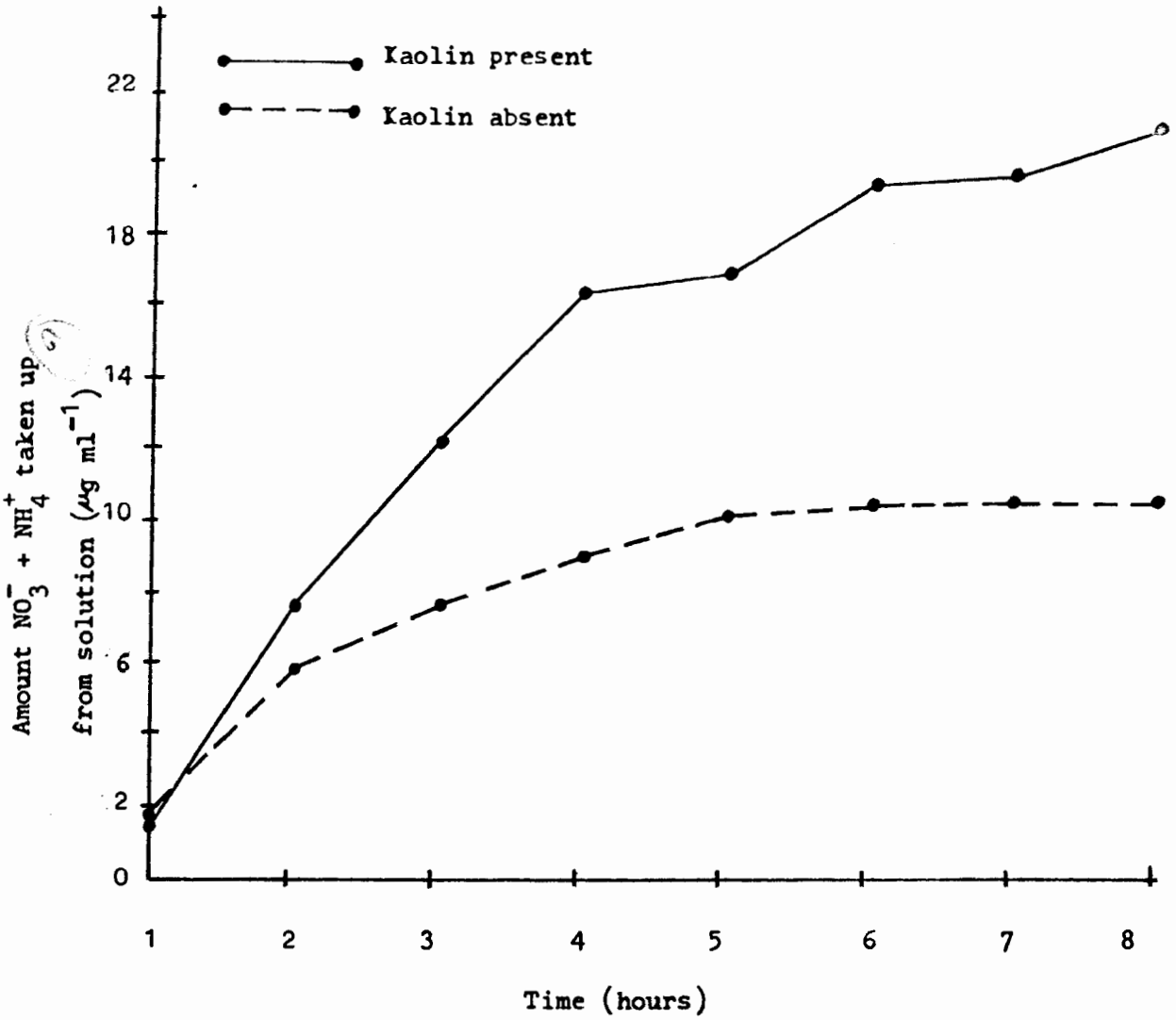

Figure 15: Amount of NH_4^+ + NO_3^- removed from 1mM NO_3^- + 1mM NH_4^+ nutrient solution by 20 day old barley plants, measured over an 8 hour period. Points represent mean values of 2 pots, 2 replicates per sample per pot.

Table 5: Mean rate of nutrient uptake ($\mu g\ ml^{-1} plant^{-1}$) by 20 day old barley plants from 3 nutrient solutions (2mM NO_3^-; 2mM NH_4^+; 1mM NO_3^- and 1mM NH_4^+) with and without 5% kaolin content, measured over 8 hour period at 20°C. Rates indicate means of 2 pots and standard errors of means.

NUTRIENT SOLUTION	SOLUTION + kaolin Rate $\mu g\ ml^{-1}_{-1}$ plant^{-1} hr^{-1}	SOLUTION- kaolin Rate $\mu g\ ml^{-1}_{-1}$ plant^{-1} hr^{-1}
1:1 Mix		
NO_3^-	0.0057 ± 0.0003 } 0.0095	0.0017 ± 0.0009 } 0.0039
NH_4^+	0.0038 ± 0.0008 }	0.0022 ± 0.002 }
2mM NO_3^-	0.0091 ± 0.003	0.0045 ± 0.0002
2mM NH_4^-	0.0047 ± 0.0006	0.0038 ± 0.0004

Results of Lord's Range Test for Small Samples performed on the above data indicated no significant differences at the 5% level between any combinations of rates, both within solutions or between kaolin and non-kaolin containing solutions.

Table 6: % of NH_4^+ ion immobilized in solutions of 2mM NH_4^+ and 1mM NO_3^- + 1mM NH_4^+ containing 5% kaolin. Results indicate means of 5 replicates, 3 readings per replicate.

2mM NH_4^+	1mM NO_3^- + 1mM NH_4^+
16,5%	8,9%

CHAPTER 4 - DISCUSSION AND CONCLUSION

The significantly higher rates of transpiration shown by plants grown with a nitrogen source when compared with the control plants (Tables 1 and 2; Figures 4 to 7), indicate that the plants perform better in the presence of nitrogen. The control plants displayed classical symptoms of nitrogen deficiency in that they were light green in colour, with the lower leaves being yellow, dry and tending to become brown. Because of this nutrient deficiency, the plants would not perform well and this would be reflected in a lower transpiration rate, compared with plants with no nutrient deficiencies.

When comparing the transpiration rates of plants grown under different nitrogen regimes, no significant differences in rates of translocation were noticed (Table 2). This would seem to indicate that nitrogen source has no significant effect on delivery rates of organic and inorganic nitrogen in barley plants. The presence and absence of kaolin in the nutrient solutions also had no significant effects on the rate of delivery of inorganic and organic nitrogen to the leaves (Table 3), and indicates that the plants did not suffer from a lack of nutrients, with the kaolin immobilizing the NH_4^+ ions. Instead, reference to Table 1 shows that the rate of delivery actually <u>increased</u> in the case of the 2mM NH_4^+ - fed plants. This provides evidence that nutrients were not lacking. It would thus appear that other factors were responsible for the differing transpiration rates observed. Since most transpiration occurs via stomata, the degree of stomatal opening is a major factor in its control (Bidwell, 1980). A possible explanation for the differing rates of transpiration observed could lie in the water contents of the plants. These may have been sufficiently low to cause

various degrees of stomatal closure, resulting in varying rates of transpiration.

Nitrogen in xylem bleeding sap.

In the plants fed with 2mM NO_3^-, the amount of nitrate present in the xylem sap was high at all times, in both kaolin-and non-kaolin-containing solutions. Similar results were obtained by Lewis, et al (1982). The amount of nitrate in the xylem sap of plants grown in solutions containing kaolin dropped after 6 hours however, and this might indicate that more NO_3^- was being reduced in the roots and incorporated into organic nitrogenous compounds. Reference to Table 4 indicates that the level of organic nitrogen in these plants did increase. In plants grown in solutions lacking kaolin, reference to Table 4 and Figure 8 shows that the levels of nitrate actually rose. This may, in fact, indicate a saturation of the nitrate reductase system of the roots (Pate, 1980), as reference to Table 4 indicates that the level of organic nitrogenous compounds remained fairly constant over the time period under consideration. From results obtained it would appear that the plants were absorbing nitrate through the roots and converting a proportion to NH_4^+ for incorporation into amino acids, while exporting the surplus to the leaves for further reduction. This supports the findings of Lewis, et al (1982) and Muhammed and Kumazawa (1974), who respectively demonstrated that NO_3^- reduction in barley and rice plants takes place mainly in the leaves. The presence of NH_4^+ - nitrogen in the xylem sap seems to indicate that some of the NO_3^- being reduced in the roots is not incorporated into organic nitrogenous compounds but is exported as NH_4^+. Another explanation for the presence of this ion could be due to a turnover of proteins or to the discharge of nitrogen storage pools in the roots (Pate, 1980).

Plants fed with a 1mM NO_3^- + 1mM NH_4^+ solution showed lower nitrate levels than those plants fed with a 2mM NO_3^- nutrient solution. In the case of solutions lacking kaolin, results indicated only traces of NO_3^- (Table 4; Fig. 8). This confirms the observations of Lewis, et al (1982), that the inclusion of NH_4^+ with NO_3^- in the nutrient solution reduces substantially the export of NO_3^- in the xylem stream, indicating repression of NO_3^- uptake by NH_4^+. This phenomenon has also been reported by other workers (Minotti, et al, 1969; Schrader, et al, 1972). In the case of nutrient solutions containing 5% kaolin however, the repression was not as marked (Table 4; Fig. 8). This can be explained by the presence of the kaolin which, in spite of having only a limited ability to immobilize NH_4^+ (see Table 6), may have produced a big enough effect to allow a certain amount of NO_3^- uptake. (The importance of this kaolin effect with regard to the use of the slurry technique will be considered later). It can also be inferred from Table 4 and Figure 10 that NO_3^- inhibited NH_4^+ uptake and assimilation, since the loading of organic nitrogen onto the xylem of plants given NO_3^- + NH_4^+ was less than in those given NH_4^+ alone. This again agrees with the conclusions of Lewis, et al (1982).

NH_4^+ transport in the xylem sap of the 1:1 mix did not differ greatly, being generally low. This can be explained by the rapid incorporation of this ion, in the root, into organic nitrogenous compounds such as glutamine (Table 4). This was also apparent in plants fed with the 2mM NH_4^+ solution. The results obtained tend to confirm the observation of Lewis, et al (1982), that the roots are the principal organs of NH_4^+ assimilation into glutamate in barley plants. No reduction of NH_4^+ is necessary and the plant can incorporate the ion directly, with the surplus (very small) being exported for further incorporation into amino

compounds in the leaves. In the case of plants fed 2mM NH_4^+, the addition of 5% kaolin appeared to have little or no effect in immobilizing the ion.

Uptake of nutrients from solution.

In looking at the nutrient solutions lacking kaolin, it can be seen by reference to Table 5 and Figures 11 to 15, that the total rates of uptake were very similar for all nutrient solutions . In the 1:1 mix NO_3^- uptake and NH_4^+ uptake were also very similar . This seems to indicate that the plants did not favour one ion above the other, and seems to be at variance with the results of Lewis and Chadwick (in press) who showed that NH_4^+ uptake in barley plants occurred at a faster rate than NO_3^- uptake. The results were similar to those obtained for corn (Zea mays) in work performed by Warncke and Barber (1973). Statistical tests showed that there were no significant differences in rates of nutrient uptake from the different solutions.

Reference to Table 5 shows that, in the kaolin-containing solutions, the total rate of uptake was very similar in the 2mM NO_3^- and 1:1 mix, but lower in the 2mM NH_4^+ solution. In the 1:1 mix however, the rate of uptake of NO_3^- was greater than that of NH_4^+ . This may have been due to the presence of kaolin which, although only immobilizing 9% of the NH_4^+ present (Table 6), may have been sufficient to effectively increase the concentration of NO_3^- available to the plants. Increased concentrations of NO_3^- in solution have been shown to reduce the uptake of NH_4^+ from the same solution (Warncke and Barber, 1973). This may well have been true in this case. In the 2mM NH_4^+ solution containing kaolin, the rate of ion

uptake was lower than that of the NO_3^- ion from both the 1:1 mix and the 2mM NO_3^- solution. Again, the presence of kaolin may have immobilized a sufficient amount of NH_4^+ to depress the uptake rate. (In this case 16.5% of NH_4^+ appeared to be immobilized -- Table 6). Results indicated satisfactory standard errors of the mean, showing that rates of uptake were fairly similar between different pots containing the same nutrient solutions. Thus it would appear, in the case of the kaolin-containing solutions, that the plant took up NO_3^- ions in preference to NH_4^+ ions and that the presence of kaolin had some effect in immobilizing the latter. It should be emphasized however, that in no case were the rates of nutrient uptake significantly different from each other.

When comparing the rates of nutrient uptake between solutions containing and lacking kaolin, it can be seen that uptake from kaolin-containing solutions was generally faster, although not significantly so. This faster rate of uptake may have been due to the presence of kaolin and the unwanted presence of micro-organisms unaffected by aeration. McCormick and Wolf (1979), have demonstrated that the addition of clay to culture media can result in a stimulation of microbial growth and metabolic activity, with the microbes removing trace elements from the clay, thus increasing their growth rate and so resulting in an increased use of nitrogen.

Reference to Figure 13 shows that nutrient uptake appeared to fluctuate at certain times in the 2mM NO_3^- solution containing kaolin. This may have been due to the kaolin masking the colour reaction of the assay and also, possibly, to the activity of micro-organisms in secreting NO_3^- back into solution.

On the basis of the results obtained, no correlation between transpiration

rate and rate of nutrient uptake was possible. Kaolin did appear to have some effect on immobilizing NH_4^+ (Table 6), but this was insufficient to justify its use in a slurry technique as envisaged. This is largely due to the fact that the cation exchange capacity of kaolin is low.

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