

## THE ABSORPTION OF AMMONIUM AND NITRATE BY PERENNIAL RYE-GRASS

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### ABSTRACT

The absorption of ammonium, nitrate, and ammonium and nitrate simultaneously as related to several external factors such as concentration, temperature, and pH, was studied in perennial rye-grass (*Lolium perenne* L.)

Although in general the absorption phenomena of ammonium could be explained in terms of the conventional carrier hypothesis, evidence was obtained that the break-down of the ammonium-carrier complex is dependent on the available amount of some enzyme.

In the same way, the absorption of nitrate was shown to fit the carrier hypothesis. It was demonstrated that the absorption of nitrate is followed by an actual accumulation of free nitrate as well as by reduction of this substance, and that both processes take place independently of each other.

Whereas the absorption of ammonium was affected by nitrate only to a small extent, the absorption rate of nitrate was considerably inhibited by the uptake of ammonium.

Because the accumulation of nitrate was not found to be influenced by ammonium in any way, the effect of ammonium uptake on the rate of nitrate absorption is ascribed to an inhibiting effect of ammonium uptake on the nitrate reduction.

As compared to nitrate, both the absorption rate of nitrite and the rate of nitrite reduction proved to be completely independent of ammonium uptake. Therefore, the effect of ammonium absorption on the uptake of nitrate is ascribed to an inhibition of the reduction process of nitrate to nitrite.

### INTRODUCTION

Experiments on ion absorption by plants have demonstrated interactions between cations as well as between anions, for example, the interaction between sodium and potassium (BANGE, 1959) and between chloride and bromide (EPSTEIN, 1953). These interactions are explained in terms of competition between ions of similar electrical charge for the same binding site.

When nitrogen is absorbed in the form of ammonium or nitrate, similar interactions are observed. For instance, when nitrogen is absorbed as ammonium, a decreased intake of potassium is seen (TROMP, 1962). Interactions have also been demonstrated between nitrate and bromide (EPSTEIN, 1953).

In addition to these interactions between ammonium and other cations or between nitrate and other anions, mutual effects of ammonium on anion absorption as well as effects of cations on nitrate absorption have been reported. The absorption of phosphate is stimulated by ammonium (ARNON, 1939) and that of nitrate is promoted by the presence of readily-absorbed cations such as potassium (SAID and EL-SHISHINY, 1949).

The effects obtained when ammonium or nitrate are absorbed together with other ions of similar electrical charge may be interpreted as competition of the ions for the same binding site (EPSTEIN, 1953). The phenomena observed when ammonium or nitrate are absorbed together with ions of opposite electrical charge can be conceived of as resulting from a tendency of the plant to maintain a constant ratio of cation: anion in its tissues (DIJKSHOORN, 1957a).

However, another kind of interaction is feasible between ammonium and nitrate in the following way. There is some evidence that when nitrogen is supplied as ammonium, it is very rapidly introduced into the assimilatory processes. Since, however, nitrate cannot be assimilated before a series of reduction processes has taken place, it is likely that after reduction the absorbed nitrate follows the same pathway in the assimilatory processes as does ammonium. In this way, competition between ammonium and the reduction products of nitrate might occur (WEISSMAN, 1950, 1951). Evidence is also available (LOOMIS, 1958) that glutamic acid forms a key compound in the incorporation of ammonium nitrogen into amino-nitrogen and that the synthesis of glutamic acid from  $\alpha$ -ketoglutaric acid and  $\text{NH}_3$  requires diphosphopyridine nucleotide, whereas the reduction of nitrate requires DPNH and/or TPNH as well (EVANS and NASON, 1953; EVANS, 1954; FEWSON and NICHOLAS, 1961). It follows that the interaction between ammonium and nitrate might be explained in terms of competition for DPNH. However, if we locate the interaction between both sources of nitrogen somewhere during assimilation, then we still do not know how the absorption rate of nitrate is influenced by this interaction. In addition, accumulation of nitrate can occur, and this process too might be affected by the simultaneous absorption of ammonium and nitrate.

The purpose of the present study was to obtain more exact and complete data concerning the absorption mechanisms of ammonium and nitrate and the type of interaction between both nitrogen sources. To this end, the influence of external factors, such as concentration, pH, temperature, and the presence of other ions in the experimental solution on  $\text{NH}_4^-$  and  $\text{NO}_3^-$  absorption was studied and compared with the results of similar experiments in which  $\text{NH}_4$  and  $\text{NO}_3$  were supplied together. Some additional experiments were performed in

order to obtain information with regard to the relation between the absorbed and the accumulated amounts of nitrate in connection with the absorption of ammonium.

## CHAPTER I

### MATERIALS AND METHODS

In the experiments use was made of intact full-grown plants and intact seedlings of perennial rye-grass (*Lolium perenne* L.). This plant material showed considerable nitrogen absorption whether supplied as ammonium or as nitrate. In some of the supplementary experiments, excised roots of perennial rye-grass seedlings were used.

#### 1. CULTIVATION OF THE FULL-GROWN PLANTS

Perennial rye-grass seeds (selected strain of the pasture type) obtained from the Institute for Biological and Chemical Research on Field Crops and Herbage, Wageningen (The Netherlands), were sown in a greenhouse on a mixture of sand and mould. The average temperature was 20° C and the seedlings were sprayed with rain water every day.

After six weeks, the material was transplanted in small batches, some in the field and some in the greenhouse, and growth was stimulated by cutting the leaves about every three weeks. During spring and summer the greenhouse plants showed the better growth and were therefore used in the absorption experiments. On the other hand, late in the summer and in the autumn the grass plants in the field were in the better condition, and in that period these plants were chosen for the experiments.

Before putting the plants on water culture, the roots were freed from soil as much as possible by washing in tap water and were cut off in such a way that only a root length of about 1 cm remained. Subsequently, the plants were mounted in square pieces of paraffined hardboard, fitting exactly on glass beakers of 600–800 ml capacity, and provided with four round holes with a diameter of 1 cm. Into each of these holes a small bundle of grass plants was put protected by a strip of cotton wool. The culture solution was continuously aerated and renewed every day. Its composition was as follows:

50 ml $\text{Ca}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$	20.4 mmol	
50 ml $\text{KNO}_3$	55.4 mmol	
50 ml $\text{MgSO}_4 \cdot 3 \text{H}_2\text{O}$	19.5 mmol	
50 ml $\text{KH}_2\text{PO}_4$	30.1 mmol	(according to WOODFORD and GREGORY, 1948)
10 ml A-Z micronutrient solution		(according to HOAGLAND and SNYDER, 1933)
20 ml sequestric acid, ferric complex	36.3 g/l	
9770 ml distilled water.		

After three weeks in the greenhouse (average temperature 20° C, average relative humidity 70 %), the root system had developed sufficiently to be used in the experiments.

## 2. CULTIVATION OF THE SEEDLINGS USED IN EXPERIMENTS WITH INTACT PLANTS

A quantity of  $0.78 \text{ g} = 500 \pm 15$  seeds of perennial rye-grass (pasture type "Barenza" of the 1956 harvest) obtained from Barenbrug's Zaadhandel N.V., Arnhem (The Netherlands), were disinfected in 10 % hydrogen peroxide for 20 minutes and rinsed. The seeds were then laid on a piece of nickel-chromium wire-gauze (area  $7 \times 5.5 \text{ cm}$ ). The gauze was placed on a glass dish containing 50 ml of 0.1 mmol  $\text{CaSO}_4$ . Desiccation of the seeds was prevented by covering the dish with a piece of moist filter paper, partly suspended in the culture solution. The  $\text{CaSO}_4$  solution was continuously aerated and renewed every day.

After six days of germination in the dark, the seedlings were transferred to a fresh complete nutrient solution and exposed for 16 hours a day to the light of one mercury lamp (Philips HO 2000, 450 W) and four 150 W Philips Comptalux lamps, mounted about 55 cm above the seedlings. The heat radiated by the lamps was absorbed by a screen of flowing water. After the first day of cultivation in the light, a solution of 0.1 mmol  $\text{CaSO}_4$  was supplied to the seedlings, and in some cases renewed every day. However, in order to prevent any deficiency, in most cases the  $\text{CaSO}_4$  solution was replaced once or more by a complete nutrient solution. The temperature was kept at 25° C and the relative humidity at 70 % during the whole period of cultivation. After 13 days, viz. six days of germination and seven days of exposure to light, the seedlings had developed sufficiently to be used in the absorption experiments.

## 3. CULTIVATION OF THE SEEDLINGS USED IN EXPERIMENTS WITH EXCISED ROOTS

Ten grams of perennial rye-grass seeds (pasture type "Barenza" of the 1961 harvest) obtained from Barenbrug's Zaadhandel N.V., Arnhem (The Netherlands) were disinfected in 10 % hydrogen peroxide for 20 minutes and rinsed in distilled water. The seeds were then spread on a stainless steel screen (area  $25 \times 30 \text{ cm}$ ) between two pieces of cheese-cloth, the edges of the cheese-cloth hanging in the culture solution. The screen was placed on a plastic tray filled with five litres of 0.1 mmol  $\text{CaSO}_4$  solution. The level of the solution was kept at about 0.5 cm under the screen. The solution was continuously and thoroughly aerated.

After six days of germination in the dark at a temperature of 25° C, the seedlings were exposed to the light of Philips TL 40 W/33 and TL 40 W/32 lamps, mounted alternately at distances of 8 cm from each other and about 50 cm above the seedlings. During the period of cultivation in the light, the 0.1 mmol  $\text{CaSO}_4$  solution was renewed

five times. The solution was continuously aerated. The temperature was kept at 25° C and the relative humidity at 70 %.

After six days of exposure to the light, the excised roots of this material proved to absorb ammonium as well as nitrate in quantities suited to the experimental requirements.

#### 4. PROCEDURE IN THE EXPERIMENTS WITH FULL-GROWN PLANTS

The absorption of ammonium- and of nitrate-nitrogen was studied in short-time experiments using static water-cultures. The method was essentially the same as that described by VAN DEN HONERT and HOOYMANS (1955). In our case the scheme was as follows:

- 30 minutes adaptation to standard conditions
- $x$  minutes absorption under standard conditions
- 30 minutes adaptation to experimental conditions
- $y$  minutes absorption under experimental conditions
- 30 minutes adaptation to standard conditions
- $x$  minutes absorption under standard conditions.

The duration of the absorption period was chosen between 30 and 105 minutes, depending on the absorption rate expected. Chosen as standard conditions were a temperature of 20° C, a pH of 5.7, and a nitrate or ammonium concentration of about 1.0 m.e./l.

The beakers with full-grown plants were placed in a water-thermostat to keep them at the desired temperature. The pH was determined with a glass electrode and kept at the desired value by the addition of a few drops of 0.1 N. NaOH or 0.1 N. HCl. The composition of the nutrient solutions used in the absorption experiments varied with the series of experiments and will be described in the respective paragraphs. During the experiments the nutrient solutions were aerated continuously. The experiments were carried out in daylight. The plants were used for two weeks and in five or six absorption experiments. Between the experiments the plants were replaced in the culture solution.

The rates of nitrate and of ammonium absorption under standard conditions and under the experimental conditions were determined by analysis of the solutions before and after absorption. The rates of nitrogen absorption from the first and second standard solution of each experiment usually differed less than 15 %, indicating thus a steady state in the absorption. An experiment was discarded if the difference exceeded 30 %. In the experimental results the absorption rates under experimental conditions are expressed as relative absorption rates with respect to the average absorption rates under standard conditions.

In the procedure described, two difficulties were encountered. In the first place, the use of the same plants in absorption experiments for several successive days induced a slight increase in the absorption of nitrate and a considerable decrease in ammonium absorption when nitrate and ammonium were supplied separately. It should be noted that the full-grown plants did not show molybdenum deficiency, as

distinct from the seedlings. In the second place, the material showed considerable seasonal variations, as will be described below. In order to avoid these difficulties, in later experiments use was made of intact grass seedlings of the same age, grown under exactly controlled conditions and used for one experiment only.

#### 5. PROCEDURE IN THE EXPERIMENTS WITH INTACT SEEDLINGS

The absorption of ammonium- and nitrate-nitrogen was studied in short-time experiments using static water-cultures. Since all the seedlings had been raised in exactly the same way, absolute rates of absorption could be compared after due corrections for rapid initial uptake had been applied. Experiments under standard conditions could therefore be omitted.

As regards ammonium-nitrogen, in a preliminary experiment lasting 5 hours, the initial rapid intake (free space) ended within 15 minutes. Consequently, in the experiments on ammonium absorption the seedlings were allowed to adapt to the experimental conditions for at least 15 minutes before being transferred to the solutions proper.

As regards the absorption of nitrate, in some preliminary experiments using 0.5 mmol  $\text{KNO}_3$  solutions buffered at a pH of 6.0–6.2, the rate of absorption was very low and variable, irrespective of the solution supplied to the material the last day of cultivation in the light, as shown in Table 1. However, the addition of 0.2 mg/l of

TABLE 1

Nitrate absorption by perennial rye-grass seedlings as influenced by the nutrient solution supplied to the material the last day of cultivation in the light.

solution supplied the last day of cultivation in the light	rate of nitrate absorption in m.e./hour-500 seedlings after an adaptation period of 15 minutes				
	during the first hour	during the second hour	during the third hour	during the fifth hour	during the sixth hour
0.1 mmol $\text{CaSO}_4$	0.0024 0.0040 0.0040	0.0036 0.0032 0.0028	— — —	— — —	— — —
1.0 mmol $\text{Ca}(\text{NO}_3)_2$	— — —	0.0007 0.0005 0.0014	0.0004 0.0009 0.0012	— — —	— — —
0.5 mmol $\text{KNO}_3$ buffered with phosphate	0.0019 0.0016 0.0014	0.0039 0.0028 0.0028	0.0035 0.0033 0.0033	— — —	— — —
complete nutrient solution	0.0023 0.0013 0.0021	0.0020 0.0037 0.0032	0.0029 0.0039 0.0024	— — —	— — —
complete nutrient solution; experimental solution contains 0.2 mg/l of Mo	0.0019 0.0018	— —	0.0050 0.0053	0.0052 0.0055	0.0051 0.0057

molybdenum in the form of ammonium molybdate to the experimental solution gave a constant rate of nitrate absorption of about 0.0053 m.e.  $\text{NO}_3$ /hour-500 seedlings after two hours, and this rate persisted within the experimental accuracy for at least 4 hours. Therefore, in the experiments on nitrate and ammonium-nitrate absorption, molybdate to an amount of 0.2 mg/l of molybdenum was added to the solution and the adaptation period was extended to 2.5 hours in order to allow the material to incorporate simultaneously a sufficient amount of molybdenum. During the experiments the glass dishes with seedlings, containing 40 ml of solution, were placed in a water-thermostat and exposed to the light of four Philips TL 40 W/55 lamps at a distance of about 35 cm. The solutions were aerated continuously. To keep the pH at the desired values, buffering of the experimental solutions with  $\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$  to a total phosphate concentration of 7–8 m.e./l proved to be effective. However, for experiments on the influence of various anions on ammonium absorption another buffer solution had to be used, consisting of triethylamine + HCl or  $\text{H}_2\text{SO}_4$  or  $\text{H}_3\text{PO}_4$ . A concentration of 3 mmol triethylamine usually proved to be effective in the pH range from 5.5–8.5. Triethylamine is supposed not to be absorbed by plants. It did not influence the ammonium and nitrate determinations. Besides, in a test using excised roots of perennial rye-grass, the rate of ammonium absorption was the same irrespective of whether the buffer solution supplied was phosphate, triethylamine +  $\text{H}_3\text{PO}_4$ , or tris-(hydroxymethylamino-methane) +  $\text{H}_3\text{PO}_4$ . The buffer solutions supplied in the individual experiments will be indicated in the respective paragraphs. The absorption rates of ammonium- and of nitrate-nitrogen were determined by analysis of the solutions before and after the experiment.

It should be noted that the rate of ammonium-nitrogen absorption at 35° C remained constant for at least six hours (Fig. 5). Referring to Table 1, it should be remarked that after an adaptation period of 2 hours a constant rate of nitrate absorption was observed for 4 hours, provided that molybdenum was supplied. Consequently, it was concluded that in the experiments using intact seedlings, there was a steady state in the absorption of ammonium- and of nitrate-nitrogen.

## 6. PROCEDURE IN THE EXPERIMENTS WITH EXCISED ROOTS OF SEEDLINGS

The experiments with excised roots were performed using static water-cultures.

Before the start of the experiment, the roots were excised just below the underlying cheese-cloth and washed in distilled water for 30 minutes. The distilled water was renewed twice in that time and was continuously aerated.

It was observed, that the removal of adhering water by centrifugation has an unfavourable effect on the absorption capacity of the perennial rye-grass roots. Therefore, the fresh weight of the quantities of roots used in the experiment was determined at the end of the procedure. Initially, estimated quantities of roots were transferred to beakers

containing 200 ml of experimental solution in some experiments and to glass tubes containing 25 ml of experimental solution in other experiments, but it became apparent that the rate of absorption was affected and that the roots were too closely packed if more than 3 g of roots were transferred into 100 ml of solution. Therefore, estimated maximum quantities of 2 g of roots per 100 ml of solution were used, and the amount of experimental solution, viz. 200–25 ml, depended on the quantities of roots needed for the determinations of nitrate and nitrite content.

During the experiment, the solution was kept at a temperature of 25° C and continuously aerated. In all experiments with excised roots, the solution was buffered at pH 6.05 with 4 mmol tris-(hydroxymethylaminomethane) and H<sub>2</sub>SO<sub>4</sub>. Although this material did not show any molybdenum-deficiency worth mentioning, ammonium molybdate to an amount of 0.2 mg Mo/l was added to all experimental solutions. (It should be noted that this material used was of a different harvest than the material with which the experiments with intact seedlings were performed). At the end of the experiment, the samples of roots were rinsed 3 times for 5 seconds, wrapped in cheese-cloth, and centrifuged for five minutes in an A.E.G. basket centrifuge, after which the fresh weight of the samples was determined.

The absorption of ammonium-, nitrate-, and nitrite-nitrogen was measured by analysis of the solutions before and after absorption had taken place. The accumulation of nitrate and nitrite was determined by analysis of the roots. As control, in each experiment the nitrate (nitrite) content was measured in two samples of roots that were not allowed to absorb nitrate (nitrite). The reduction of nitrate and of nitrite was calculated by subtraction of the amount of free nitrate (nitrite) detected in the root material from the amount of nitrate (nitrite) absorbed.

It was ascertained that in an experiment lasting 5 hours, ammonium-nitrogen was absorbed at a constant rate. If nitrate was supplied alone, under the described experimental conditions the rate of nitrate absorption mostly remained constant within the range of experimental accuracy for 6 hours. However, in some experiments the rate of nitrate absorption was found to increase with time.

## 7. ANALYTICAL METHODS

a. For the determination of ammonium concentrations, the Nessler colorimetric method was used (ALLPORT, 1947). The measurements of colour intensity were performed at a wavelength of 440 m $\mu$  with a Unicam absorptiometer.

b. Nitrate concentrations were determined colorimetrically according to the phenol disulphonic acid method (ALLPORT, 1947) at a wavelength of 420 m $\mu$ . The nitrate concentration of the standard KNO<sub>3</sub> solutions was controlled by determination of the *K* concentration with the aid of a flame photometer.

c. The concentrations of nitrite were measured at a wavelength



of 546  $m\mu$  and determined according to the colorimetric method of Parker with  $\alpha$ -naphthylamine (LANGE, 1956).

d. Chloride concentrations were determined by means of a conductometrical titration with a 0.01 N.  $\text{AgNO}_3$ -solution at  $0^\circ \text{C}$  with the aid of a Philips conductometer (GM 4249).

e. The determination of the nitrate content of the excised roots was performed in essentially the same way as described by ALLPORT (1947). Weighed portions of excised roots were minced and transferred to an 100 ml Erlenmeyer flask together with 20 ml of water and the flask immersed in a boiling water-bath for 15 minutes. The mixture was allowed to cool, rendered just acid to bromocresol green with dilute sulphuric acid, and any nitrites which might be present were oxidized by adding potassium permanganate until a faint pink colour persisted for 1 minute. Chlorides which might be present were precipitated by adding a few drops of a saturated solution of silver sulphate. Proteins were precipitated by adding 2.5 ml of a solution of lead subacetate (the excess lead was precipitated by addition of solid sodium oxalate in order to prevent turbidity of the end solution), and by adding 2.5 ml of a solution of ammonia and potassium aluminium sulphate. The mixture was shaken, filtered, and sufficient distilled water was passed through the filter to produce 100 ml of filtrate. The nitrate concentration of the filtrate was determined by the method described above.

f. The determination of the nitrite content of the excised roots was performed in the following way: weighed samples of excised roots were minced and transferred to an 100 ml Erlenmeyer flask together with 20 ml of water and the flask immersed in a boiling water-bath for 15 minutes. The mixture was then allowed to cool. Subsequently, proteins were precipitated by means of lead subacetate and a solution of ammonia and potassium aluminium sulphate, as

TABLE 2

Nitrate and nitrite content of the roots with and without addition of a known quantity of nitrate or nitrite.

m.e. $\text{NO}_3$ added	m.e. $\text{NO}_3$ detected	m.e. $\text{NO}_2$ added	m.e. $\text{NO}_2$ detected
0.00000	0.00000	—	—
0.00000	0.00000	—	—
0.00047	0.00038	—	—
0.00047	0.00046	—	—
0.00094	0.00077	—	—
0.00141	0.00130	—	—
0.00188	0.00150	—	—
0.00188	0.00150	—	—
0.00235	0.00180	—	—
0.00235	0.00200	—	—
—	—	0.0000	0.0001
—	—	0.0000	0.0001
—	—	0.0015	0.0015
—	—	0.0015	0.0015

described for nitrate determination. After treatment with sodium oxalate, the mixture was shaken, filtered, and sufficient water was passed through the filter to produce 100 ml of filtrate. The nitrite concentration of the filtrate was determined by the method described above.

The reliability of the determinations of nitrate- and nitrite contents was evaluated in the following way. The nitrate or nitrite content of excised roots that had not absorbed these nitrogen sources was measured, and the nitrate or nitrite content was determined in samples of excised roots to which a known quantity of nitrate or nitrite was added after the material was minced but before the treatment in boiling water had taken place. As shown in Table 2, consistent values were obtained. However, in the case of nitrate determination a constant fraction of about 20 % of the added quantity of nitrate was not recovered, whereas all of the added amount of nitrite was detected in the roots.

## CHAPTER II

### THE ABSORPTION OF AMMONIUM-NITROGEN

Experiments concerning the influence of external factors on the absorption rate of ammonium-nitrogen may elucidate the way in which this source of nitrogen enters the roots of perennial rye-grass. Some information as to the mechanism by which ammonium-nitrogen is absorbed can be obtained by experiments on the relation between external concentration of ammonium salt and rate of absorption. The question of whether or not the absorption of ammonium-nitrogen is dependent on metabolic processes can be studied, for example, by experiments on the influence of temperature and of concentration. The characteristic influence of pH on the dissociation of  $\text{NH}_4\text{OH}$  may provide a basis for a conclusion as to the form in which ammonium-nitrogen enters the grass roots.

#### 1. EXPERIMENTS ON THE INFLUENCE OF CONCENTRATION ON THE ABSORPTION RATE OF AMMONIUM-NITROGEN

##### 1.1. *Experiments on the influence of external ammonium salt concentration in full-grown plants*

The influence of ammonium concentration was studied in the range of 0.0–1.0 m.e./l  $\text{NH}_4$ . Three series of experiments were involved: two series were carried out in the spring and one in the winter.

*Experimental conditions.* The experimental solutions under standard and experimental conditions had the following composition:

$\text{CaSO}_4$	0.1	mmol
$\text{MgSO}_4$	0.1	mmol
$\text{KH}_2\text{PO}_4$	0.15	mmol
KCl	0.275	mmol
$(\text{NH}_4)_2\text{SO}_4$	0.0–0.5	mmol

The temperature at standard conditions was the same as in the experiment. The experiments in the spring were performed at 20° and 35° C, those in the winter at a temperature of 35° C. The pH was maintained at 5.7. The absorption times were chosen between 60 and 105 minutes.

*Results.* The absorption curves obtained with full-grown plants were of the type of a Langmuir adsorption isotherm (Fig. 1). During the spring, a half-value (50 % of the maximum rate of absorption) of 0.04 m.e./l  $\text{NH}_4$  was found at both 20° and 35° C. However, in the winter, a half-value as high as 0.2 m.e./l was found.

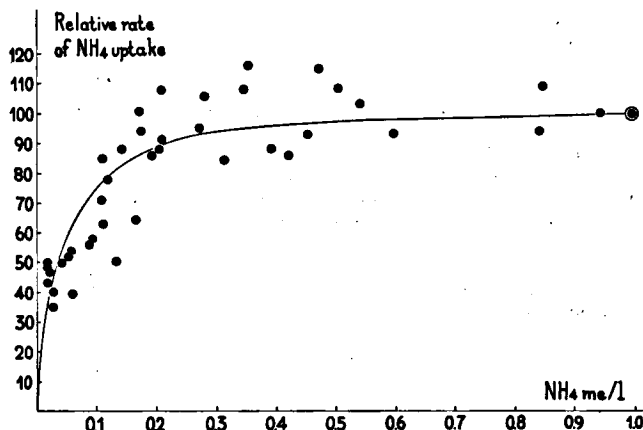


Fig. 1a. Absorption of  $\text{NH}_4$  in relation to  $\text{NH}_4$  concentration in the spring at a temperature of 20° C and a pH of 5.7 (full-grown plants). The curve is a Langmuir adsorption isotherm with a half-value of 0.04 m.e./l

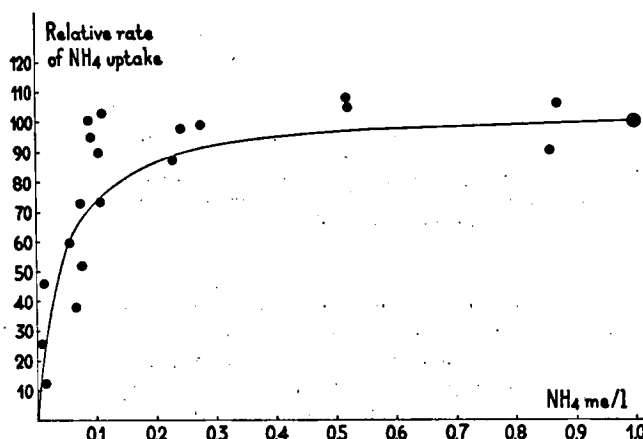


Fig. 1b. Absorption of  $\text{NH}_4$  in relation to  $\text{NH}_4$  concentration in the spring at a temperature of 35° C and a pH of 5.7 (full-grown plants). The curve is a Langmuir adsorption isotherm with a half-value of 0.04 m.e./l

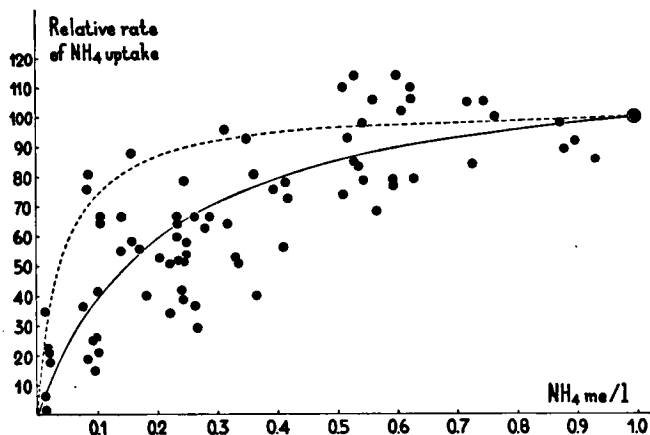


Fig. 1c. Absorption of  $\text{NH}_4$  in relation to  $\text{NH}_4$  concentration in the winter at a temperature of  $35^\circ\text{C}$  and a pH of 5.7 (full-grown plants). The curve is a Langmuir adsorption isotherm with a half-value of 0.2 m.e./l. For comparison, the dotted line shows a Langmuir adsorption isotherm with a half-value of 0.04 m.e./l

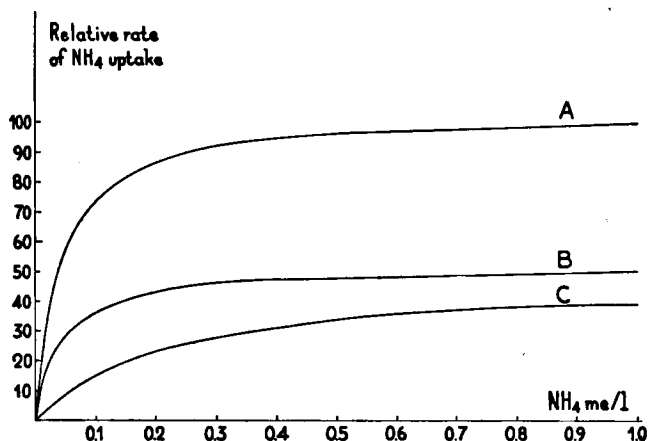


Fig. 1d. The curves of Figs. 1a, b, and c, representing the roughly estimated effect of temperature and season on the absorption of  $\text{NH}_4$  in relation to  $\text{NH}_4$  concentration. A. absorption of  $\text{NH}_4$  in the spring at a temperature of  $20^\circ\text{C}$ ; B in the spring at  $35^\circ\text{C}$ , and C in the winter at  $35^\circ\text{C}$ .

From the shape of the absorption curves and the estimated ratio of the absorption rates at a concentration of 1.0 m.e./l  $\text{NH}_4$ , the ratio of the maximum rates of absorption in the spring at  $20^\circ$ , in the spring at  $35^\circ$ , and in the winter at  $35^\circ\text{C}$  was derived as 1.0:0.5:0.4 (Fig. 1d). It should be borne in mind that these results were obtained with material of different origin: the spring material was grown in the greenhouse, whereas the winter material was obtained from the field. Moreover, the light conditions in the period of cultivation on water-

culture and during the experiments differed. Thus, it should be emphasized that the above comparison of the maximum rates of ammonium-nitrogen absorption is not actually justified and is intended only to roughly represent the effect of temperature and season on the relation between ammonium-nitrogen concentration in the medium and the rate of absorption.

### 1.2. *Experiments on the influence of external ammonium salt concentration in intact seedlings*

The experiments on the influence of ammonium-nitrogen concentration on the rate of absorption were repeated, using intact seedlings grown under standardized conditions in order to obtain comparable results in the individual experimental series.

The seedlings had been supplied with a complete nutrient solution as mentioned in Chapter I sub 1 during the whole period of growth in the light. The influence of ammonium-nitrogen concentrations in the medium in the range of 0.0–1.0 m.e./l  $\text{NH}_4$  was investigated. Two series of experiments were carried out with this material, the only difference being the accompanying anion.

*Experimental conditions.* In the first series,  $(\text{NH}_4)\text{H}_2\text{PO}_4$  concentrations of 0.0–1.0 mmol. were used, buffered with 2.5 mmol triethylamine and  $\text{H}_3\text{PO}_4$ . In the second series,  $(\text{NH}_4)_2\text{SO}_4$  0.0–0.5 mmol solutions were supplied, buffered by means of 2.5 mmol triethylamine and  $\text{H}_2\text{SO}_4$ . The experiments were performed at a pH of 6.1 and a temperature of 25° C. The duration of the experiments was chosen between 30 and 40 minutes.

*Results.* The same sort of relation between concentration and rate of ammonium-nitrogen absorption was found with intact seedlings as with full-grown plants (Fig. 2). The half-value of about 0.1 m.e./l

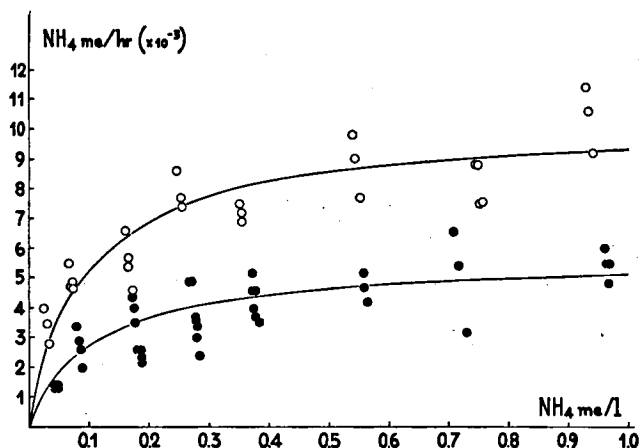


Fig. 2. Absorption of  $\text{NH}_4$  in relation to  $\text{NH}_4$  concentration from (○)  $(\text{NH}_4)\text{H}_2\text{PO}_4$  and (●)  $(\text{NH}_4)_2\text{SO}_4$  solutions (500 intact seedlings). The curves are Langmuir adsorption isotherms with a half value of about 0.1 m.e./l ( $t = 25^\circ \text{C}$ ,  $\text{pH} = 6.1$ ).

proved to be unaffected by the accompanying anion. It should be noted that this half-value differs significantly from those obtained with full-grown plants.

On the other hand, the maximum rate of absorption was affected to a considerable extent by the nature of the accompanying anion. In the experiments with  $(\text{NH}_4)\text{H}_2\text{PO}_4$  this maximum rate amounted to about 0.010 m.e./hour-500 seedlings, whereas in the case of  $(\text{NH}_4)_2\text{SO}_4$  this value did not exceed 0.0056 m.e./hour-500 seedlings.

## 2. EXPERIMENTS ON THE INFLUENCE OF TEMPERATURE ON THE RATE OF AMMONIUM-NITROGEN ABSORPTION

### 2.1. *Experiments on the influence of temperature on the ammonium uptake by full-grown plants*

Two series of experiments were carried out in the temperature range from 2.5°–35° C. In the first series a one-salt solution was used; in the second, the plants were supplied with a balanced solution.

*Experimental conditions.* The experiments were performed in the summer. The one-salt solution consisted of a 0.55 mmol  $(\text{NH}_4)_2\text{SO}_4$  solution. The complete nutrient solution was as mentioned in section 1.1 of this Chapter. It should be kept in mind that the absorption rate of ammonium-nitrogen approximates the maximum rate of absorption at the ammonium concentration used in these experiments (Fig. 1). The temperature at standard conditions was 20° C. The pH was kept at 5.7. The absorption periods were chosen between 60 and 90 minutes.

*Results.* The influence of temperature on the rate of ammonium-nitrogen absorption can be described by an optimum curve with the optimum at about 22° C (Fig. 3). Within the experimental accuracy,

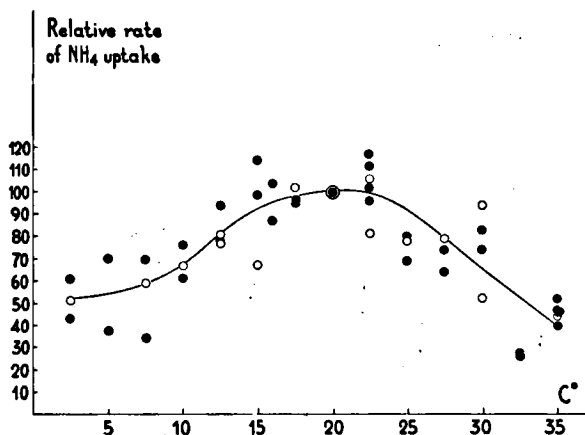


Fig. 3. Absorption of  $\text{NH}_4$  from (○) complete nutrient solutions and from (●) one-salt solutions at an  $\text{NH}_4$  concentration of 1.1 m.e./l and a pH of 5.7 in relation to temperature (full-grown plants).

quantitatively the same results were obtained in the experiments with a one-salt solution as in the experiments in which balanced solutions were supplied.

It should be noted that the absorption rate under standard conditions after exposure to temperatures between 20° and 35° C for 90 minutes did not differ appreciably from that before exposure.

## 2.2. *Experiments on the influence of temperature on the rate of ammonium-nitrogen absorption by intact seedlings*

In experiments with intact seedlings, the influence of temperature in the range from 5° to 35° C was studied. The rate of ammonium absorption was determined, and also that of the accompanying anion. The seedlings had been supplied with a complete nutrient solution only during the first day of growth in the light.

*Experimental conditions.* The experimental solution consisted of a 1.0 mmol  $\text{NH}_4\text{Cl}$  solution buffered at pH 6.2 with phosphate. The absorption rates of both  $\text{NH}_4$  and Cl were determined after absorption periods between 120 and 150 minutes.

*Results.* The temperature curve for ammonium-nitrogen absorption has the shape of an optimum curve, resembling those obtained with full-grown plants, and an optimum temperature of 27° C was found (Fig. 4). Although the rate of ammonium absorption decreased with

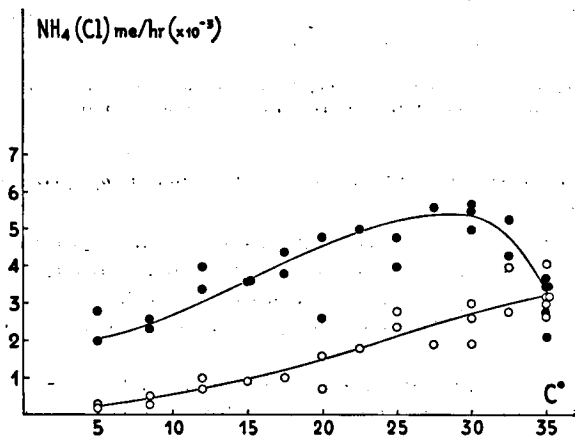


Fig. 4. Absorption of  $\text{NH}_4$  (●) and Cl (○) at an  $\text{NH}_4\text{Cl}$  concentration of 1.0 mmol and a pH of 6.2 in relation to temperature (500 intact seedlings).

increasing temperature above 27° C, the rate of Cl absorption continued to increase.

## 2.3. *The influence of time on the temperature effect*

The influence of time on the absorption rate of ammonium-nitrogen by intact seedlings was studied at 35° C. The seedlings had been supplied with complete nutrient solutions during the whole period of growth in the light.

*Experimental conditions.* The experimental solution was 0.5 mmol  $(\text{NH}_4)\text{H}_2\text{PO}_4$ , buffered at pH 6.0 by means of 6 mmol triethylamine and phosphoric acid. The rate of ammonium-nitrogen uptake was determined after 2, 3, 4, 5, and 6 hours of absorption.

*Results.* Within the experimental accuracy, the rate of ammonium-nitrogen absorption at 35° C was observed to remain constant for at least six hours (Fig. 5).

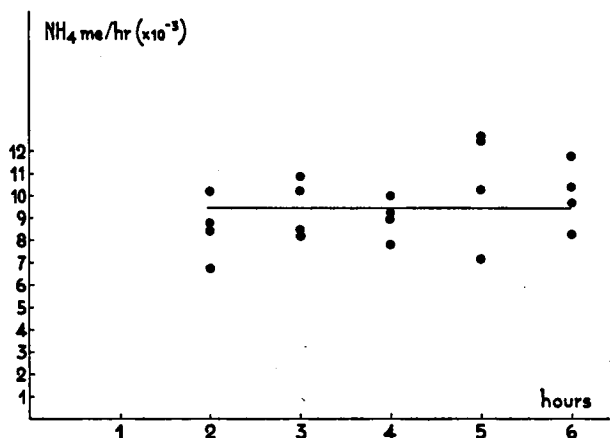


Fig. 5. Absorption of  $\text{NH}_4$  at an  $\text{NH}_4$  concentration of 1.0 m.e./l, a temperature of 35° C, and a pH of 6.0 in relation to time (500 intact seedlings).

#### 2.4. The influence of temperature on the growth of perennial rye-grass seedlings

The low temperature optimum observed for ammonium-nitrogen absorption raised the question of whether this temperature optimum is specific for the uptake of ammonium or related to an influence of temperature on the growth and, connected with this, on the assimilation of ammonium.

The following experiment was performed in order to obtain more exact information about growth at different root temperatures.

*Experimental conditions.* After a six-day period of germination, groups of seedlings were allowed to grow in artificial light for 4 days at a constant root temperature of 5, 15, 22.5, 25, 30, and 35° C per ten groups. During the light period, a complete dilute nutrient solution was supplied and renewed twice. The nutrient solution was as in Chapter I sub 1, and thus contained nitrate as the only source of nitrogen. At the end of this period, the seedlings were dried at 95° C for 24 hours and the weight of the dry matter determined.

*Results.* As shown in Fig. 6, the relation between temperature and dry weight can be again described in an optimum curve. The optimum temperature was found at about 20° C.



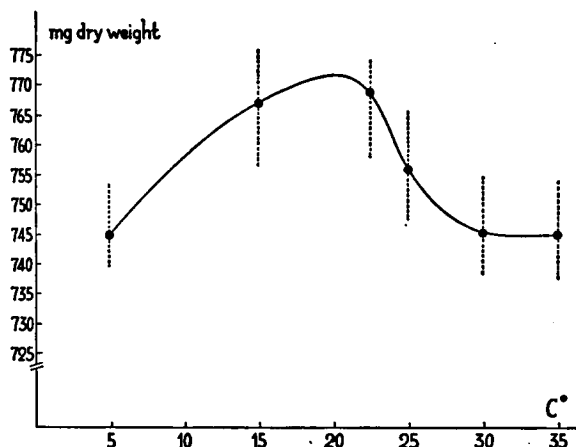


Fig. 6. Influence of temperature on the growth of seedlings. The nutrient solution contained nitrate as the only source of nitrogen. The points represent the mean values of dry weight; the dotted lines indicate the range of values of dry weight determined for ten groups of 500 seedlings at each experimental temperature.

### 3. EXPERIMENTS ON THE INFLUENCE OF THE pH OF THE SOLUTION ON THE RATE OF AMMONIUM-NITROGEN ABSORPTION

#### 3.1. *The influence of the pH of the solution in experiments with full-grown plants*

The influence of pH on the rate of ammonium-nitrogen absorption was studied in full-grown plants over a pH-range of pH 4.0–8.5 at two temperatures.

*Experimental conditions.* The experiments were performed during the summer in daylight; the air temperature in the hot-house varied between 20 and 25° C. Balanced experimental solutions were supplied as described in section 1.1 of this Chapter. The solution contained ammonium sulphate in a concentration of 0.55 mmol. The pH was checked every 5 minutes and kept at the desired value by the addition of a few drops of NaOH 0.1 N.

In two series of experiments the influence of pH at root temperatures of 20° and 35° C was studied. In the first series, at standard conditions the pH was kept at 6.0 and the temperature at 20° C. In the second series, at standard conditions the pH was again kept at 6.0 but the temperature at 35° C. In an additional experiment at pH 6.0, the ratio of the absorption rate at 20° to the absorption rate of ammonium-nitrogen at 35° C was determined. In this way it was possible to express all data in terms of absorption unities under standard conditions at 20° C.

*Results.* In both series of experiments it was observed that the rate of ammonium-nitrogen absorption is hardly affected by pH within the range of pH 4.0–6.5, but that the rate of absorption is stimulated by an increase of the pH between pH 6.5 and 8.5 (Fig. 7).

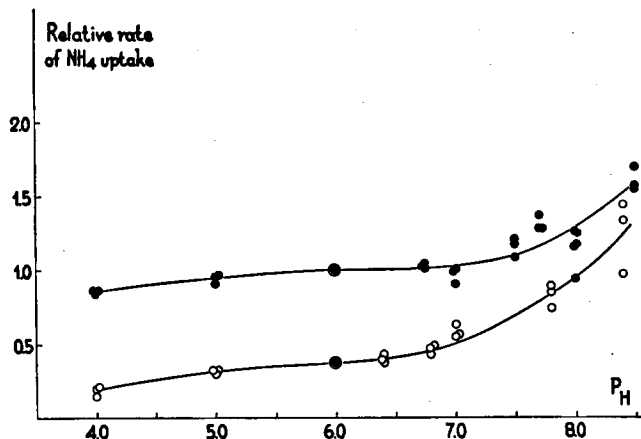


Fig. 7. Absorption of  $\text{NH}_4$  from balanced solutions at an  $\text{NH}_4$  concentration of 1.1 m.e./l and at temperatures of 20° C (●) and 35° C (○) in relation to pH (full-grown plants).

The same temperature effect as described in section 2.1 and 2.2 of this Chapter was seen. This effect of temperature on the rate of ammonium absorption appeared to be nearly constant in the range of pH 4.0–6.0. However, at higher pH values the ratio, uptake at 20° C: uptake at 35° C shows a considerable decrease from 3 to about 1.3.

### 3.2. *The influence of pH on the rate of ammonium-nitrogen absorption by intact seedlings*

The influence of pH on the rate of ammonium-nitrogen absorption was studied on intact seedlings over a pH-range from 5.5–8.5 at a constant temperature and using mono-salt solutions. As in the experiments with full-grown plants, the seedlings were supplied with ammonium-nitrogen solutions whose concentration did not limit the rate of absorption at temperatures of 20° and 35° C and at a pH of about 6.0 (Chapter II, section 1.1 and 1.2, Figs. 1 and 2). The seedlings had been given a fresh complete nutrient solution each day of growth in the light.

**Experimental conditions.** The experiments were performed at a temperature of 25° C. Three series of experiments were performed, differing in the anion supplied in the ammonium salt and the triethylamine buffer as follows:

- 1)  $(\text{NH}_4)_2\text{SO}_4$  0.5 mmol + 12 mmol triethylamine +  $\text{H}_2\text{SO}_4$ .
- 2)  $(\text{NH}_4)_2\text{HPO}_4$  1.0 mmol + 3 mmol triethylamine +  $\text{H}_3\text{PO}_4$ .
- 3)  $\text{NH}_4\text{Cl}$  1.0 mmol + 3 mmol triethylamine +  $\text{HCl}$ .

At pH values above 7.0, especially in the ammonium sulphate series, the triethylamine buffer proved to be ineffective for keeping the pH at a constant value even when the concentration of  $(\text{C}_2\text{H}_5)_3\text{N}$  was increased to 12 mmol. Therefore, it should be kept in mind that

for the experiments above pH 7.0, the pH given is the average of the pH at the beginning and at the end of the experiment, the difference not exceeding half a pH-unit. The duration of the absorption from the experimental solution was chosen between 30 and 60 minutes.

*Results.* In the experiments with seedlings, the effect of pH was seen to vary with the nature of the accompanying anion (Fig. 8).

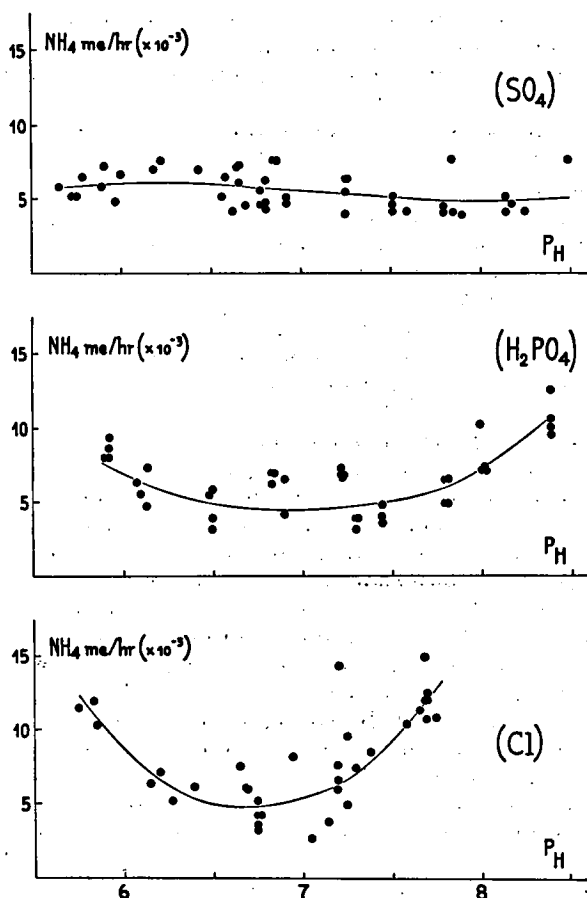


Fig. 8. Absorption of  $\text{NH}_4$  from one-salt solutions of  $(\text{NH}_4)_2\text{SO}_4$ ,  $(\text{NH}_4)\text{H}_2\text{PO}_4$  or  $\text{NH}_4\text{Cl}$ , at an  $\text{NH}_4$  concentration of 1.0 m.e./l and a temperature of  $25^\circ\text{C}$  in relation to pH (500 intact seedlings).

A considerable effect was found if ammonium was supplied as  $\text{NH}_4\text{Cl}$ , whereas the rate of absorption was affected by pH to a lesser extent when  $(\text{NH}_4)\text{H}_2\text{PO}_4$  was used. Hardly any effect was observed when ammonium-nitrogen was given as  $(\text{NH}_4)_2\text{SO}_4$ . At pH 6.2–6.7, no influence of the accompanying anion was detected. A decrease of the pH from 6.7 to 5.5 or an increase from 6.7 to higher values enhanced

the ammonium absorption in the sequence  $\text{NH}_4\text{Cl} > (\text{NH}_4)\text{H}_2\text{PO}_4 > (\text{NH}_4)_2\text{SO}_4$ .

#### 4. CONCLUSION

1. It was established that the relation between ammonium-nitrogen concentration in the solution and the rate of ammonium-nitrogen absorption by perennial rye-grass can be described by a Langmuir adsorption isotherm. The characteristics of such a curve are the maximum rate of absorption and the concentration in the medium at which half the maximum absorption rate is reached (half-value).

The ammonium-absorption curves obtained with full-grown plants and with intact seedlings varied quantitatively such that the *maximum rate* of absorption

is dependent on temperature;

is dependent on the accompanying anion;

is indicated to be dependent on seasonal factors;

and that the *half-value*

is independent of temperature;

is independent of the accompanying anion;

is dependent on the experimental procedure and/or the material used;

is dependent on seasonal factors.

The influence of ammonium-nitrogen concentration on the rate of absorption by perennial rye-grass shows a qualitative resemblance to that observed in experiments with maize. VAN DEN HONERT and HOOYMANS (1961) obtained an ammonium-absorption curve resembling a Langmuir adsorption isotherm with a half value of 0.11 m.e./l in experiments with static water-cultures, while BECKING (1956), using a continuous-flow technique, computed a half-value of 0.013 m.e./l. Similarly, it was shown by TROMP (1962) that with a continuous-flow technique, at very low concentrations the influence of ammonium concentration on the rate of absorption by intact wheat can be described by a Langmuir adsorption isotherm with a half-value of 0.007 m.e./l.

2. It became apparent that the maximum rate of ammonium-nitrogen absorption is dependent on temperature in such a way that there is an optimum temperature for ammonium absorption between 22° and 27° C.

In full-grown plants the influence of temperature on the rate of ammonium-nitrogen absorption was the same whether ammonium was supplied in the form of a one-salt solution or as a balanced solution.

At 35° C the rate of ammonium-nitrogen absorption by intact seedlings shows a steady state during a period of at least six hours.

Exposure of full-grown plants to temperatures above 20° C did not influence the absorption rate of ammonium after subsequent transfer to a temperature of 20° C.

While the rate of ammonium-nitrogen absorption in the temperature range from 5° to 35° C shows an optimum, the Cl absorption increases

with increasing temperature over the whole range of temperatures investigated.

The relation between temperature and the dry weight of seedlings shows an optimum at a temperature of about 20° C.

In contrast to the temperature-optimum curves obtained for perennial rye-grass, a temperature curve for ammonium absorption by maize was found (VAN DEN HONERT and HOOYMANS, personal communication), showing a  $Q_{10}$  as high as 7 between 3° and 5° C and a  $Q_{10}$  of 1.1 between 20° and 40° C, and no optimum was observed. On the other hand, WANNER (1948) did not observe a change in the rate of absorption at 15° and 25° C for ammonium-nitrogen by intact wheat seedlings, whereas TROMP (1962) obtained a pronounced temperature optimum curve with an optimum temperature of 24° C for ammonium absorption by excised wheat roots.

3. In experiments with full-grown plants, the rate of ammonium-nitrogen absorption from complete nutrient solutions was observed to be practically independent of the external pH in the range from pH 4.0 to 6.5, but to be stimulated by an increase of the pH between pH 6.5 and 8.5.

A constant effect of temperature on the rate of ammonium absorption appeared in the range of pH 4.0 to 6.0, but the ratio, uptake at 20°: uptake at 35° showed a considerable decrease at higher pH values.

In the experiments with seedlings, the absorption rate of ammonium-nitrogen from unbalanced solutions decreases with increasing pH between 5.5 and 6.7 and increases with increase of the pH from 6.7 to higher values. The effect of pH was observed to be dependent on the accompanying anion.

The effect of pH on the rate of ammonium absorption by full-grown plants (obtained with a balanced solution) is very similar to the effect of pH on the rate of ammonium-nitrogen absorption by maize (also on complete nutrient solutions) as reported by VAN DEN HONERT and HOOYMANS (1961). On the other hand, in experiments using complete nutrient solutions but with respect to wheat seedlings WEISSMAN (1950) observed that the absorption of ammonium-nitrogen was increased by increasing the initial pH from 4.3 to 5.3 and from 5.3 to 6.3.

Very similar effects of pH using unbalanced solutions were obtained by BURSTRÖM (1940) with respect to the absorption of K and Mg in experiments with wheat seedlings. TROMP (1962) observed the absorption of ammonium-nitrogen by excised roots of wheat from unbalanced solutions to increase with increasing values of pH from 4.5 to 6.5 and from 7.5 to higher values, whereas no effect of pH between 6.5 and 7.5 on the absorption velocity of ammonium-nitrogen was apparent.

MORTON and MACMILLAN (1954), in experiments with fungi, observed an increased rate of ammonium-nitrogen absorption with increase of the pH over the whole range of pH 4 to 8 investigated.

## CHAPTER III

## THE ABSORPTION OF NITRATE-NITROGEN

As for ammonium-nitrogen absorption, information about the way in which nitrate-nitrogen enters the roots of perennial rye-grass was obtained by studying the influence of several external factors on the rate of nitrate uptake. Thus, the influence of the external nitrate concentration, temperature, pH, and of the accompanying cation (potassium and calcium) on nitrate absorption by intact seedlings was investigated. Attention was also given to the influence of the presence of molybdenum on nitrate absorption.

#### 1. THE INFLUENCE OF MOLYBDENUM ON THE RATE OF NITRATE ABSORPTION

In a preliminary experiment using seedlings supplied with a complete nutrient solution the first day of growth in the light and a 0.1 mmol  $\text{CaSO}_4$  solution each of the other days of cultivation, the absorption rate of nitrate from a 0.5 mmol  $\text{KNO}_3$  solution buffered at pH 6.0 by phosphate, was low and variable. Supply of calcium nitrate, potassium nitrate and phosphate buffer, or a complete nutrient solution the last day of cultivation in the light did not improve the rate of nitrate absorption. This complete nutrient solution, composed as mentioned in section 1 of Chapter I, did not contain molybdenum. Addition of ammonium molybdate to an amount of 0.2 mg/l of molybdenum to the experimental solution resulted in an appreciably increased and, between 2–6 hours after exposure to this solution, constant rate of nitrate absorption (Chapter I, section 5, Table 1).

Although these experiments were preliminary and intended merely to ascertain optimum conditions for nitrate absorption in experiments with perennial rye-grass seedlings, it seems worth-while mentioning here the observation that when using intact seedlings in short-time experiments, the rate of nitrate absorption is markedly increased by molybdenum.

In the earlier experiments using full-grown plants, a steady state in the considerable rate of nitrate absorption was observed after cultivation of the material as described in Chapter I, sub 1. These results gave no reason to investigate a possible favourable effect of molybdenum on the rate of  $\text{NO}_3$  absorption.

In a later stage of the present study, the question as to the effect of molybdenum in the above preliminary experiments with seedlings became important. Therefore, using samples of excised roots, the influence of ammonium molybdate at a concentration of 0.2 mg./l of molybdenum on the rate of nitrate absorption was investigated. As contrasted with the results obtained with intact seedlings, nitrate absorption was only slightly improved by molybdenum, but in most cases the effect was distinct. It should be kept in mind that the excised roots were obtained from seeds of different harvest than the intact seedlings in question.

## 2. EXPERIMENTS ON THE INFLUENCE OF EXTERNAL NITRATE CONCENTRATION ON THE RATE OF NITRATE ABSORPTION

The influence of nitrate concentrations from 0.0 to 1.0 m.e./l  $\text{NO}_3$  on the rate of nitrate absorption was studied in intact seedlings. Two series of experiments were conducted, differing in the nature of the accompanying cation. The seedlings had been provided with a renewed complete nutrient solution each day of cultivation in the light.

*Experimental conditions.* In one of the series, the experimental solutions consisted of 0.0–1.0 mmol.  $\text{KNO}_3$  solutions, buffered at pH 6.2 with triethylamine and phosphoric acid, and in the other series solutions of 0.0–0.5 mmol.  $\text{Ca}(\text{NO}_3)_2$ , buffered at pH 6.2 by means of the same buffer, were used. There was a drift in the pH values from 6.2 to 5.9 in the case of  $\text{KNO}_3$  and from 6.2 to 6.5 in the case of  $\text{Ca}(\text{NO}_3)_2$ . The temperature was maintained at 25° C and the experiments lasted 10 to 60 minutes after an adaptation period of 2.5 hours.

*Results.* The influence of nitrate concentration on the rate of  $\text{NO}_3$  absorption can be described by a Langmuir adsorption isotherm (Fig. 9). No influence of the cation supplied (K or Ca) was apparent. The maximum rate of absorption amounted to 0.0057 m.e./hour-500 seedlings, and the half-value was 0.033 m.e./l.

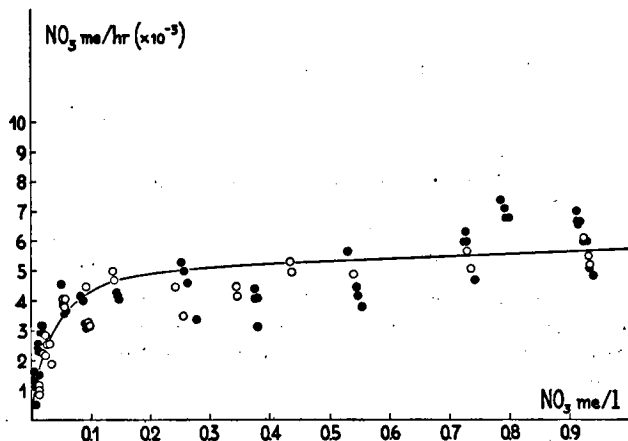


Fig. 9. Absorption of  $\text{NO}_3$  in relation to  $\text{NO}_3$  concentration from (○)  $\text{KNO}_3$  and (●)  $\text{Ca}(\text{NO}_3)_2$  solutions at a temperature of 25° C and a pH of about 6.2 (500 intact seedlings). The curve is a Langmuir adsorption isotherm with a half-value of 0.033 m.e./l.

## 3. THE INFLUENCE OF TEMPERATURE ON THE ABSORPTION OF NITRATE

The influence of temperature in the range from 5° to 35° C on the rate of nitrate absorption was investigated in intact seedlings. The seedlings had been supplied with a complete nutrient solution on the first day of growth in the light, and a daily-renewed  $\text{CaSO}_4$  0.1 mmol solution was given during the rest of cultivation.

*Experimental conditions.* The experiments were performed in 1.0 mmol  $\text{KNO}_3$  solutions, buffered at pH 6.3 with phosphate. At the nitrate concentration used in these experiments, the maximum rate of absorption is approached according to section 2 of this Chapter (Fig. 9). The experiments lasted 120 to 210 minutes.

*Results.* It was observed (Fig. 10) that a rise in temperature resulted in an increased rate of  $\text{NO}_3$  absorption over the whole range of temperatures investigated. However, this increase in absorption rate was reduced by raising the temperature above 25° C. Temperature was found to have the same effect on the rate of absorption of  $\text{NO}_3$  and of Cl (Figs. 4 and 10).

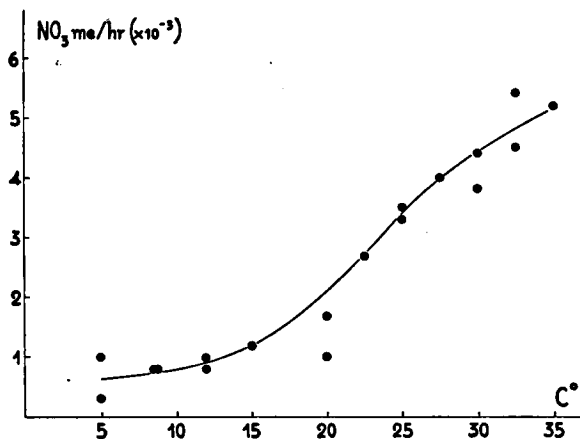


Fig. 10. Absorption of  $\text{NO}_3$  at a  $\text{NO}_3$  concentration of 1.0 m.e./l and a pH of 6.3 in relation to temperature (500 intact seedlings).

#### 4. THE INFLUENCE OF pH IN THE ABSORPTION OF NITRATE

The influence of pH between values of 4.5 and 7.7 on the rate of nitrate absorption was studied for intact seedlings. The seedlings had been supplied with a complete nutrient solution each day of cultivation in the light.

*Experimental conditions.* The experiments were conducted with 0.4 mmol  $\text{KNO}_3$  solutions, buffered with phosphate. During the experiments, low pH values tended to rise and high pH values tended to decrease, the maximum deviation being 0.24 in the former case and 0.29 in the latter. The temperature was maintained at 25° C and the experiments lasted 30 to 60 minutes.

*Results.* The influence of the pH on the rate of nitrate absorption is represented by a typical optimum curve with an optimum at pH 6.2 (Fig. 11). At pH values below 4.7, nitrate was released. Between pH 4.7 and 6.2 an increase of the pH value resulted in a higher rate of nitrate absorption, and a further increase of the pH from 6.2 to 7.7 caused a decrease in the absorption rate.



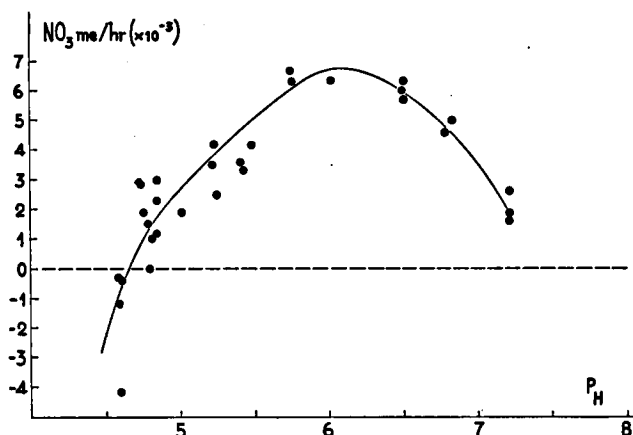


Fig. 11. Absorption of  $\text{NO}_3$  at a  $\text{NO}_3$  concentration of 0.4 m.e./l and a temperature of  $25^\circ \text{C}$  in relation to pH (500 intact seedlings).

## 5. CONCLUSION

1. In experiments with intact perennial rye-grass seedlings and excised roots, it became apparent that nitrate absorption is hampered by molybdenum deficiency.

2. It was observed that the relation between external  $\text{NO}_3$  concentration and rate of absorption can be described by a Langmuir adsorption isotherm. The same sort of relation between nitrate concentration and rate of absorption was found by VAN DEN HONERT and HOOYMANS (1955) with respect to maize.

3. It was seen that the *maximum rate* of absorption  
 is dependent on root-temperature and is increased by any increase in temperature between  $5^\circ$  and  $35^\circ \text{C}$ ;  
 is dependent on the pH of the solution, showing an optimum at pH 6.2;  
 is independent of the accompanying cation K or Ca.

The observed effect of temperature on nitrate absorption is comparable to the influence of temperature on the uptake of nitrate by maize (VAN DEN HONERT and HOOYMANS, 1955).

As contrasted with the pH-optimum curve obtained for perennial rye-grass, VAN DEN HONERT and HOOYMANS (1955) observed a considerable decrease of nitrate absorption by maize with increasing pH values above 6, whereas between pH 6 and 4.5 the results on the whole showed a pronounced increase, sometimes a decrease of absorption as the pH values became lower. WEISSMAN (1950) did not observe any influence of pH between pH 4.3 and 6.3 on the rate of nitrate absorption by wheat seedlings. The pH-optimum curve with a maximum at pH 6.2 for nitrate absorption by perennial rye-grass is comparable with the pH-optimum curve for Br ions by barley (HOAGLAND and BROYER, 1940; JACOBSON *et al.*, 1957) and for Cl ions by maize (HOOYMANS, personal communication).

A pH-optimum curve with a maximum rate of nitrate absorption at pH 6.0 was reported by MORTON and MACMILLAN (1954) with respect to fungi.

4. The *half-value* of nitrate absorption amounting to 0.033 m.e./l was unaffected by the accompanying cation K or Ca. The influence of temperature and pH on this half-value was not investigated.

According to VAN DEN HONERT and HOOYMANS (1955), the half-value of nitrate absorption by maize is not influenced by temperature and pH within the experimental error.

#### CHAPTER IV

### THE INTERACTION BETWEEN AMMONIUM AND NITRATE

Chapters II and III dealt with experiments on nitrate- and ammonium-nitrogen absorption in which either nitrate or ammonium was supplied as the only source of nitrogen. These experiments showed that both the absorption rate of nitrate and the rate of ammonium-nitrogen uptake from solutions containing either nitrate- or ammonium-nitrogen are dependent on the external concentration of the nitrogen source, root-temperature, and the pH of the solution. With respect to the influence of temperature and pH, the rate of ammonium absorption was affected differently than the absorption rate of nitrate.

Therefore, it was supposed that the investigation of a possible interaction between ammonium and nitrate in the absorption by perennial rye-grass would be most successful if the investigation were not restricted to experiments in which the concentrations of the nitrogen sources in question are varied simultaneously or with respect to each other, but extended to include experiments concerning the influence of temperature and pH on the simultaneous absorption of both nitrogen sources.

#### 1. EXPERIMENTS BASED ON VARIATION OF THE CONCENTRATION OF ONE OR BOTH NITROGEN SOURCES

##### 1.1. *The influence of $\text{NH}_4\text{NO}_3$ concentration on the rate of ammonium- and of nitrate-nitrogen absorption*

The effect of varied equal concentrations of ammonium and nitrate on the rate of ammonium- and nitrate-nitrogen absorption was investigated on intact seedlings in the concentration range from 0.025 to 1.0 mmol  $\text{NH}_4\text{NO}_3$ . During the period of growth in the light, the material had been supplied with a daily-renewed complete nutrient solution. Before the experiments, the material was adapted to the final experimental solution for 2.5 hours.

*Experimental conditions.* The experimental solution contained besides  $\text{NH}_4\text{NO}_3$ , ammonium molybdate to an amount of 0.2 mg./l of molybdenum, and was buffered with 3 mmol triethylamine + phosphoric

acid at pH 6.2. The temperature was 25° C. The experiments lasted 30–60 minutes.

*Results.* The influence of ammonium nitrate concentration on the rate of ammonium and nitrate absorption is shown in Fig. 12. Although

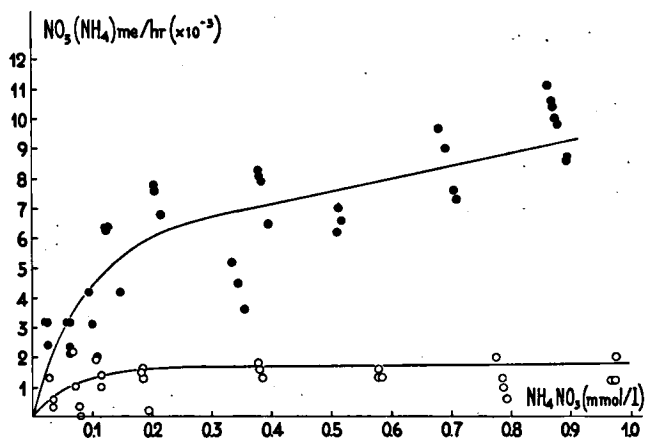


Fig. 12. Absorption of  $\text{NH}_4$  and  $\text{NO}_3$  combined (● and ○ respectively) in relation to  $\text{NH}_4\text{NO}_3$  concentration at a temperature of 25° C and a pH of 6.2 (500 intact seedlings).

the data obtained for ammonium show considerable scattering, the curve is thought to approach the form of a Langmuir adsorption isotherm with about the same maximum rate of uptake and a somewhat higher half-value as compared to the one obtained from ammonium phosphate solutions (Fig. 2).

Although nitrate absorption was low, the data suggest an absorption curve of the same type as found in  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$  solutions (Fig. 9). However, the maximum rate of nitrate absorption (0.0017 m.e./hour-500 seedlings) was reduced to 30 % by the presence of ammonium. As a result of the low rates of nitrate absorption observed, it was impossible to determine the half-value with sufficient accuracy.

#### 1.2. *The absorption of ammonium from solutions of a constant ammonium-nitrogen concentration and varied concentrations of nitrate*

The influence of concentrations of up to 3 m.e./l  $\text{NO}_3$  on the rate of ammonium-nitrogen absorption from 0.2 m.e./l  $\text{NH}_4$ -solutions was investigated. The intact seedlings had been supplied with a daily-renewed complete nutrient solution during the growth in the light. The experiments were performed after an period of adaptation to the experimental solution of 2.5 hours.

*Experimental conditions.* The solution contained 0.2 mmol  $\text{NH}_4\text{Cl}$  and 0.0–3.0 mmol  $\text{KNO}_3$ , and was buffered at pH 6.0 by means of 3 mmol triethylamine and hydrochloric acid. The absorption experiments lasted 30–75 minutes at a temperature of 25° C.

*Results.* Evidence was obtained (Fig. 13) that the rate of ammonium absorption is hampered by the presence of  $\text{NO}_3$  but only to a very small extent, the reduction of the absorption amounting to about 3 % for each 0.2 m.e./l  $\text{NO}_3$  added.

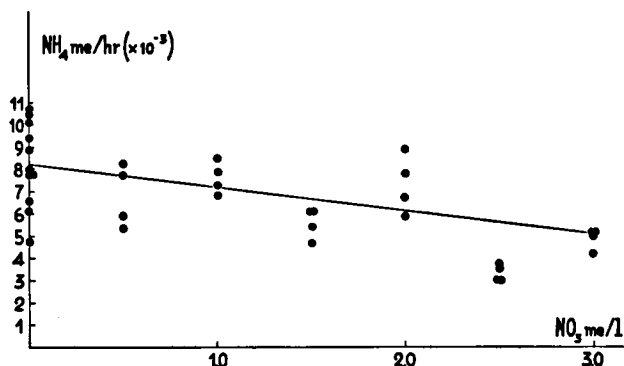


Fig. 13. Absorption of  $\text{NH}_4$  at a constant  $\text{NH}_4$  concentration of 0.2 m.e./l in relation to  $\text{NO}_3$  concentration (500 intact seedlings).

It should be mentioned that with the 0.2 m.e./l  $\text{NH}_4$  solution used, in experiments without nitrate the maximum rate of ammonium-nitrogen absorption is not yet reached.

1.3. *The absorption of nitrate from solutions of a constant nitrate concentration and varied concentrations of ammonium-nitrogen*

The influence of 0.0–1.0 m.e./l  $\text{NH}_4$  on the nitrate absorption from a 1.0 m.e./l  $\text{NO}_3$  solution was determined. The intact seedlings had been supplied with daily-renewed complete nutrient solutions during

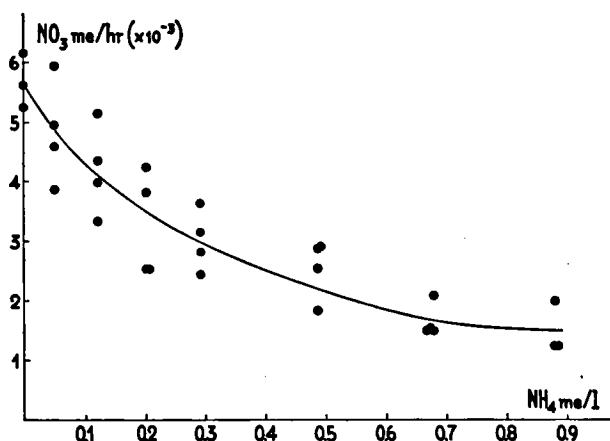


Fig. 14. Absorption of  $\text{NO}_3$  at a constant  $\text{NO}_3$  concentration of 1.0 m.e./l in relation to  $\text{NH}_4$  concentration (500 intact seedlings).

growth in the light. Before the experiments, the material was adapted to the experimental solution for 2.5 hours.

*Experimental conditions.* The solutions supplied contained 1.0 mmol  $\text{KNO}_3$  and 0.0–1.0 mmol  $(\text{NH}_4)\text{H}_2\text{PO}_4$ . They were buffered at pH 6.3 with 3 mmol triethylamine and phosphoric acid. The experiments lasted 60 minutes and the temperature was kept at 25° C.

*Results.* It became apparent (Fig. 14) that the rate of  $\text{NO}_3$  absorption (0.0057 m.e./hour-500 seedlings in the absence of ammonium) decreased markedly with rising ammonium concentrations approaching a minimum absorption of only 0.0016 m.e.  $\text{NO}_3$ /hour-500 seedlings. At any increase of the ammonium concentration, nitrate absorption seemed to be inhibited proportionally to the increase of ammonium-nitrogen absorption.

## 2. EXPERIMENTS ON THE INFLUENCE OF TEMPERATURE ON THE SIMULTANEOUS ABSORPTION OF AMMONIUM AND NITRATE

### 2.1. *Experiments with full-grown plants*

The influence of temperature on the simultaneous absorption of ammonium and nitrate from  $\text{NH}_4\text{NO}_3$  solutions was investigated on full-grown plants in the range from 2.5° to 35° C.

*Experimental conditions.* The experiments were performed in the summer. Ammonium nitrate was given in a concentration of 1.0 mmol  $\text{NH}_4\text{NO}_3$  and 0.1 mmol  $\text{CaSO}_4$  was added to this solution. The pH was 6.0 and the experiments lasted 90 minutes.

*Results.* The results, shown in Fig. 15, will be reported together with those of the next experiments.

### 2.2. *Experiments with seedlings*

The influence of temperature on the simultaneous absorption of nitrate and ammonium from  $\text{NH}_4\text{NO}_3$  solutions was investigated for intact seedlings in the temperature range from 5° to 35° C. In these experiments the material had been supplied with a complete nutrient solution on the first day of growth in the light only. Prior to the experiments, the material was adapted to the experimental solution and to the experimental temperature for 2.5 hours.

*Experimental conditions.* The experiments were performed with a 1.0 mmol  $\text{NH}_4\text{NO}_3$  solution buffered at pH 6.2 with phosphate, and the duration of the experiments was chosen between 120 and 150 minutes.

*Results.* Identical results were obtained with the full-grown plants and with the seedlings (Figs. 15 and 16). The influence of temperature on the rate of absorption of ammonium from  $\text{NH}_4\text{NO}_3$  solutions appeared to be hardly or not at all affected by the presence of nitrate and curves with a distinct optimum at 24–25° C for full-grown plants and seedlings were again obtained. As already mentioned, optimal  $\text{NH}_4$  absorption by full-grown plants from  $(\text{NH}_4)_2\text{SO}_4$  solutions occurred at 22–23° C and by seedlings from  $\text{NH}_4\text{Cl}$  solutions at about 27° C (Figs. 3 and 4).

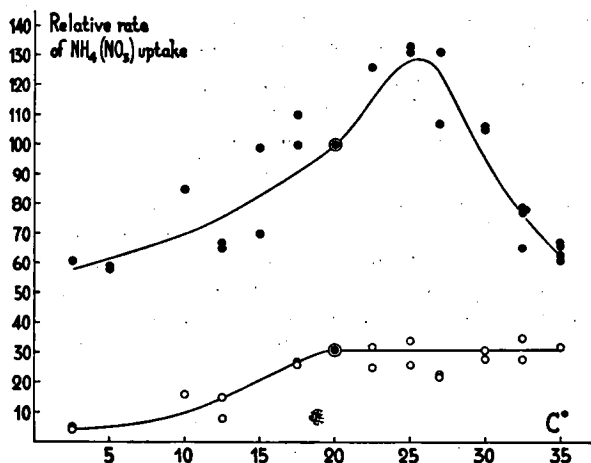


Fig. 15. Absorption of  $\text{NH}_4$  and  $\text{NO}_3$  combined (● and ○ respectively) at an  $\text{NH}_4\text{NO}_3$  concentration of 1.0 mmol and at a pH of 6.0 in relation to temperature (full-grown plants).

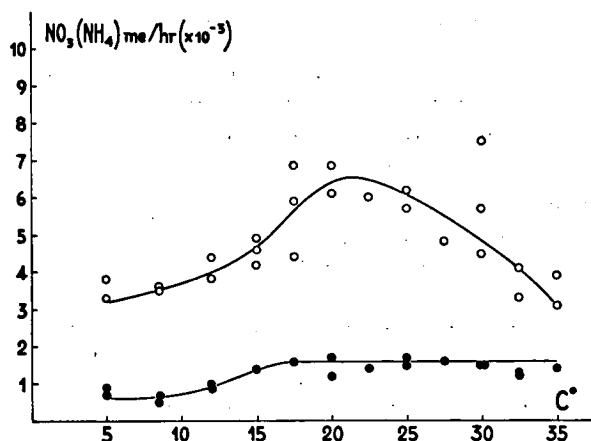


Fig. 16. Absorption of  $\text{NH}_4$  and  $\text{NO}_3$  combined (○ and ● respectively) at an  $\text{NH}_4\text{NO}_3$  concentration of 1.0 mmol and a pH of 6.2 in relation to temperature (500 intact seedlings).

The influence of temperature on the nitrate absorption proved on the contrary to be greatly affected by the presence of ammonium. In comparing Figs. 10, 15, and 16, it becomes evident that the rate of nitrate absorption increases with increasing temperatures up to 15° C whether or not  $\text{NH}_4$  is present. However, by raising the temperatures above 15–20° C to 35° C, the absorption of nitrate is further stimulated when  $\text{NH}_4$  is absent, whereas in the presence of ammonium the rate of nitrate absorption is not affected by temperatures in the

range from 20°–35° C after a rate of nitrate absorption of 0.0016 m.e./hour-500 seedlings is reached.

The absorption ratio  $\text{NH}_4:\text{NO}_3$  at 20° C amounted to 3 for full-grown plants and 4 for seedlings.

### 3. EXPERIMENTS ON THE INFLUENCE OF pH ON THE SIMULTANEOUS ABSORPTION OF AMMONIUM AND NITRATE

The influence of pH in the range from pH 5.3 to 8.2 on the simultaneous absorption of ammonium and nitrate was determined for intact seedlings supplied with a daily-renewed complete nutrient solution during growth in the light.

*Experimental conditions.* The solutions used were 1.0 mmol  $\text{NH}_4\text{NO}_3$  solutions buffered with 3.0 mmol triethylamine and hydrochloric acid. The temperature was 25° C, and the experiments lasted 45 minutes after an adaptation period of 2.5 hours in an experimental solution buffered at pH 6.0.

*Results.* Concerning the influence of pH on the rate of  $\text{NH}_4$  absorption from  $\text{NH}_4\text{NO}_3$ , the minimum curve obtained resembled those obtained in  $\text{NH}_4\text{Cl}$  and  $(\text{NH}_4)_2\text{HPO}_4$  solutions (Figs. 8 and 17). However, the minimum was less pronounced and occurred at a slightly lower pH value (pH 6.2) than in case of  $\text{NH}_4\text{Cl}$  and  $(\text{NH}_4)_2\text{HPO}_4$  (pH 6.7 and 6.9 respectively).

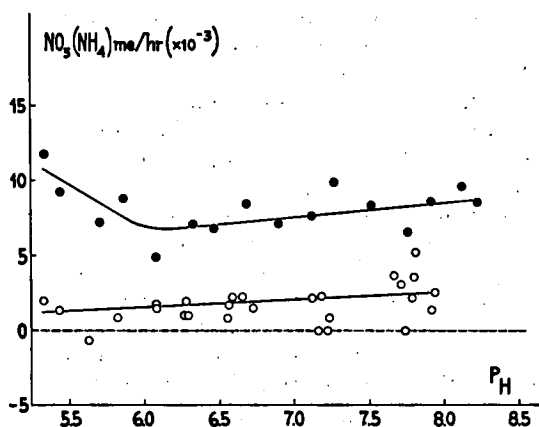


Fig. 17. Absorption of  $\text{NH}_4$  and  $\text{NO}_3$  combined (● and ○ respectively) at an  $\text{NH}_4\text{NO}_3$  concentration of 1.0 mmol and at a temperature of 25° C in relation to pH (500 intact seedlings).

With respect to the influence of pH on the nitrate absorption from  $\text{NH}_4\text{NO}_3$ , the results were completely different from those obtained when  $\text{KNO}_3$  was supplied (Figs. 11 and 17). In the latter case, a curve with a distinct maximum at pH 6.2 was found. Contrarily, if  $\text{NO}_3$  was supplied as  $\text{NH}_4\text{NO}_3$ , an increase in pH resulted in a weak increase in the rate of nitrate absorption over the whole range of pH values investigated.

#### 4. CONCLUSION

In experiments on the simultaneous absorption of ammonium-nitrogen and nitrate, the following phenomena were observed:

1. The absorption of ammonium is only slightly affected by the presence of nitrate in all relevant experiments.

2. If both nitrogen sources were absorbed from solutions in which the nitrate- and ammonium-nitrogen concentrations were varied equally, the nitrate absorption was greatly inhibited by the presence of ammonium, but a relation between ammonium nitrate concentration and rate of nitrate absorption that can be described by a Langmuir adsorption isotherm was indicated.

If both nitrogen sources were taken up from solutions of a constant nitrate concentration and varied concentrations of ammonium nitrogen, the absorption of nitrate is heavily but not completely inhibited by increased concentrations of ammonium-nitrogen in the external solution. The extent to which the nitrate absorption was inhibited seemed to be proportional to the increase of ammonium absorption.

As compared to these observations, WEISSMAN (1950) concluded that when ammonium and nitrate are present simultaneously, the absorption of either by wheat seedlings is less than when present alone. EL-SHISHINY (1955) on the other hand, observed that storage tissues of sweet potato tubers and radish and carrot roots absorbed both nitrogen sources at more or less equal rates from 5 mmol  $\text{NH}_4\text{Cl}$ -,  $\text{KNO}_3$ -, or  $\text{NH}_4\text{NO}_3$ -solutions.

For mould fungi, MORTON (1956) obtained results comparable to those for perennial rye-grass; he showed that nitrate assimilation in his material is inhibited to a very large extent by the presence of ammonium-nitrogen.

The widely differing results from experiments on the simultaneous absorption of the two nitrogen sources reported by many workers have been ascribed to such factors as the concentration of ammonium nitrate in the experimental solution (PRIANISCHNIKOV, 1934), the ratio  $\text{NH}_4:\text{NO}_3$  supplied (WEISSMAN, 1950), the species of plant investigated (STREET and SHEAT, 1958), and also to the stage of plant development (PRIANISCHNIKOV, 1934; STREET and SHEAT, 1958).

3. In the presence of equal amounts of ammonium-nitrogen and nitrate, any rise in temperature between 5° and 15–20° C promoted the maximum rate of nitrate absorption independent of the presence of ammonium. Between 15–20° and 35° C, ammonium inhibited the rate of nitrate absorption to a constant low value and in consequence, between 15–20° and 35° C the absorption rate of nitrate was not seen to be favoured by raising the temperature.

4. In the presence of equal amounts of ammonium-nitrogen and nitrate, the effect of pH on the absorption rate of ammonium from solutions buffered with triethylamine and HCl, did not appear to be qualitatively influenced by the presence of nitrate. Quantitatively, the effect of pH on the absorption rate of ammonium proved to depend



on the accompanying anion in the sequence:  $\text{Cl} > \text{H}_2\text{PO}_4 > \text{SO}_4$ , and the influence of  $\text{NO}_3$  together with a triethylamine +  $\text{HCl}$  buffer resembled that of  $\text{Cl}$  and  $\text{H}_2\text{PO}_4$ .

Whereas in the absence of ammonium a pH-optimum curve was obtained for nitrate absorption, the rate of nitrate absorption in the presence of ammonium was hardly affected by pH over the whole range of pH values investigated, showing only a slight increase at increasing pH.

WEISSMAN (1950) observed for wheat seedlings that the rate of ammonium absorption is stimulated by increased pH from 4.3 to 6.3 whether or not nitrate is present. However, the nitrate absorption that proved to be independent of the pH between values of pH 4.3 to 6.3 if supplied alone, showed a decrease with increasing pH from pH 4.3 to 6.3 in the presence of ammonium-nitrogen.

## CHAPTER V

### EXPERIMENTS ON THE RATE OF NITRATE ACCUMULATION AND NITRATE REDUCTION IN EXCISED ROOTS

It is a known fact that the nitrate absorbed by the cell can either be accumulated as such or reduced and assimilated into amino acids.

Because the results reported in the preceding Chapter showed a pronounced effect of ammonium on the absorption rate of nitrate, it became interesting to investigate the effect on the rates of nitrate accumulation and nitrate reduction of a change in the rate of absorption due to the presence of ammonium.

It is common knowledge that a high capacity to reduce nitrate can be present in the leaves. However, a possible reduction process in the leaves is of no importance to the present problem because we are interested in accumulation and reduction only in so far as these processes are linked with the absorption process in the roots. It follows that the experiments described here could not be performed with intact plants but had to be carried out with excised roots.

#### 1. EXPERIMENTS ON THE OCCURRENCE OF NITRATE ACCUMULATION AND NITRATE REDUCTION IN EXCISED ROOTS

Firstly, it had to be ascertained whether in perennial rye-grass part of the absorbed nitrate is accumulated and part of the nitrate taken up is indeed reduced. This question can be answered by the simple investigation of the amount of nitrate absorbed by the roots in a given space of time and the amount of nitrate within the roots that can subsequently be demonstrated in this form. The difference between the absorbed quantity of nitrate and the amount of free nitrate within the roots represents the quantity of nitrate that has been reduced.

*Experimental procedure.* Samples of about 0.3 g of excised roots were allowed to absorb nitrate for 5.5 hours from 25 ml of a solution con-

taining 0.45 mmol  $\text{KNO}_3$ , ammonium molybdate to an amount of 0.2 mg/l of Mo, and buffered at pH 6.05 by 4 mmol Tris +  $\text{H}_2\text{SO}_4$ . The solution was kept at 25° C and continuously aerated. At the end of the absorption period, the decrease of nitrate concentration in the solutions was determined and the samples of roots were analysed for nitrate content.

*Results.* The results, summarized in Table 3, show that after an absorption period of 5.5 hours a considerable part, although not all, of the absorbed nitrate was recovered from the material. So it must be assumed that in the above experiment, on the average 45 % of the absorbed quantity of nitrate was reduced and 55 % of this absorbed quantity was accumulated in the roots.

TABLE 3

The amounts of nitrate absorbed, accumulated, and reduced after an absorption period of 5.5 hours (excised roots).

sample	m.e. $\text{NO}_3$ per gram of roots absorbed	m.e. $\text{NO}_3$ per gram of roots accumulated	m.e. $\text{NO}_3$ per gram of roots reduced (deduced values)
1	0.0154	0.0085	0.0069
2	0.0125	0.0080	0.0045
3	0.0127	0.0068	0.0059
4	0.0134	0.0073	0.0061
5	0.0155	0.0068	0.0087
mean	0.0139	0.0075	0.0064

## 2. EXPERIMENTS WITH RESPECT TO NITRATE ABSORPTION, NITRATE ACCUMULATION, AND ACCUMULATED NITRATE RETAINED FOR TWO HOURS

If nitrate accumulation is followed by the process of nitrate reduction and assimilation, the quantity of nitrate accumulated could be expected to decrease if absorption of nitrate is not continued. On the other hand, if the accumulation and reduction of nitrate are independent processes, the content of free  $\text{NO}_3$  ions in the roots could be expected to remain constant if further absorption is prevented. Experiments were therefore conducted in which the quantities of nitrate absorbed and nitrate accumulated were measured. The excised roots were then transferred to distilled water, after which the release of absorbed nitrate to distilled water and the quantity of free nitrate retained in the roots were determined.

*Experimental procedure.* In two series of experiments, using 3.40 and 2.24 grams of excised roots respectively, the material was allowed to absorb nitrate from 200 ml of a 0.45 mmol  $\text{KNO}_3$  solution, buffered at pH 6.05 by means of 4 mmol Tris and sulphuric acid, and to which ammonium molybdate to an amount of 0.2 mg/l of Mo was added.

The absorption period at 25° C was continued for 4.5 hours. Subsequently, the quantity of nitrate absorbed was determined by analysis of the solutions. In both series of experiments the nitrate content of part of the material was determined, while the other part of the root material was transferred to distilled water for two hours. The distilled water was analysed for nitrate content, and the nitrate content of the roots was also determined. The solutions were aerated continuously.

*Results.* The results are given in Table 4; they again show that a considerable part of the absorbed nitrate can be detected in the roots as accumulated nitrate. Practically no loss of accumulated nitrate to distilled water was demonstrated. It was apparent that within the experimental accuracy, accumulated nitrate was not assimilated in a period of two hours.

TABLE 4

Quantities of nitrate absorbed, nitrate accumulated, and accumulated nitrate retained for two hours.

experimental series	m.e. NO <sub>3</sub> per gram of roots absorbed	m.e. NO <sub>3</sub> per gram of roots accumulated	m.e. NO <sub>3</sub> per gram of roots released to distilled water	m.e. NO <sub>3</sub> per gram of roots retained
1	0.0140	0.0073 0.0074	0.0005	0.0064 0.0065
2	0.0136	0.0065 0.0073	0.0000	0.0067 0.0066
mean	0.0138	0.0071	0.0003	0.0066

### 3. INFLUENCE OF AMMONIUM-NITROGEN ON THE ACCUMULATION AND REDUCTION OF NITRATE

To study this question, the absorption and accumulation of nitrate from nitrate and ammonium + nitrate solutions was determined.

*Experimental conditions.* The material was allowed to absorb nitrate from 200 ml of a solution of 0.45 mmol KNO<sub>3</sub>, buffered at pH 6.05 by means of 4 mmol Tris + sulphuric acid and containing ammonium molybdate to an amount of 0.2 mg/l of Mo. The influence of 0.15 and of 0.75 mmol (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> on the amount of nitrate absorbed and nitrate accumulated was investigated.

The solutions were aerated continuously. The experiments lasted 5 hours and the temperature was held at 25° C.

*Results.* It will be recalled that in previous experiments (Chapter II, section 1.2, Fig. 2) the rate of ammonium absorption by intact seedlings approached its maximum value at a concentration of 0.3 m.e./l of NH<sub>4</sub>. In agreement with this observation, the rate of ammonium absorption in the present experiments using excised roots was

not accelerated by an increase of the external ammonium-nitrogen concentration from 0.3 m.e./l of  $\text{NH}_4$  to 1.5 m.e./l of  $\text{NH}_4$  (Table 5).

TABLE 5

The influence of ammonium-nitrogen on the absorption, accumulation, and reduction of nitrate in excised roots.

experimental series	m.e./l of $\text{NH}_4$ supplied	m.e. $\text{NH}_4$ absorbed per gram of material	m.e. $\text{NO}_3$ absorbed per gram of material	m.e. $\text{NO}_3$ accumulated per gram of material	m.e. $\text{NO}_3$ reduced per gram of material
1	0.0	—	0.0115	0.0039 0.0042	0.0076 0.0073
2	0.0	—	0.0118	0.0044 0.0042	0.0074 0.0076
mean	no $\text{NH}_4$ added		0.0117	0.0042	0.0075
3	0.30	0.0150	0.0088	0.0040 0.0042	0.0048 0.0046
4	1.50	0.0128	0.0091	0.0042 0.0043	0.0049 0.0048
mean	$\text{NH}_4$ added		0.0090	0.0042	0.0048

As for intact plants (Chapter IV), evidence was obtained that the absorption rate of  $\text{NO}_3$  by excised roots is reduced by ammonium (Table 5), and that the inhibition of nitrate absorption is not proportional to the external ammonium concentration but to the rate of ammonium absorption (cf. Chapter IV, section 1.3).

Whereas nitrate absorption was markedly reduced by  $\text{NH}_4$ , the accumulation proved to be completely unaffected. It follows that the reduction of nitrate is inhibited to a large extent if nitrate absorption is affected by ammonium.

#### 4. THE INFLUENCE OF AMMONIUM-NITROGEN ON THE ABSORPTION, ACCUMULATION, AND REDUCTION OF NITRATE IN EXCISED ROOTS AS COMPARED TO THE INFLUENCE OF AMMONIUM-NITROGEN ON THE ABSORPTION, ACCUMULATION, AND REDUCTION OF NITRITE

In the preceding section it was shown that if nitrate absorption is reduced as result of the presence of ammonium, the reduction of nitrate is simultaneously inhibited to a large extent, whereas nitrate accumulation is not affected. It is common knowledge that the reduction of nitrate, preceding assimilation, is a complicated process. Therefore, in order to obtain data as to the stage of nitrate reduction at which ammonium-nitrogen exerts its influence, the effect of supply of ammonium to the experimental solution on the absorption and accumulation of nitrate in this experiment was compared with that on the absorption and accumulation of the first intermediate in the reduction process of nitrate, nl. nitrite.

*Experimental procedure.* Forty samples of excised roots with a fresh

weight of between 0.15 and 0.46 grams were allowed to absorb either nitrate or nitrite from 25 ml of a solution containing the respective nitrogen sources for 5.5 hours. Use was made of  $\text{NO}_3$ ,  $\text{NO}_3 + \text{NH}_4$ ,  $\text{NO}_2$ , and  $\text{NO}_2 + \text{NH}_4$  solutions, and each of these solutions was supplied to 10 samples of roots. These solutions contained ammonium molybdate to an amount of 0.2 mg/l of Mo and were buffered at pH 6.05 with 4 mmol Tris +  $\text{H}_2\text{SO}_4$ . The solutions also contained either 0.45 mmol  $\text{KNO}_3$  or 0.35 mmol  $\text{KNO}_2$  and  $(\text{NH}_4)_2\text{SO}_4$  to a concentration of 0.3 mmol was either added or omitted. The temperature was kept at 25° C and the solutions were aerated continuously.

After the absorption period, the decrease in the nitrate or nitrite concentration of the solutions was determined and the samples of roots were analysed for nitrate or nitrite content. The quantities of absorbed and accumulated nitrate or nitrite were computed per gram of fresh root weight, and by subtraction of these values the amounts of reduced nitrate or nitrite per gram of fresh weight of matter was obtained.

*Results.* The results are shown in Fig. 18. With respect to nitrate, it is again seen that ammonium supply results in reduced rates of both absorption and reduction, but that ammonium does not affect nitrate accumulation.

With respect to nitrite, it is established that ammonium does not reduce the rate of nitrite absorption and that both nitrite accumulation and nitrite reduction are independent of the presence of ammonium-nitrogen.

It was also observed that in this experiment the rate of nitrate reduction in the absence of ammonium equals approximately the rate of nitrite reduction.

##### 5. THE INFLUENCE OF AMMONIUM-NITROGEN ON THE ABSORPTION OF NITRATE AND NITRITE IN INTACT SEEDLINGS

These experiments were conducted with excised roots, which raises the question of whether the experimental results with respect to the influence of ammonium on the absorption of nitrite in excised roots would also hold for intact perennial rye-grass. An additional experiment with intact seedlings was therefore performed. In this experiment the absorption rates of nitrate and of nitrite were determined and the influence of ammonium on these absorption rates was investigated.

*Materials.* Samples of 0.5 gram of seeds (of the 1961 harvest) were allowed to germinate and then grown in artificial light as described for the cultivation of the intact seedlings used in previous experiments (Chapter I, section 2).

*Experimental procedure.* Each of 10 samples of seedlings was allowed to adapt to 90 ml of the experimental solution for 2.5 hours. This solution contained 0.5 mmol  $\text{KNO}_3$ , ammonium molybdate to an amount of 0.2 mg/l of Mo, and was buffered at pH 6.05 by 4 mmol Tris +  $\text{H}_2\text{SO}_4$ . Five of the 10 samples were supplied with this solution,

and 5 samples were supplied with this solution plus  $(\text{NH}_4)_2\text{SO}_4$ , added to a concentration of 0.3 mmol.

After the adaptation period, the experimental absorption period followed, lasting 3 hours. Subsequently, the absorption of  $\text{NO}_3$  for 3 hours was determined by analysis of the experimental solutions.

Each of 12 other samples of seedlings was allowed to absorb nitrite from 90 ml of a solution containing 0.35 mmol  $\text{KNO}_2$  and ammonium molybdate to an amount of 0.2 mg/l of Mo, buffered at pH 6.05 by

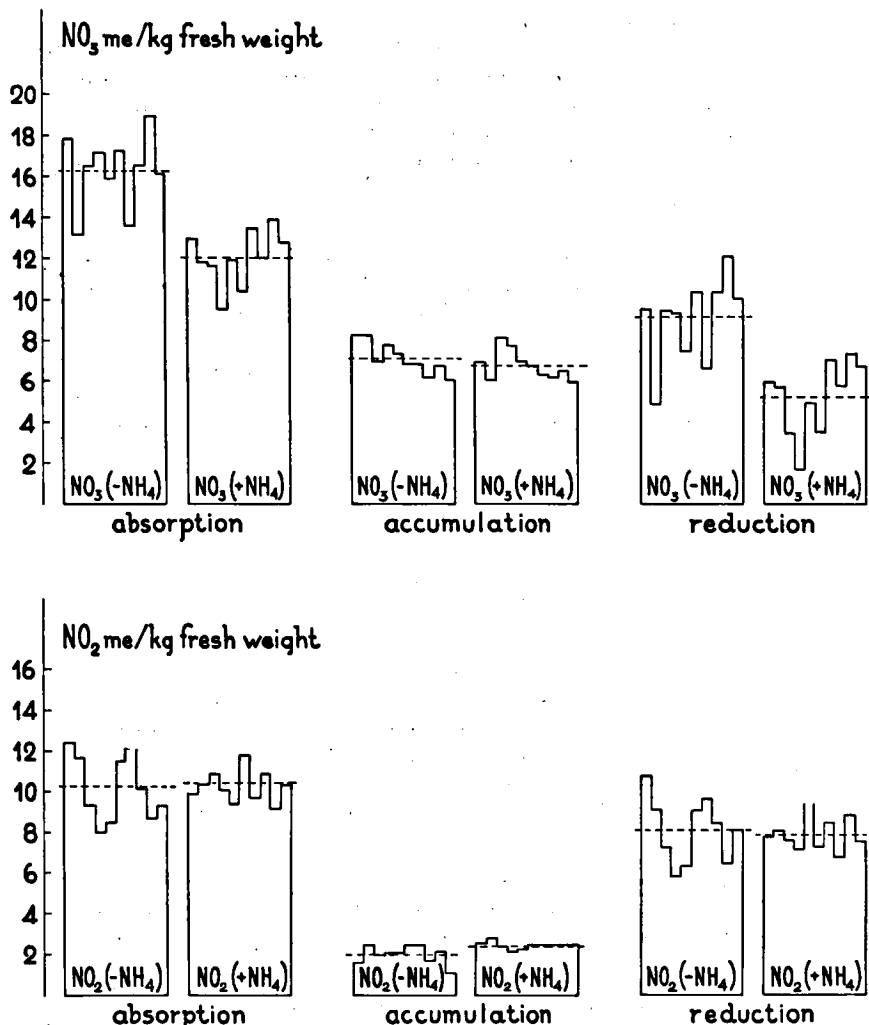


Fig. 18. Effect of  $\text{NH}_4$  on the absorption, accumulation, and reduction of  $\text{NO}_3$  and  $\text{NO}_2$  (excised roots). Either  $\text{NO}_3$  or  $\text{NO}_2$  was supplied and  $\text{NH}_4$  was either added or omitted. Absorption period: 5½ hours. The dotted lines represent the mean values of 10 experiments.

means of 4 mmol Tris +  $\text{H}_2\text{SO}_4$ . This solution, containing in addition 0.3 mmol  $(\text{NH}_4)_2\text{SO}_4$ , was supplied to 6 of the 12 samples.

There was no period of adaptation and the absorption period was 5.5 hours. The absorption of nitrite for 5.5 hours was determined by analysis of the experimental solutions.

The temperature of all solutions in this experiment was  $25^\circ\text{C}$  and the solutions were aerated continuously.

*Results.* The results are shown in Fig. 19. Evidence was obtained that in this material (seeds of the 1961 harvest) the absorption rate of nitrate was inhibited by about 50 % in the presence of ammonium. In previous experiments with intact seedlings (seeds of the 1956 harvest), nitrate absorption was inhibited by about 75 % in the presence of ammonium nitrogen.

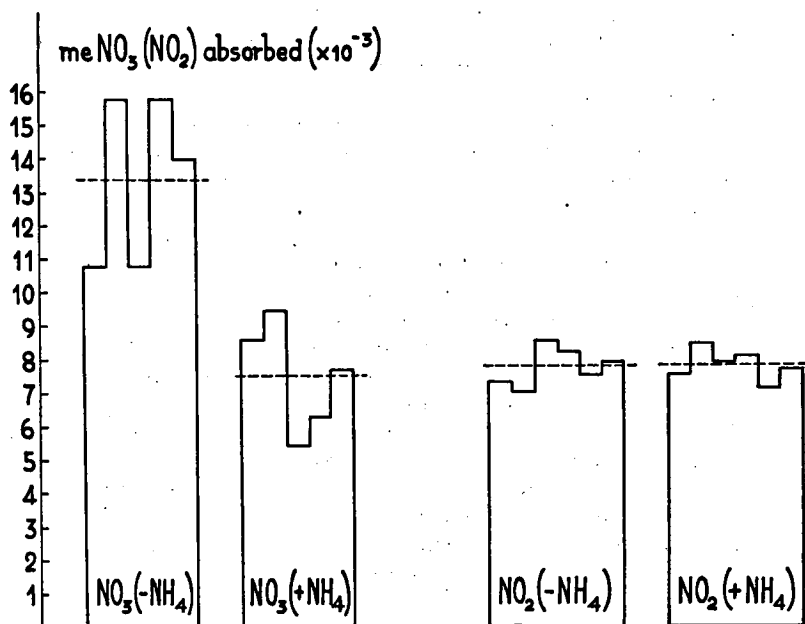


Fig. 19. Effect of  $\text{NH}_4$  on the absorption of  $\text{NO}_3$  and  $\text{NO}_2$  (about 300 intact seedlings). Either  $\text{NO}_3$  or  $\text{NO}_2$  was supplied and  $\text{NH}_4$  was either added or omitted.  $\text{NO}_3$  was absorbed for 3 hours after an adaptation period of 2.5 hours.  $\text{NO}_2$  was absorbed for  $5\frac{1}{2}$  hours. There was no period of adaptation. The dotted lines represent the mean  $\text{NO}_3$  absorption of 5 experiments or the mean  $\text{NO}_2$  absorption of 6 experiments.

In agreement with the experiments with excised roots, the rate of nitrite absorption was found to be independent of the presence of ammonium-nitrogen.

## 6. CONCLUSION

The experiments on absorption, accumulation, and reduction in excised roots show that:

1. The absorbed nitrate is both accumulated and reduced.

2. Accumulated nitrate is retained quantitatively in that form for at least 2 hours. It follows that accumulated nitrate is not involved directly in nitrate reduction. Consequently, an actual accumulation takes place independently of the reduction process.

In contrast to this observation, YEMM and WILLIS (1956) reported that during the absorption of ammonium-nitrogen by barley there is an initial increase of free ammonium content in the roots, followed by a decrease. In this case the accumulation of free ammonium results from a rate of ammonium assimilation that is inadequate with respect to the rate of ammonium absorption in the first period of uptake. Therefore, I prefer to call the above accumulation of ammonium-nitrogen in barley an *ammonium block* as distinct from the *true accumulation* of nitrate in perennial rye-grass.

3. If nitrate absorption is affected by the presence of ammonium, nitrate accumulation is not influenced but the rate of nitrate reduction is reduced markedly.

In contrast with this result, from experiments lasting 4 days it was shown by WEISSMAN (1951) that ammonium nitrogen affects both the accumulation and the reduction of nitrate in wheat seedlings.

4. At ammonium concentrations at which the rate of ammonium absorption approaches its maximum value, the inhibition of nitrate absorption and nitrate reduction is not increased at increased concentration of ammonium-nitrogen in the solution.

5. Nitrite absorption and nitrite accumulation and reduction are completely independent of the presence of ammonium. In agreement with this observation, nitrite absorption by intact seedlings was found to be independent of ammonium supply to the medium.

These results are in good agreement with those obtained by MORTON and MACMILLAN (1954) and by MORTON (1956) with mould fungi, showing that nitrate assimilation is nearly completely inhibited by ammonium assimilation, whereas nitrite absorption is practically unaffected. Similarly, it was shown by SYRETT and MORRIS (1963) that nitrate assimilation in *Chlorella* is inhibited completely by ammonium assimilation. However, the nitrite assimilation was also inhibited to a large extent, although not completely (80 % inhibition), by ammonium.

## CHAPTER VI

## DISCUSSION

The experiments, described in this thesis have demonstrated the existence of interaction between ammonium and nitrate in the absorption by perennial rye-grass. The problem is now, in what way and in which phase of the absorption process this interaction takes place. The first point here is to understand how either one of the two nitrogen sources is absorbed when supplied alone. The interaction between ammonium and nitrate will therefore be discussed after these questions have been answered:



1. In what form are ammonium- and nitrate nitrogen absorbed by perennial rye-grass?
2. Is the absorption of ammonium- and nitrate-nitrogen an active or a passive process?
3. What do we know about the absorption systems by which ammonium- and nitrate-nitrogen are absorbed?

# 1. THE FORM IN WHICH AMMONIUM- AND NITRATE-NITROGEN ARE ABSORBED BY PERENNIAL RYE-GRASS

## 1.1. *The form in which ammonium-nitrogen is absorbed*

Concerning the form in which ammonium-nitrogen enters the plant roots, differences of opinion are evident from literature. In principle there are three possibilities:

- a. ammonium is absorbed as cations.
- b. ammonium-nitrogen is absorbed in the form of undissociated  $\text{NH}_4\text{OH}$  molecules.
- c. ammonium-nitrogen is absorbed both as ions and in the form of  $\text{NH}_4\text{OH}$  molecules.

With respect to these possibilities, the form in which ammonium-nitrogen is most probably absorbed can best be ascertained on basis of the observed relation between rate of absorption of ammonium-nitrogen and the pH of the solution, in view of the fact that the ratio undissociated  $\text{NH}_4\text{OH}$  molecules: $\text{NH}_4$  cations increases with increased pH value.

Of the pH curves for absorption of ammonium-nitrogen obtained in the present work, only these curves resulting from experiments in which a complete nutrient solution was supplied will be used for discussion in this problem. In the following it will be shown that the shape of the pH curves obtained with one-salt solutions is different as a result of complications.

If it is assumed that ammonium-nitrogen is absorbed only in the form of ions, it becomes difficult to explain the observation that in our experiments the rate of ammonium-nitrogen absorption was hardly affected by the  $\text{H}^+$  concentration in the pH range of pH 4.0–6.5 but was markedly influenced by pH values of 6.5 to 8.5 such that an increased pH value above 6.5 resulted in an increased rate of absorption (Fig. 7). Moreover, it is evident from the same experiments that the rate of absorption of ammonium nitrogen is strongly dependent on temperature in the pH range from 4.0 to 6.5: at a temperature of  $35^\circ\text{C}$  the absorption is inhibited to a large extent. On the other hand, the increase in the absorption rate in the pH range of pH 6.5 to 8.5 proved to be independent of temperature.

The experiments with full-grown plants (Fig. 7) showed that at increasing concentrations of  $\text{H}^+$  ions in the medium the absorption of ammonium-nitrogen decreases and approaches asymptotically a definite rate of absorption. A similar observation was made by VAN DEN HONERT and HOOYMANS (1961) in experiments with maize.

Since at increasing concentrations of  $H^+$  ions in the medium the concentration of  $NH_4OH$  molecules decreases to zero, it seems to be excluded that ammonium-nitrogen is only absorbed by perennial rye-grass in the form of molecules.

From the foregoing it is evident that the influence of pH on the absorption rate of ammonium-nitrogen cannot be explained conclusively in terms of absorption of ammonium-nitrogen either in the form of ions only or in the molecular form.

In consequence of the different relations between  $NH_4$  concentration and rate of  $NH_4$  absorption by maize plants at pH 6.0 and pH 7.4, VAN DEN HONERT and HOOYMANS (1961) hypothesized that ammonium-nitrogen is absorbed in the form of  $NH_4$  ions by a process that is independent of pH values above 6.0 and that, in addition, ammonium-nitrogen enters the plant roots in the molecular form at a rate proportional to the amount of  $NH_4OH$  present in the solution. The following fact supports the hypothesis of van den Honert and Hooymans: in the case of perennial rye-grass, the influence of pH on the absorption of ammonium-nitrogen from complete nutrient solutions can be seen as follows: absorption independent of pH but dependent on temperature, and an additional absorption above pH 6.3 that is dependent on pH (as is the concentration of  $NH_4OH$  in the solution) but independent of temperature. Therefore, the assumption seems warrantable that ammonium-nitrogen is absorbed in the form of ions and is also taken up in the molecular form at pH values above 6.3.

As will be discussed later in this paper, the influence of pH on the absorption of ammonium-nitrogen from one-salt solutions is of a more complicated nature. Nevertheless, the results obtained do not weaken this hypothesis.

It should be noted that with the exception of the experiments concerning the influence of pH, all experiments with regard to the absorption of ammonium-nitrogen were performed at pH values of between 5.7 and 6.0. Because of this condition, we can more or less confine the discussion of the absorption of ammonium-nitrogen to a discussion of the absorption of ammonium ions.

### 1.2. *The form in which nitrate-nitrogen is absorbed*

The question of the form in which nitrate-nitrogen is absorbed by perennial rye-grass offers few difficulties because in solutions of nitrate the concentration of undissociated nitrate is negligible.

*Summarizing*, it is assumed that ammonium-nitrogen is absorbed by perennial rye-grass in the form of ions. In addition, it is also assumed that ammonium-nitrogen enters the roots of perennial rye-grass in the molecular form on condition that the pH value of the medium surpasses 6.3.

With the exception of the influence of pH on the rate of ammonium-nitrogen absorption, the uptake of  $NH_4OH$  molecules is beyond the scope of this paper.

It is concluded that nitrate-nitrogen is absorbed in the form of ions.

## 2. ARE AMMONIUM AND NITRATE IONS ABSORBED IN AN ACTIVE OR IN A PASSIVE WAY?

The concepts passive and active absorption can be handled only with great difficulty. For this reason, the concepts '*metabolically independent*' and '*metabolically dependent*' absorption are suggested. Under *metabolically independent* absorption is understood absorption that, in the existing system, is not supported by metabolic processes. In such absorption a steady state will not be attained: with respect to the ions to be absorbed, the concentrations at the outside and the inside of the plant will establish an equilibrium.

Under *metabolically dependent* absorption is understood absorption that, in the existing system, is supported by metabolic processes, either because the metabolism interferes with the absorption mechanism itself, or because with respect to the ions to be absorbed the metabolism maintains a gradient of concentrations at the outside and the inside of the plant. Both forms of metabolically dependent absorption are characterized by the achievement of a steady state. A typical example of a *metabolically dependent* absorption in which the metabolism maintains a gradient of concentrations is given by the observation of VAN DEN HONERT and HOOYMANS (1961) with regard to the uptake of  $\text{NH}_4\text{OH}$  by maize: a rectilinear relation between the  $\text{NH}_4\text{OH}$  concentration in the medium and the rate of absorption was found, and a steady state was also demonstrated. In terms of the definition, these data point to a *metabolically dependent* absorption whose rate is limited by a prior diffusion.

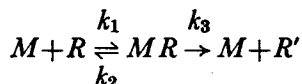
A steady state in the absorption of ammonium by intact seedlings was demonstrated in experiments in which the rate of  $\text{NH}_4$  absorption at  $35^\circ\text{C}$  was traced for six hours (Fig. 5). Similarly, a steady state in the absorption of nitrate by intact seedlings appeared after 2.5 hours in experiments in which the rate of  $\text{NO}_3$  absorption from solutions containing molybdenum and buffered by Tris and  $\text{H}_2\text{SO}_4$  was observed for 6 hours. Moreover, it will be recalled that in the experiments with full-grown plants, the rates of nitrate and ammonium absorption under standard conditions before exposure to the experimental conditions and under standard conditions after exposure usually differed less than 15 %. In consequence, it may be concluded that both the absorption process of ammonium and the absorption process of nitrate by perennial rye-grass is supported by a metabolic process.

*Summarizing*, the question of whether or not the uptake of ammonium and nitrate by perennial rye-grass takes place by an active process can be answered in this way, that both the absorption of  $\text{NH}_4$  and the uptake of  $\text{NO}_3$  in the consisting systems are maintained by a metabolic process.

## 3. THE SYSTEM BY WHICH AMMONIUM AND NITRATE ARE ABSORBED

It is common knowledge that in many experiments the relation between the rate of the *metabolically dependent* absorption and the concentration of ions in the solution can be described by a Langmuir

adsorption isotherm. VAN DEN HONERT (1936) arrived at the 'conveyor belt' theory, later evolved into the 'carrier' theory. In this theory it is assumed that ions are bound to a more or less specific carrier substance, thus forming a complex that is able to penetrate into the root cells and, after entrance, is subsequently broken down, leaving the free ions. JACOBSON *et al.* (1950) formulated the carrier theory as a chemical reaction:



in which  $M$  = ion,  $R$  = carrier,  $MR$  = ion-carrier complex, and  $R'$  = carrier more or less transformed.

On the analogy of the Michaelis-Menten equation for enzyme reactions, EPSTEIN and HAGEN (1952) derived for this reaction the rate of absorption

$$v = \frac{V_{\max} \cdot (M)}{K_m + (M)} = \frac{k_3 \cdot (R_t) \cdot (M)}{\frac{k_2 + k_3}{k_1} + (M)}$$

in which  $v$  = rate of absorption,  $V_{\max}$  = maximum rate of absorption,  $(M)$  = the ion concentration in the medium,  $K_m = (k_2 + k_3)/k_1$  = Michaelis-Menten constant = concentration at which  $v = \frac{1}{2} V_{\max}$  = half-value, and  $(R_t)$  = the total quantity of  $R$  and  $MR$ .

However, two points should be noted here: one, that the above scheme is presumably a considerable simplification of what happens in reality, and two, that if the ions are taken up anyhow, for example by simple diffusion, and are subsequently involved in an enzymatic process of the metabolism, an identical relation can be still found since the same kinetic operation holds both for carrier-systems and for enzyme-systems.

In the following discussion of our experimental data we will start from the formula according to Epstein and Hagen and attempt to determine how far it can explain the observed facts.

### 3.1. *The mechanism for absorption of ammonium*

All experiments with respect to the relation between the  $\text{NH}_4$  concentration in the solution and the rate of absorption by perennial ryegrass gave absorption curves that, within the experimental accuracy, conform the equation:

$$v = \frac{V_{\max} \cdot (M)}{K_m + (M)}$$

(c.f. Figs. 1 and 2). Although each of the concentration-absorption curves obtained can be described by the above equation, quantitative differences among these curves were observed. These differences are defined as follows:

- $V_{\max}$  is dependent on temperature,  
 is dependent on the accompanying anion,  
 is dependent on the pH,  
 (with respect to the effect of the material used and the experimental procedure, comparable data are lacking),  
 is dependent on season.
- $K_m$  is independent of temperature,  
 is independent of the accompanying anion,  
 (with respect to the influence of the pH, data are lacking),  
 is dependent on the experimental procedure and the material used,  
 is dependent on season (as was established with regard to full-grown plants).

3.1.1.  $V_{\max}$  is dependent on temperature. The relation between root temperature and the maximum rate of ammonium absorption found expression in typical optimum curves showing unusually low optimum temperatures ( $22^{\circ}$ – $27^{\circ}$  C). The suggestion that the unbalanced solutions used in these experiments were responsible for this low temperature optimum must be dismissed. For, from the Figs. 3 and 4 it follows that identical results were obtained irrespective of the composition of the experimental solution supplied, nl., a solution containing only ammonium sulphate, or a solution of  $\text{NH}_4\text{Cl}$  and phosphate buffer, or a complete nutrient solution, containing  $(\text{NH}_4)_2\text{SO}_4$ .

According to Fig. 4, the uptake of chloride is, unlike the rate of  $\text{NH}_4$  absorption, favoured by higher temperatures. Because there is obviously a continuous and gradual increase in the *metabolically dependent* absorption of chloride in response to a rise in temperature, it seems very unlikely that the reduced absorption of  $\text{NH}_4$  is to be explained in terms of injury of the grass tissues by high temperatures.

In the experiments with full-grown plants it was observed that the rates of  $\text{NH}_4$  absorption under standard conditions at  $20^{\circ}$  C, before and after exposure to higher temperatures, did not differ worth mentioning. In consequence, the supposition of either a damaged carrier system or an injured enzyme system seems highly improbable. Moreover, at  $35^{\circ}$  C a constant rate of  $\text{NH}_4$  absorption was found for at least 6 hours.

According to Fig. 6, the optimum for  $\text{NH}_4$  absorption approximately coincides with the temperature optimum for growth, measured by the dry weight of the material. The materials concerned were cultivated at different root temperatures and were supplied with complete nutrient solutions in which ammonium was omitted, but which contained nitrate as the sole nitrogen source. Because in short-time experiments both the absorption of chloride and that of nitrate are promoted if the temperature is raised to  $35^{\circ}$  C, the decrease in dry weight at high temperatures is probably not to be ascribed to decreased absorption of nutrient salts. The suggestion that the decreased dry weight of perennial rye-grass is due to the inhibition of some metabolic

process by higher temperatures seems to be the most satisfying explanation. Probably, the effect of temperature above 25° C on the rate of  $\text{NH}_4$  absorption can be described in terms of a direct or indirect influence of the temperature on the rate of ammonium assimilation.

In this connection it must be assumed that ammonium is hardly or not at all accumulated. This assumption is supported by STREET and SHEAT (1958): 'Numerous observations indicate that, in the presence of adequate respirable carbohydrates, ammonium assimilation proceeds so rapidly that, with a high level of nitrogen uptake, only a trace of free ammonium can be detected in the tissues', while a number of cases are mentioned which support poisoning by ammonium if the available amount of carbohydrates is inadequate. On the other hand, YEMM and WILLIS (1956), in their experiments with barley plants, observed that the assimilation of ammonium lags behind the rate of ammonium absorption during the first hours of uptake, resulting in a "block" of free  $\text{NH}_4$ . How can this idea be incorporated into the scheme of Epstein and Hagen? From the above it appears that the influence of temperature is probably due to an effect of temperature on ammonium assimilation. Therefore, the change in  $V_{\max}$  as a result of a temperature effect on  $(R_t)$  seems to be unlikely. On the other hand, the change of  $V_{\max}$  could be due to an effect of temperature on  $k_3$ , allowance being made for the fact that in the scheme of Epstein and Hagen,  $k_3$  represents the rate of a complicated process involving not only the simple break-down of the ammonium-carrier complex but also the total of assimilatory processes of ammonium.

Because  $K_m$  is not affected in a perceptible manner, in case of an influence of temperature on  $V_{\max}$  by way of  $k_3$ , it follows that the value of  $k_3$  must be very much lower than that of  $k_2$ .

3.1.2.  $V_{\max}$  is dependent on the accompanying anion. From Fig. 2 it can be seen that the rate of ammonium absorption is approximately doubled if ammonium is supplied not in the form of the sulphate salt but as ammonium phosphate.

The first question to be answered is: does the influence of the anion depend on its physico-chemical properties, on the rate at which it is absorbed, or on the properties of the products arising metabolically from these anions? Secondly, the question arises of how the absorption mechanism for  $\text{NH}_4$  is affected by this influence.

With respect to perennial rye-grass, it was observed by DIJKSHOORN (1959) that about twice as much phosphate is absorbed as sulphate. In manurial experiments concerning perennial rye-grass (DIJKSHOORN, 1957c) in which  $\text{KCl}$ ,  $\text{KH}_2\text{PO}_4$ , and  $\text{K}_2\text{SO}_4$  were supplied respectively, the cation content of the plants attained a lower level in the same sequence while the ratio, cation: anion content was remained constant. From the above it follows that the influence of the accompanying anion is not specific for the absorption of ammonium but is operative on the absorption of cations on the whole. Moreover, from the above

it seems probable that the influence of the accompanying anion on the absorption of ammonium is due in the first instance to the rate at which the accompanying anion is absorbed.

In consequence of the observed effect of anions on the absorption of ammonium, one inclines to the assumption that the absorption of  $\text{NH}_4$  is not performed by an absorption mechanism of its own, but is coupled with the uptake of anions, according to the theory of LUNDEGÅRDH (1954). This theory even seems to be supported by the observation that the uptake of nitrate is not affected by the accompanying cation (either K or Ca) as follows from Fig. 9. On the other hand, this theory is not supported by the fact that in our experiments the half-value of  $\text{NH}_4$  absorption was not influenced by the accompanying anion supplied either as phosphate or as sulphate. The half-values of phosphate and of sulphate absorption need not be identical. More convincing arguments can be advanced in favour of the opinion that the process of cation absorption is principally independent of the anion uptake. With respect to barley, JACOBSON and ORDIN (1954) concluded that the cation absorption in excess of anion uptake can be counterbalanced either by exchange for previously absorbed cations or by the formation of organic acid anions within the cell, the latter being confined largely to the malic acid fraction. According to DIJKSHOORN (1958) the absorption of ammonium by perennial ryegrass is accompanied by exchange for  $\text{H}^+$  and free cations (mainly K). As soon as the possibility of exchange becomes inadequate, the absorption of cations will keep pace with an equivalent absorption of anions.

As a result of these considerations, it becomes likely that the absorption of ammonium, although it is a separate process, is related to the maintenance of the balance of the cation and the anion content. However, the mechanism of this coupling is completely unknown. Therefore, it remains uncertain how the dependence of  $V_{\text{max}}$  of ammonium absorption on the accompanying anion fits into the scheme of Epstein and Hagen.

3.1.3.  *$V_{\text{max}}$  is dependent on pH.* For the sake of clarity, the experiments on the relation between the pH value of the solution and the rate of ammonium absorption will be discussed in two groups. These groups are distinguished according to the experiments with reference to the absorption of ammonium from one-salt solutions, i.e. solutions containing only  $(\text{NH}_4)_2\text{SO}_4$ ,  $(\text{NH}_4)\text{H}_2\text{PO}_4$ , or  $\text{NH}_4\text{Cl}$  plus buffer as triethylamine with  $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$  or  $\text{HCl}$ . The other experiments concern the absorption of ammonium from complete nutrient solutions as described in Chapter II section 1.1, containing  $\text{SO}_4$ ,  $\text{H}_2\text{PO}_4$ ,  $\text{HPO}_4$ , and Cl anions and  $\text{NH}_4$ , Ca, Mg, and K cations.

a) With respect to absorption from one-salt solutions; according to the preceding section,  $V_{\text{max}}$  is determined in large measure by the rate of organic acid formation within the cell if the accompanying anion ( $\text{SO}_4$ ) is absorbed slowly. It can be supposed that the pH values

in the interior of the cell in short-time experiments and within certain limits are rather independent of the  $H^+$  concentration in the medium. On the basis of this supposition, it follows that hardly any influence of the external pH on dissimilation is to be expected. Therefore, the absorption of ammonium from a one-salt solution of  $(NH_4)_2SO_4$  will not be affected markedly by pH (Fig. 8). If at pH values above pH 6.3 undissociated  $NH_4OH$  enters the roots, as a result of the internal pH values this  $NH_4OH$  will dissociate immediately as soon as entry into the protoplasm has taken place. The moment the production of  $H^+$  ions has become inadequate to neutralize the liberated  $OH$  ions, an increase of pH within the cell will take place, resulting in a retrogression of the  $NH_4OH$  dissociation and in equilibration of the  $NH_4OH$  concentration in the solution and in the protoplasm.

If ammonium is supplied as  $(NH_4)H_2PO_4$  or  $NH_4Cl$ , the rate of  $H^+$  production need not be a limiting factor in the rate of ammonium absorption, because the accompanying anions are readily taken up. Therefore, provided that the absorption of  $H_2PO_4$  and  $Cl$  ions is not affected by the pH to a large extent, hardly any effect of pH on the absorption of  $NH_4$  is to be expected and an increased rate of ammonium-nitrogen absorption as a result of  $NH_4OH$  intake is expectable at increased pH values above 6.5 as long as the maximal capacity of ammonium assimilation within the cell is not reached and the formation of  $H^+$  ions and the uptake of anions is adequate for neutralizing. Contrary to expectation, in this case a decreased rate of ammonium absorption was observed with increased pH values between pH 4 and 6.5 (Fig. 8), indeed an increased rate of ammonium absorption was found with increase of the pH values above 6.7. It is possible, that the absorption of ammonium-nitrogen is influenced by pH as a result of an effect of pH on the absorption rates of the accompanying phosphate or chloride ions. In addition, these observations show a striking resemblance to the pH curves for Mg and K absorption by wheat plants, as obtained by BURSTRÖM (1940). These pH curves reflect a decreased rate of Mg and K absorption at increased pH values between 6 and 7 and an increased rate of uptake at increased pH values between 7 and 8. Burström ascribed the limiting effect of pH between pH 6 and 7 to supply of an unbalanced solution, i.e. a solution of a much higher concentration of monovalent cations in proportion to the concentration of polyvalent cations. Unbalanced solutions were thought to destabilize the surface of the protoplasm. On the other hand, using balanced solutions Burström found a continuously increased rate of K and Mg absorption at increased pH values, from 6 to 8, so an inhibiting effect was absent. As distinct from the above results with unbalanced solutions, TROMP (1962), in experiments with excised wheat roots, observed that the rate of ammonium absorption was about 30 % lower at pH 4.5 than at pH 6.0. A rise in pH from about 6.0 to 7.5 caused an increase in the absorption rate of only 10 %. Above a pH of 7.5 the results suggested a rather strong rise in absorption rate. As a result of the addition of potassium to the unbalanced solution, over the whole range of pH



values investigated a continuously increased rate of  $\text{NH}_4$  absorption at increased pH values became apparent. It should be kept in mind that Tromp's results derive from excised roots.

b) With respect to the absorption from complete nutrient solutions, no real inhibitory effect of pH values between 4 and 6.5 on the absorption rate of ammonium was found. The increased rate of  $\text{NH}_4$  absorption accompanying an increase in pH between pH 6.5 and 8.5 were ascribed again to an additional uptake of ammonium-nitrogen in the form of  $\text{NH}_4\text{OH}$ . These experimental results are very comparable to the observed influence of pH on the absorption rate of ammonium by maize, using balanced nutrient solutions (VAN DEN HONERT and HOOYMANS, 1961).

It should be kept in mind that the experiments using unbalanced solutions were performed with intact seedlings, whereas the complete nutrient solutions were supplied to intact full-grown plants.

3.1.4.  $V_{\max}$  is dependent on season. This topic will be discussed together with the seasonal influence on the  $K_m$  values of  $\text{NH}_4$  absorption.

3.1.5. The  $K_m$  values of ammonium absorption. The question is, how far can the obtained half-values of  $\text{NH}_4$  absorption be interpreted on the basis of the scheme of Epstein and Hagen, in which

$$K_m = \frac{k_2 + k_3}{k_1}.$$

On the one hand, a number of data point to a constant value of  $K_m$  for ammonium absorption under external conditions that do affect  $V_{\max}$ . On the other, in some cases changes of  $K_m$  values have become apparent.

The data in question are:

a) As shown by Fig. 1, in experiments with full-grown plants during the spring, a  $K_m$  value of about 0.04 m.e./l was obtained both at 20° and at 35° C although  $v_{\max}$  was observed to be markedly dependent on temperature. These data correspond with the observation made by VAN DEN HONERT (1936) with regard to the uptake of phosphate by sugar cane and with the experimental results obtained by VAN DEN HONERT and HOOYMANS (1955) as to nitrate absorption by maize.

b) From experiments using intact seedlings, evidence was obtained of a constant  $K_m$  value irrespective of whether the accompanying anion was phosphate or sulphate, whereas  $V_{\max}$  was obviously affected by the nature of the accompanying anion. In the discussion on the effect on  $V_{\max}$  of temperature it is argued that the influence of temperature on  $V_{\max}$  can be ascribed to a change of  $k_3$  but not to an effect on ( $R_t$ ). Because  $K_m$  is not perceptibly affected by temperature, it follows that in the formula of  $K_m$  the value of  $k_3$  must be negligible with respect to that of  $k_2$ . In consequence, if the absorption of ammonium takes place according to the scheme of Epstein and Hagen,

the equilibrium in the first reaction, i.e. the affinity of the carrier to ammonium, is affected neither by temperature nor by the accompanying anion.

c) Comparison of the ammonium absorption by intact full-grown plants with that by intact seedlings showed a considerable difference of  $K_m$  value.

In this case, as a result of the experimental procedures applied we cannot compare the values of  $V_{\max}$ . The change of  $K_m$  could be ascribed either to an alteration of the affinity of the carrier to ammonium or to a change of  $k_3$ . However, a change of  $k_3$  is only perceptible if the  $k_3$  value is not exceeded to a large extent by  $k_2$  and from the above it seems that  $k_3$  is negligible with respect to  $k_2$ .

In the experiments concerned, the factors influencing the value of  $K_m$  may be related to the different samples of seeds used for the cultivation of full-grown plants and seedlings, with the differing methods of cultivation, and with the differing experimental procedures. For comparison, it should be remarked, that with regard to the ammonium absorption by maize VAN DEN HONERT and HOOYMANS (1961) obtained a half-value of about 0.11 m.e./l by means of a technique in which static water-cultures were used, whereas BECKING (1956) with a continuous flow technique computed a  $K_m$  of about 0.013 m.e./l.

d) It is demonstrated by Figs. 1 and 2 that for full-grown plants as well as for intact seedlings,  $V_{\max}$  at 20° considerably exceeds  $V_{\max}$  at 35° C. For the full-grown plants no influence of temperature on  $K_m$  was found in the spring-time experiments. However, in the winter  $V_{\max}$  was reduced at a temperature of 35° C compared to  $V_{\max}$  at 35° C in the spring, and it is noteworthy that  $K_m$  also changed.

In the foregoing there was doubt as to whether  $K_m$ , as proposed by Epstein and Hagen, changed via the affinity of the carrier or by way of  $k_3$ . But with regard to the influence of the season, the observed change of  $K_m$  (0.04 m.e./l in the spring and 0.2 m.e./l in the winter) certainly cannot be ascribed to an effect on  $k_3$  because  $K_m$  and  $V_{\max}$  are altered in opposite directions. It would be tempting to relate this seasonal effect to the experiments of HELDER (1952) on the influence of the available quantity of carbohydrate on the uptake of phosphate and to ascribe the observed seasonal effect to the available amount of carbohydrate.

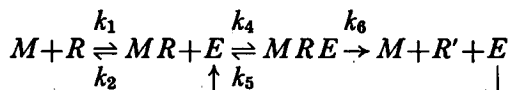
If the change of  $K_m$  under the influence of the conditions mentioned is interpreted according to the scheme of Epstein and Hagen, it must be assumed either that the affinity of the primary binding substance can alter or that several compounds can act as primary binding substance. Both suppositions seem to be rather unlikely, although the first possibility cannot be excluded (DIXON and WEBB, 1958).

The observed opposite alteration of  $V_{\max}$  and  $K_m$  can be explained in other ways:

a) The ability to assimilate ammonium can be reduced by some factor and become inadequate to the absorption capacity, resulting

in a release to the solution of the previously absorbed  $\text{NH}_4$  ions. In this case the change in the  $K_m$  value is only apparent, for it is not the amount of absorbed  $\text{NH}_4$  which is determined but the difference between the absorbed and the released quantities of ammonium. This explanation seems unlikely because it was observed that the  $K_m$  value was not changed by a rise of temperature up to  $35^\circ \text{C}$ . Nevertheless, on the basis of dry weight determinations, it was assumed that the assimilation is inhibited by high temperatures.

b) In the scheme of Epstein and Hagen, limiting factors in the absorption must influence  $k_3$  and/or  $(R_t)$  only. An extension of the possibilities is proposed by BANGE (1962). This author suggested that the ion-carrier complex is broken down by an enzyme and that this enzyme ( $E_t$ ) is available only to a small extent in respect of the total amount of carrier ( $R_t$ ). This according to the scheme:



in which  $M$  = ion,  $R$  = carrier,  $MR$  = ion-carrier complex, and  $E$  = the enzyme *carrierase* for the complex  $MR$ .

According to this scheme, the rate of ion absorption is approximated by the equation:

$$v = \frac{(M) \cdot V_{\max}}{(M) + H} = \frac{(M) \cdot \frac{k_6 \cdot (R_t) \cdot (E_t)}{(R_t) + \frac{k_5 + k_6}{k_4}}}{(M) + \frac{k_2 \cdot \frac{k_5 + k_6}{k_4}}{\frac{k_1}{k_4} \cdot \frac{k_5 + k_6}{(R_t) + \frac{k_5 + k_6}{k_4}}}$$

This equation not only fulfils the condition that the absorption rate can be described by a Langmuir adsorption isotherm, but can also explain a change of  $V_{\max}$  and  $K_m$  in opposite directions as observed in the experiments in different seasons. For, in the Bange's equation a decrease of  $(R_t)$  results in a decrease of the numerator and an increase of the denominator. Because in our experiments  $K_m$  proved to be dependent on the season and on one or more of the following factors: seed sample, methods of cultivation, and experimental procedure, for that very reason it becomes tempting to ascribe this change of  $K_m$  to the variability of the amount of available  $(R_t)$  in Bange's scheme.

Similarly, it has been suggested that the ammonium absorption by wheat may take place according to this scheme (TROMP, 1962) and that the same holds with respect to the  $\text{NH}_4$  uptake by barley (BANGE and TROMP, in preparation).

Summarizing, it follows from the discussion of the absorption of ammonium by perennial rye-grass that:

1. The relation between  $\text{NH}_4$  concentration in the solution and rate of absorption can be thought to be in accordance with the carrier hypothesis and can be described by the conventional formula of Epstein and Hagen.
2.  $V_{\max}$  is dependent on temperature and on the ionic balance. In the scheme of Epstein and Hagen this means that  $R_t$  and/or  $k_3$  is influenced by these factors. An influence of  $k_3$  is conceivable only if  $k_3$  is not the reaction constant of a simple process in which the ion-carrier complex is broken down, but is the expression of the rate of a complicated process including both the breakdown of the  $\text{NH}_4$ -carrier complex and the assimilation of  $\text{NH}_4$ .
3. The observation that  $K_m$  is dependent on one or more of the factors: seed sample, experimental procedure, and season, and that  $V_{\max}$  is at least dependent on the season, can be explained by the assumption that
  - a. either the absorption of  $\text{NH}_4$  takes place according to the scheme of Epstein and Hagen by means of a carrier of which the affinity for  $\text{NH}_4$  is influenced by some factors;
  - b. or, the absorption of  $\text{NH}_4$  takes place according to the scheme of Epstein and Hagen, but in the absorption mechanism a variable system of carriers is involved;
  - c. or, the absorption of  $\text{NH}_4$  takes place according to the scheme of Bange, i.e.  $\text{NH}_4$  is absorbed by one carrier substance, the available quantity of which is variable, and the ammonium-carrier complex is broken down by an enzyme present only in a very small amount as compared to that of the carrier substance.

Of these three hypotheses, the last seems to be the most probable.

### 3.2. *The mechanism for absorption of nitrate*

The experiments performed to investigate the relation between the nitrate concentration in the solution and the absorption rate, resulted with respect to perennial rye-grass in absorption curves that conform to a Langmuir adsorption isotherm (Fig. 9). Apart from the influence of the nitrate concentration, the effect of other external factors was investigated yielding the following quantitative details for the concentration-absorption curves:

- $V_{\max}$  is dependent on temperature;
- is independent of replacement the accompanying cation K by Ca;
- is dependent on pH;
- is dependent on the presence of molybdenum.
- $K_m$  is independent of whether the accompanying cation is K or Ca;
- (no experiments were made on the influence of temperature and pH).

These details will be discussed in connection with the question of whether in perennial rye-grass the absorption mechanism for nitrate

can be explained plausibly by the scheme of EPSTEIN and HAGEN (1952).

3.2.1.  *$V_{\max}$  is dependent on temperature.* The absorption rate of nitrate at a concentration at which  $V_{\max}$  is approached was found to increase at each increase in temperature between 5° and 35° C (Fig. 10). This observation with regard to nitrate corresponds qualitatively with that concerning the metabolically inert chloride (Fig. 4) but differs essentially from the observed influence of temperature on the uptake of ammonium (Figs. 3 and 4).

The temperature effect on the absorption of chloride and nitrate can be interpreted easily in view of the scheme of Epstein and Hagen, in which scheme both  $k_3$  and the available quantity of ( $R_t$ ) can be conceived to be dependent on temperature. However, the question arises, why the optimum curve for ammonium shows a much lower temperature optimum than the temperature curve for nitrate. Starting from the supposition that ammonium is not accumulated whereas nitrate both can be assimilated and accumulated, it is conceivable that in case of ammonium the absorption rate is limited by higher temperatures as a result of an effect of temperature on the assimilation. In case of nitrate, the same limitation on the assimilatory process is to be expected, because after the reduction of nitrate, the assimilation will follow the same reactions as does ammonium. However, there is the additional possibility of nitrate accumulation. This explanation is supported by the fact that the effect of temperature on nitrate absorption corresponds qualitatively with that on the absorption of the chloride ion that is not involved in the assimilation.

3.2.2.  *$V_{\max}$  is independent of the accompanying cation whether given in the form of K or in the form of Ca.* Fig. 9 shows that if nitrate is given in the form of  $\text{KNO}_3$  a relation is found between the nitrate concentration in the medium and its rate of uptake and also that this relation is completely identical with the observed relation between nitrate concentration and absorption rate after supply of  $\text{Ca}(\text{NO}_3)_2$ . And this relation can be described by a Langmuir adsorption isotherm.

As is supposed with respect to ammonium absorption, it also seems obvious with regard to nitrate absorption that the plant tissues try to maintain equilibrium in the content of positively and negatively charged ions.

If nitrate absorption were dependent on the absorption rate of the accompanying cation, then it was to be expected that in the presence of the rapidly absorbed potassium, the nitrate uptake would exceed the nitrate absorption in the presence of the slowly absorbed calcium. Because the experimental solutions contained only the cations potassium or calcium, and triethylamine which is supposed not to be absorbed, the inconsiderable intake of calcium ions was not compensated by the absorption of other cations as it was in the experiments of DIJKSHOORN (1957b). In consequence, nothing remains to us but to assume that the independence of the accompanying cation in our experiments is related to one or more of the theoretical possibilities:

the absorption of nitrate in excess of the cation uptake is compensated by release of OH ions, by release of  $\text{HCO}_3$  ions, or by absorption of  $\text{H}^+$  ions. However, the actual course of events in the balancing of the electrical charges by perennial rye-grass is far from clear.

At present, it is sufficient to stress that under the described conditions nitrate absorption was independent of the supply of either K or Ca as the accompanying cation, and that the relation between the nitrate concentration of the solution and the rate of absorption in this respect shows no complications that prevent an interpretation of the nitrate absorption process on basis of the Epstein and Hagen scheme.

**3.2.3.  $V_{\max}$  is dependent on pH.** The influence of pH on the rate of nitrate absorption found expression in an optimum curve with an optimum at pH 6.2 (Fig. 11). The explanation of BURSTRÖM (1940), according to which this effect of pH on the absorption of nitrate results indirectly from the simultaneous effect of pH and unbalanced solutions on the assimilation of nitrate, is less probable. For, results comparable to ours were obtained with respect to the influence of pH on the absorption of the metabolically inert bromide ion (HOAGLAND and BROYER, 1940; JACOBSON *et al.*, 1957) and also with respect to the influence of pH on the uptake of the metabolically inert chloride (HOOYMANS, personal communication).

So it seems that the observed influence of pH in some way results from an effect of pH on the absorption process itself and not from some effect of the pH on a process that follows the absorption. This suggestion essentially opens the possibility of explaining the pH effect on nitrate absorption by perennial rye-grass on the basis of the Epstein and Hagen scheme in which the ion-carrier complex is broken down in an irreversible reaction.

The pH-optimum curve obtained implies either a pH effect of a complicated nature or a pH effect somewhere in the absorption process analogous to the influence of pH on enzyme reactions.

It is true that with regard to the pH effect on nitrate absorption by maize VAN DEN HONERT and HOOYMANS (1955) adduced arguments in support of the opinion that the  $V_{\max}$  of nitrate absorption is affected by pH via ( $R_t$ ) in the scheme of Epstein and Hagen. Nevertheless, with respect to perennial rye-grass, the available experimental data are insufficient to permit even a tentative explanation of the influence of pH on  $V_{\max}$  either by an effect on the reaction velocity  $k_3$  or by an influence on the amount of carrier ( $R_t$ ). It can only be concluded that the above discussion has not adduced arguments refuting the absorption of nitrate according to the scheme of Epstein and Hagen.

**3.2.4.  $V_{\max}$  is dependent on the presence of molybdenum.** From preliminary investigations using intact seedlings to determine the experimental conditions to be chosen for nitrate absorption, it became evident that nitrate absorption by perennial rye-grass is dependent on the presence of molybdenum (Table 1). Also, in experiments with excised roots nitrate absorption, although only slightly, was substantially improved

by the presence of molybdenum. These observations conform with the conclusion of MULDER (1948) that plants fed with nitrate as nitrogen source require more molybdenum than plants having ammonium-nitrogen at their disposal. SPENCER and WOOD (1954) noted that molybdenum-deficient plants accumulate large amounts of nitrate but that their protein content remains abnormally low. With respect to this, our experiments using excised roots not deficient in molybdenum showed separate nitrate accumulation and nitrate assimilation processes (Chapter V, section 2). EVANS and NASON (1953), NICHOLAS, NASON and McELROY (1953) isolated the enzyme nitrate reductase which reduces nitrate to nitrite and contains molybdenum as an essential metallic component. Thus, it seems very probable that the influence of molybdenum on  $V_{\max}$  must be ascribed to the available quantity of nitrate reductase.

The question arises of what information the influence of molybdenum on the rate of nitrate absorption can give about the absorption mechanism for nitrate. Starting from the scheme of Epstein and Hagen the influence of molybdenum on  $V_{\max}$  is accounted for by an effect on  $(R_t)$  and/or an effect on  $k_3$ .

An influence on  $(R_t)$  can be visualized:

a. if the carrier substance contains molybdenum as an essential component, just as does nitrate reductase. This view is not to be excluded, although it seems improbable. Unless nitrate is taken up by way of more than one carrier, the improbability is enhanced by the above observation of Spencer and Wood that accumulation is not hampered by molybdenum deficiency but that it is the assimilation that is influenced.

b. if the carrier substance and nitrate reductase are the same. Unless additional nitrate absorption takes place by means of a carrier and results in nitrate accumulation, under these circumstances nitrate accumulation is not possible. For, the reduction of nitrate must be considered an irreversible process (KINSKY, 1961), so absorption of nitrate by nitrate reductase cannot result in nitrate accumulation. As will be discussed later in connection with the influence of ammonium on the nitrate absorption, the activity of nitrate reductase at the outer side of the protoplasm seems less probable.

Likewise, an influence on  $k_3$  can be conceived in two ways:

a. if  $k_3$  is the reaction constant of a simple reaction, namely the irreversible break-down of the nitrate-carrier complex, any influence of molybdenum on  $k_3$  should be accountable only if nitrate reductase itself is involved in the break-down of the ion-carrier complex. However, under such conditions it is difficult to see how nitrate accumulation takes place.

b. Therefore, if it is supposed that  $k_3$  is influenced by molybdenum,  $k_3$  is more likely to be a measure for the total rate of the processes in which  $\text{NO}_3$  is detached from the carrier, reduced, and assimilated. In this way  $k_3$  reflects the velocity of nitrate reduction, and thus can

be influenced by the available amount of nitrate reductase dependent on the molybdenum content of the plants.

In consequence, either more than one nitrate carrier must be assumed (if  $(R_t)$  is dependent on molybdenum) or it must be concluded that  $k_3$  reflects a complicated process (if  $k_3$  is dependent on molybdenum).

On the assumption that nitrate is taken up by two carriers, at least in the absorption process resulting in nitrate reduction the formation of the nitrate-carrier complex and subsequent entrance of this complex into the cells is followed by an enzyme reaction. On the assumption that  $k_3$  reflects a complicated process, it follows from the fact that nitrate accumulation and reduction take place independently of each other (Chapter V, section 2) that the nitrate-carrier complex is broken down by a carrierase, resulting in nitrate accumulation and that the carrier complex also has to do with the action of nitrate reductase, resulting in nitrate reduction and assimilation. Whether the nitrate-carrier complex is broken down by a carrierase or is exposed to the action of nitrate reductase, in both cases the absorption process can be described by the scheme of BANGE (1962). In the discussion of the interaction between ammonium and nitrate, further attention will be given to this subject.

3.2.5.  $K_m$  is independent of the accompanying cation potassium or calcium. The observation that  $K_m$  is unaffected by replacement of the accompanying cation potassium by calcium is easily explained on the basis of the scheme of Epstein and Hagen if it is assumed that these cations do not influence the affinity of the carrier substance for nitrate. For, as  $V_{\max}$  is no more influenced than  $K_m$  by replacement of K by Ca,  $k_3$  is not affected.

Summarizing, it follows from the discussion of the absorption of nitrate by perennial rye-grass that:

1. The relation between  $\text{NO}_3$  concentration in the medium and rate of absorption can be thought to be in accordance with the carrier hypothesis and can be described by the scheme of Epstein and Hagen.
2.  $V_{\max}$  is dependent on temperature and pH. In the scheme of Epstein and Hagen this means that  $(R_t)$  and/or  $k_3$  is influenced by these factors.
3. With respect to nitrate, under the described conditions the absorption phenomena are not complicated by the processes contributing to the ionic balance.
4.  $V_{\max}$  is dependent on the molybdenum content of the material. This can be explained in terms of a nitrate absorption according to Epstein and Hagen's scheme either on the assumption that two distinct carrier substances are involved or on the supposition that  $k_3$  does not reflect a simple, irreversible reaction, but reflects a complicated process. In case of a break-down of the nitrate-carrier complex by a carrierase and in case of a reaction between the carrier complex and nitrate reductase, the Epstein and Hagen's scheme requires an extension according to the scheme of Bange.



### 3.3. *The interaction between ammonium and nitrate*

Essentially, there are several phases of the nitrate absorption process in which an interaction between ammonium and nitrate can be realized. At present these possibilities will be discussed in the light of the experimental data.

3.3.1. *The interaction between ammonium and nitrate takes place at the carrier system of nitrate during the formation of the nitrate-carrier complex (ion-competition).* A competitive inhibition according to EPSTEIN and HAGEN (1952) is very improbable because an interaction between the  $\text{NH}_4$  ion and the  $\text{NO}_3$  ion is in question. So, in this case a carrier would be required whose binding-site has a strong affinity to both a positively charged and a negatively charged ion. With respect to an un-competitive and a non-competitive inhibition, grave objections can be raised to the assumption of interaction at the carrier system.

In the first place, from the observed influence of temperature on the interaction between ammonium and nitrate (Figs. 15 and 16), it follows that nitrate absorption is independent of  $\text{NH}_4$  between temperatures of 5° to 15–20° C, but that in the presence of  $\text{NH}_4$  nitrate absorption is not favoured by raising the temperature from 15–20° up to 35° C. In case of interaction at the carrier system, it is difficult to see why  $\text{NH}_4$  at some temperatures does not influence nitrate absorption and does at other temperatures. It is also evident from the experiments that at increased  $\text{NH}_4$  concentrations the absorption rate of nitrate is not decreased until a complete inhibition is approached (Fig. 14). This observation conflicts with all three schemes of ion competition according to Epstein and Hagen, namely of competitive, un-competitive, and non-competitive inhibition, unless nitrate is absorbed by two carrier systems, one of which system is inhibited by ammonium and the other not.

3.3.2. *The interaction between ammonium and nitrate takes place during the break-down of the ion-carrier complex (carrier-competition).* In this case, the nitrate-carrier complex and the ammonium-carrier complex would be broken down by a common enzyme (BANGE, 1962; TROMP, 1962). This idea would explain the limited inhibition of nitrate absorption (Fig. 14) because in Bange's scheme the amount of competing material is restricted. Nevertheless, it might be considered surprising that carriers of such distinct ions, having opposite electrical charges, would be related in such a way that the very same enzyme breaks down their complexes. This would require the assumption that the nitrate and ammonium carriers are amphoteric compounds. This possibility cannot be excluded.

3.3.3. *The interaction between ammonium and nitrate takes place in the nitrate reduction process.* The possibility to be discussed now rests on the observation that in some experiments, i.e. those using intact seedlings, the addition of molybdenum to the solution resulted in a markedly increased rate of nitrate absorption. In other experiments, i.e. those with excised roots, supply of molybdenum accelerated the rate of nitrate absorption only slightly. This differing influence of

molybdenum is comprehensible because a supply of it only takes effect if the material is deficient in this metal, and the content of molybdenum can differ in diverse samples of seeds.

With respect to the above, it is known that molybdenum constitutes the essential metal component of nitrate reductase. It is true that there is also a theoretical possibility—which cannot be completely excluded—that molybdenum forms a component of the nitrate carrier. Nevertheless, the observed effect of molybdenum indicates that the rate of nitrate absorption may be also dependent on the reduction process of nitrate. Besides, data from the literature (KINSKY, 1961; MORTON, 1956) point to the fact that ammonium can exercise an influence on the formation and activation of nitrate reductase. And it is conceivable that not only an effect of molybdenum deficiency on nitrate reduction but also inhibition of nitrate reductase by ammonium, affects the rate of  $\text{NO}_3$  absorption.

In the experiments with excised roots, part of the absorbed quantity of nitrate could be detected in the roots while another part had disappeared and was thus probably reduced (Chapter V, 1). The amount of nitrate recovered from the material proved to be actually accumulated, since the excised roots, after an absorption period of 4.5 hours, were kept under circumstances where nitrate cannot be absorbed, and the content of nitrate in the roots remained practically constant for two hours (Chapter V, 2). Therefore, in all probability with respect to perennial rye-grass we have to do with a process of nitrate reduction in addition to a process of actual nitrate accumulation.

From the experiments mentioned in Chapter V, 3, it became apparent that the decreased absorption of nitrate in the presence of ammonium can be ascribed completely to a decreased reduction of nitrate within the experimental period and under the prevailing experimental conditions.

On the other hand, in experiments continued for 5.5 hours and performed under conditions comparable to those in the experiments on the influence of ammonium in nitrate absorption, neither the absorption nor the reduction of nitrite was affected by ammonium (Chapter V, 4). The observation that the nitrate reduction is hampered by the presence of ammonium whereas the nitrite reduction is not influenced, can be adduced as a tenable argument in support of the view that it is the presence or the activity of nitrate reductase that is affected by ammonium.

*Summarizing*, we may conclude that:

1. Nitrate reduction does not follow nitrate accumulation; the processes of nitrate accumulation and reduction take place simultaneously and independently of each other.
2. Nitrate reduction is markedly affected by the presence of ammonium but nitrate accumulation is not influenced within the experimental period and under the described conditions.
3. The decreased absorption of nitrate in the presence of ammonium cannot be accounted for by an influence of ammonium on the

absorption mechanism itself of nitrate; the absorption of nitrate is decreased by an inhibited reduction of nitrate to nitrite.

#### 3.4. *The interrelationship of nitrate absorption, accumulation, and reduction.*

From the above it follows that nitrate is absorbed from the medium and can be both accumulated and reduced by distinct processes that are independent of each other. Evidence was obtained that under the experimental conditions and within the experimental period ammonium in some way inhibits the reduction of nitrate to nitrite but does not affect the nitrate accumulation. In addition, it was observed that at the ammonium concentrations of 0.3 and 1.5 m.e./l of  $\text{NH}_4$ , the reduction of nitrate was inhibited to the same extent (Chapter V, 3). At the concentrations mentioned,  $V_{\max}$  of ammonium absorption is approached (Chapter II, 1 and Chapter V, 3). It is indicated that it is not the ammonium concentration in the medium but the rate of ammonium absorption that is related to the inhibition of nitrate reduction. This points to the fact that ammonium does not exert its influence at the outer surface of the protoplasm, but after penetration into the cells.

In consequence, with respect to the connection between nitrate absorption out of the medium, accumulation, and reduction, the following possibilities can be conceived:

If we localize the absorption process at the outer surface of the protoplasm, it is possible that:

1. the separate processes of accumulation and reduction are preceded by separate processes of nitrate absorption from the medium, or

2. the separate processes of accumulation and reduction are preceded by a common process of nitrate absorption from the medium.

1. With respect to the first possibility, it should be remarked that the assumption of two distinct processes of absorption from the medium implies that this absorption is not a simple diffusion process. The relation between nitrate concentration in the medium and rate of nitrate absorption was found within the experimental accuracy to be described quite well by a Langmuir adsorption isotherm. It follows that the relation between nitrate concentration and rate of absorption both in the absorption-accumulation process and in the absorption-reduction process can be described by a Langmuir adsorption isotherm. Therefore, with respect to the rate-limiting step of the accumulation process of nitrate, the possibility that nitrate enters the roots by means of a simple diffusion process is excluded.

Should the  $K_m$  of the absorption-accumulation process differ in a large measure from the  $K_m$  of the absorption-reduction process, no simple Langmuir adsorption isotherm would be obtained. Consequently, the  $K_m$  value of both processes must be of approximately the same magnitude.

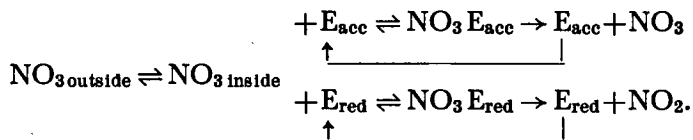
The absorption-accumulation process can be described in terms of the carrier hypothesis, whether or not in terms of the modified carrier hypothesis of BANGE (1962).



Because the rate-limiting step in the absorption, accumulation and reduction of nitrate is in question, the possibility must also be kept open that

3. nitrate enters the protoplasm by a diffusion process in such a way that an equilibrium is arrived at between the nitrate content of the protoplasm and the nitrate concentration of the medium. In this case, subsequently the internal nitrate can be both reduced, if active nitrate reductase is present, and excreted into the vacuole or via the endodermis into the xylem by means of an accumulation process.

This possibility can be represented by the scheme



In this case the relation between  $NO_3$  concentration in the medium and rate of absorption can again be described by a Langmuir adsorption isotherm.

At present, on the basis of the available data, it cannot be decided whether only one absorption process is involved or two. And if one absorption process precedes the accumulation and reduction, we cannot say with certainty whether this process takes place by means of a diffusion process or a carrier mechanism. In principle, these questions may be elucidated by experiments with respect to competition between nitrate and other anions.

Until now, the influence of pH and the effect of temperature on the absorption of nitrate and in the presence of ammonium have been more or less ignored. The influence of pH on the absorption of either ammonium or nitrate when supplied alone is already complicated (Chapter II, 3, Chapter III, 4) and not fully understood. With respect to both the influence of pH and the combined influence of pH and ammonium on the absorption of nitrate (Chapter III, 4, Chapter IV, 3), a better understanding of these points will require investigation into the influence of pH on the relation between the rates of uptake, accumulation, and reduction of nitrate.

It is to be expected, that when the reduction of nitrate is inhibited by the presence of ammonium, any rise of temperature within the temperature range of 5–35° C would at the same time result in an increased rate of nitrate accumulation. Therefore, it was expected that in the presence of ammonium, the rate of nitrate absorption would increase with rising temperature as observed with respect to chloride. (Chapter III, 3, Chapter II, 2). However, the results obtained seem to demonstrate the temperature effect on the rate of nitrate absorption to be independent of the presence of ammonium within the temperature range of 5–15° C. On the other hand, in the temperature range of 15–35° C any increase of nitrate absorption with rising temperature is inhibited by ammonium. The experimental

results can perhaps be explained in terms of an increased rate of nitrate accumulation and an increased inhibition of nitrate reduction by ammonium with rising temperature. Obviously, this explanation requires experimental evidence.

In the meantime, the investigations with respect to the absorption mechanism of nitrate and the interaction between ammonium and nitrate in perennial rye-grass are being continued.

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