THE INFLUENCE OF THE PLANT NUTRITION STATUS ON BLEEDING AND SALT UPTAKE

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SUMMARY

When tomato plants were kept in a high-salt condition by changing the nutrient solution daily, the rate of bleeding after decapitation and the nitrate concentration of the bleeding sap dinimished rapidly. Practically all the nitrate taken up from the nutrient solution was transported to the xylem vessels. When decapitation was preceded by a period during which the nutrient solution was replaced by tap water the rate of bleeding was low immediately after cutting but it increased rapidly to a more or less constant value, which declined again after some time. The nitrate concentration of the sap after a short initial period of low concentration was much higher than with high-salt plants. The rate of nitrate uptake from the solution was greater than the nitrate excretion into the vessels; the size of this difference increased with the length of the pretreatment period. When the content of water soluble carbohydrates was determined in the roots at the end of the experiment, this was always much higher in plants with a pretreatment on tap water than with the high-salt controls.

With high-salt plants both the nitrate concentration of the bleeding sap as well as the rate of bleeding could be increased by adding sugars to the external solution. Glucose was more effective than sucrose, which was better than fructose. As isotonic mannitol solution had no effect.

These results are discussed in relation to earlier findings and results of other workers in this field. It is concluded that the rate of bleeding is dependent on the rate of salt excretion into the vessels and that this excretion, in turn, depends on the amount of metabolites present in the root. High salt roots have practically no sugar reserves and are dependent on transport from the shoot. A pretreatment on tap water causes an accumulation of sugars in the root and a depletion of nitrate. For these reasons salt excretion after decapitation can go on for a much longer period than with high salt plants, while also the nitrate level in the root tissue is restored again, so that the nitrate intake from the solution is larger than the excretion into the vessels.

1. INTRODUCTION

In a preceding paper (ALBERDA, LOUWERSE & BROUWER 1964) it was demonstrated that tomato plants take up nitrate at an enhanced rate when this uptake was hampered during a preceding period. When, for instance, nitrogen was withheld from the nutrient solution for one day the rate of nitrate uptake almost doubled when it was administred again. Also if the nitrate uptake was diminished due to lack of aeration, it resumed at an enhanced rate upon aeration.

These facts demonstrate the importance of the nutritional status of the plants when the salt uptake is studied. In the earlier experiments the nitrate concentration in the bleeding sap was usually between 20 and 25 me/l and it was supposed that this was the highest concentration that could be reached by

the active mechanism that transfers nitrate ions to the xylem sap against a concentration gradient. With these cut plants, the rate of bleeding diminished rapidly. This was attributed to the interruption of the downward sugar transport from the shoot. This could diminish the rate of nitrate transport into the xylem and, consequently, the rate of bleeding, but the concentration should remain approximately the same. In subsequent experiments (LOUWERSE & ALBERDA 1965) sometimes much higher concentrations were found. Therefore, the effect of a difference in sugar content, caused by a temporary withdrawal of nitrate from the culture solution was studied in more detail.

2. MATERIAL AND METHODS

Seeds of the tomato variety Ailsa Craig were germinated in fine moist gravel. The seedlings were transplanted to 1 litre pots filled with a half strength Hoagland solution and grown in a green house at 20°C. The nutrient solution was aerated continuously. After 2 to 3 weeks the plants were transferred to 10 litres plastic buckets and brought into a growth room at 20°C with artificial illumination during 17 hours per day with 400 Watt Philips HPL-lamps, giving a light intensity at plant height of 7 cal dm^{-2} min^{-1}.

In most of the experiments a part of the plants got a pretreatment in which the nutrient solution was changed for tap water (-N plants) during different lengths of time. The plants that stayed on the nutrient solution (+N plants) had their solution changed daily during the pretreatment period. All solutions were changed daily during the experimental period.

For bleeding experiments the plants were cut at the stem base, leaving a stump of $\pm$ 5 cm with the root system. A rubber tube was tightened around the stump and the sap was collected by bending the other end of the short tube into an erlemeyer flask. The amount of bleeding sap was determined periodically by weighing and the nitrate content by the method of SNELL & SNELL (1949). The water loss and the nitrate uptake were determined over the same period by weighing the bucket with plant and solution before and after the uptake period and taking a sample for nitrate determination. Loss of water due to aeration was neglegible. In one experiment also the chloride uptake from the medium and excretion into the bleeding sap was determined, using the method of Mohr.

In another experiment different sugars were added to the external solution in such an amount that the concentration was 0.02 molar. The effect of these sugars was studied in comparison to an isoosmotic solution of mannitol. To determine the amount of water soluble carbohydrates in the plant the dried and ground material was extracted following the method of DE MAN & DE HEUS (1949) and the sugars determined with the VAN DER PLANK (1936) method.

3. RESULTS

3.1. Experiment 1

Of 12 nine weeks old tomato plants 6 got a pretreatment on tap water during 12 days; the other 6 remained on nutrient solution. After this pretreatment period all plants were cut and of the -N plants the tap water was replaced by the nutrient solution (-N+N). The other group remained on the solution (+N+N). The water and nitrate uptake from the nutrient solution was determined periodically as well as the amount of bleeding sap and its nitrate concentration. The experiment lasted three days. At the end the total water soluble sugars were determined in the roots and also the nitrate content. The results are given as mean figures for 6 plants. *Fig. 1a* gives the accumulated amount of bleeding sap plotted against time. At first the rate of bleeding was highest with the +N+N plants but it diminished fairly rapidly during the experiment. The rate of the -N+N plants was rather low during the first few hours but then changed to a rapid and fairly constant rate during the rest of the time. It exceeded that of the +N+N plants about 15 hours after cutting.

In *fig. 1b* the amounts of nitrate excreted as well as that taken up from the solution are plotted against time. With the +N+N plants the nitrate excretion into the vessels practically stopped after the first 10 hours. The uptake from the solution seemed to be even somewhat lower and this also practically stopped after 10 hours. With the -N+N plants the situation was comoletely different. During the first 10 hours there was hardly any nitrate found in the bleeding sap but the uptake from the solution was already considerable. Thereafter both uptake and excretion proceeded first at an increasing rate and after about 30 hours at a gradually decreasing rate. The difference between the amount taken up from the solution and excreted into the vessels remained approximately the same after the first day *(fig. 1c)*.

Of the +N+N plants the decrease in the rate of nitrate excretion was greater than the decrease in the rate of bleeding so that the concentration gradually decreased. The same occurred with the -N+N plants, especially during the second half of the experiment. This can be seen from *table 1*.

Table 1. Nitrate concentration in me/l in the bleeding sap of high-nitrate and low-nitrate tomato plants.

hours after cutting	+N+N	—N+N	duration of bleeding period
8	19.4	14.5	8 hours
24	9.3	43.8	16 „
72	0.0	19.4	24 „

At the end of the experiment a total amount of 27.8 me nitrate had been taken up from the nutrient solution against 18.1 me excreted into the vessels. Of the

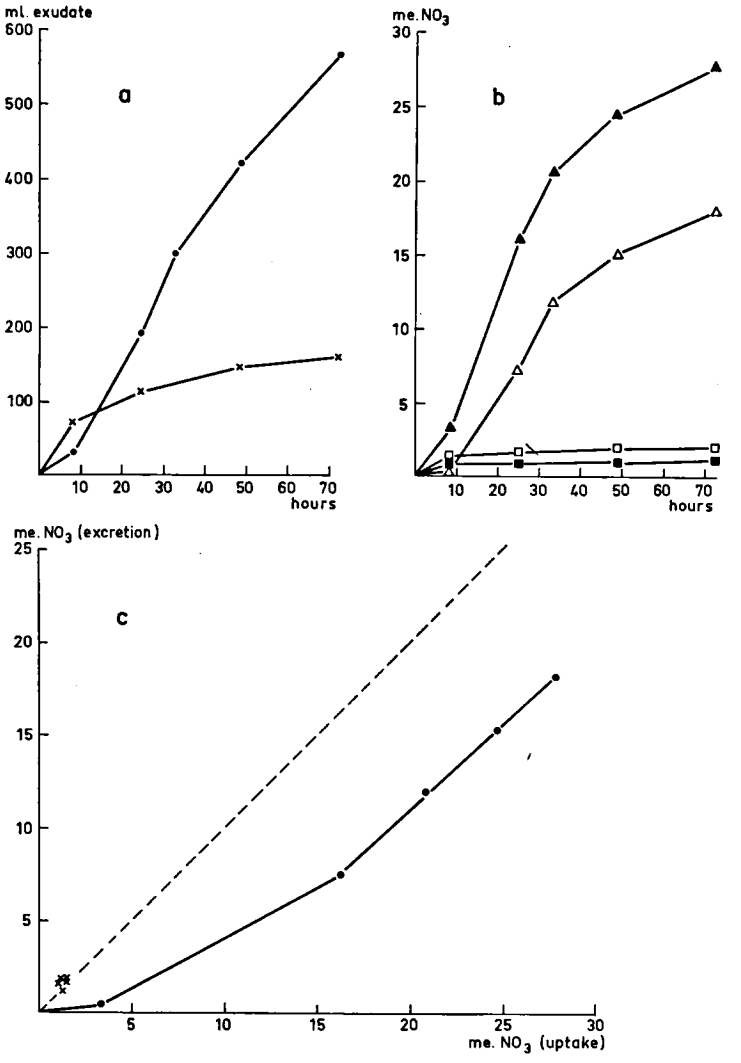

Fig. 1.a. Accumulated amount of exudate from cut tomato stems against time (hours).
●——● -N+N; x——x +N+N

b. Accumulated amount of nitrate taken up from the nutrient solution as well as excreted with the bleeding sap.
■——■ +N+N uptake □——□ +N+N excretion
▲——▲ —N+N △——△ —N+N

c. Amount of nitrate taken up by the plant plotted against the amount of nitrate excreted with the bleeding sap.
●——● —N+N; x——x +N+N

difference, 9.7 me, only 5.3 me was found as nitrate in the plant. It can be supposed that the rest was elaborated into organic form.

The sugar content was also determined at the end of the bleeding period. The content of all +N+N plants was less than 0.1% of the dry weight, that of the -N+N plants varied between 4 and 5%.

3.2. Experiment 2

Twelve 10 weeks old tomato plants were given the following pretreatment during 15 days: 4 plants stayed on the nutrient solution and the other 8 were transferred to tap water. Due to this pretreatment the plants differed in weight. At the time of cutting the +N plants stayed on nutrient solution (+N+N); half of the -N plants was also placed on nutrient solution (-N+N) and the other half was placed on a solution in which nitrate was replaced by an equivalent amount of chloride (-N+Cl). The duration of the bleeding period was again 72 hours.

The bleeding rate of the +N+N plants was lower than that of the -N+N plants *(fig. 2a)*. The former decreased continuously, whereas the latter remained constant for two days with a slight decrease during the third day. The -N+Cl plants had the lowest bleeding rate at the beginning but this remained constant so that it surpassed that of the +N+N plants during the third day.

The nitrate uptake of the +N+N plants during the three days was very low and equal to the amount of nitrate excreted into the vessels. With the -N+N plants the rate of nitrate uptake was much higher than that of nitrate excretion *(fig. 2b)*. Similar results were obtained for the Cl-uptake and excretion of the -N+CI plants. It should be noted that the rate of uptake and excretion of chloride is about 10 times smaller than that of nitrate.

Fig. 2c demonstrates that it was especially in the beginning of the experiments that with low salt plants the uptake exceeded the excretion. Later on the difference was much less (NO_3) or neglegible (Cl).

At the end of the experiment 38.9 me No_3 were taken up and 24.7 me excreted. Of the difference, 14.2 me, 7.2 me were found in the plant as nitrate. With Cl these figures were 4.29 and 2.92 for uptake and excretion respectively. The amount of chloride in the plant was 3.36 me instead of the 1.37 difference value. Since the nutrient solutions were prepared with tap water some Cl could have been taken up from this. The concentration of Cl in the -N+N plants was 1.32 me. The agreement between 3.36 me found and $1.37+1.32=2.69$ is reasonably good.

Table 2. Nitrate concentration in the bleeding sap of high-nitrate and low-nitrate tomato plants (me/l).

group	+N+N	—N+N
after 24 hours	11.3	43.4
„ 48 „	5.1	42.8
„ 72 „	1.4	24.7

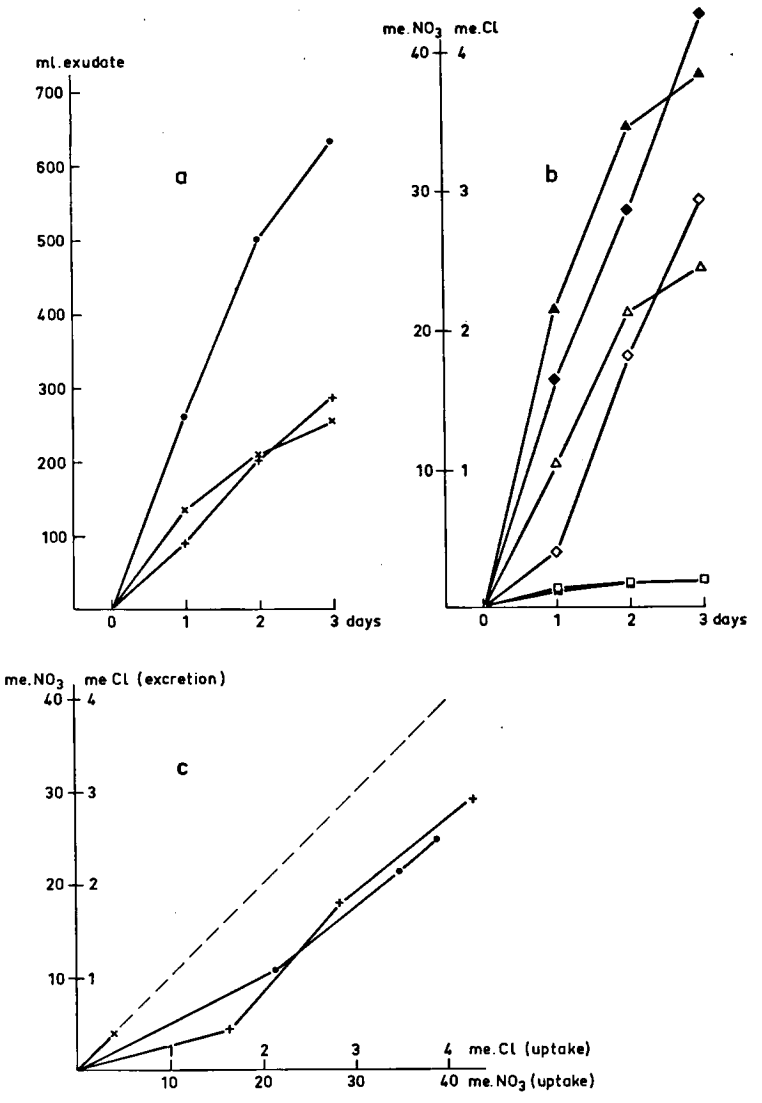

Fig. 2.a. Accumulated amount of exudate from cut tomato stems against time (days).
●——● —N+N; x——x +N+N; +——+ —N+Cl

b. Accumulated amount of nitrate, resp. chloride, taken up from the nutrient solution as well as excreted with the bleeding sap.
■——■ +N+N □——□ +N+N
▲——▲ —N+N uptake △——△ —N+N excretion
◆——◆ —N+Cl ◇——◇ —N+Cl

c. Amount of nitrate, resp. Cl taken up by the plant plotted against the amount excreted with the bleeding sap.
●——● —N+N; x——x +N+N; +——+ —N+Cl

The water soluble sugar content after bleeding amounted to 5.39% of the dry weight of the -N+N plants; 7.33% of the -N+Cl plants and less than 0.3% of the +N+N plants. The nitrate concentrations in the bleeding sap are given in *table 2.*

3.3. Experiment 3

Eleven weeks old tomato plants, 24 in number, were divided into 6 groups of four plants each. The plants of all groups were cut at the same time but the length of the pretreatment period on tap water was different, namely 21, 14, 7, 4, 1, and 0 days. At the moment of cutting all plants were replaced on the nutrient solution. The great differences in length of the pretreatment caused differences in shoot dry weight between the groups. The experiment lasted four days.

In *fig. 3* the amount of bleeding sap is plotted against time. With minor deviations which are probably of no significance the rate of bleeding increased with the length of the pretreatment. Of all groups, except the 21 days pretreatment, the bleeding rate declined during the experiment. Of the 1 and 0 days pretreatment the rate was already rather low after one day.

Fig. 4 shows the amount of nitrate taken up and excreted for the six different groups. Both the rate of uptake and excretion increased with increasing length of the pretreatment. With the 21 and 14 days groups the difference between uptake and excretion increased during the whole experimental period; with the 7 and 4 days pretreatment there was only a difference during the first day and with the 1 and 0 days pretreatment the differences were small but increased slightly during the experiment.

The nitrate concentrations in the bleeding sap are given in *table 3.*

Table 3. Nitrate concentration in the bleeding sap of tomato plants with a different length of pretreatment on water (me/l).

days of pretreatment:	21	14	7	4	1	0
after 24 hours	43.3	31.6	18.7	13.9	7.9	7.2
„ 48 „	27.6	19.2	11.8	8.4	3.9	4.1
„ 72 „	17.6	11.3	8.8	5.1	2.1	2.5
„ 96 „	13.8	7.6	7.5	1.4	1.2	2.0

The concentration was higher with the longer pretreatment and decreased with time. The water soluble carbohydrates were determined in the roots at the end of the experiment. For the 21, 14, 7, 4, 1, and 0 days pretreatment the values were 3.13, 1.00, 0.27, 0.22, 0.21, and 0.19 per cent of the dry weight, i.e. sugars decreased with decreasing pretreatment time but for pretreatments shorter than 4 days, there was no difference in final sugar content.

3.4. Experiment 4

Six tomato plants, about 6 weeks old, which had been on nutrient solution

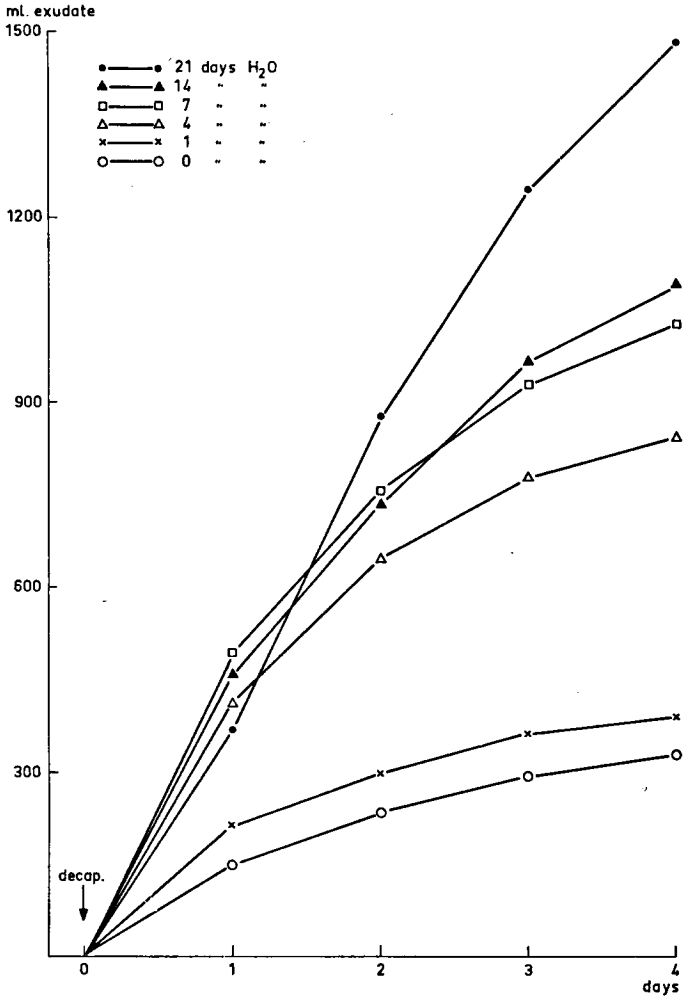

Fig. 3. Accumulated amount of exudate from cut tomato stems with different pretreatments, plotted against time (days).

throughout, were all cut at the same time. At that moment 1 plant got glucose in the nutrient solution, a second one fructose, a third sucrose and a fourth mannitol; the two remaining plants served as control. The three sugars and mannitol were added in such quantities to bring the total concentration of the solution on a 0.02 mol value. All solutions were changed daily. The bleeding sap was sampled periodically, weighed and the nitrate content determined. It was not possible to measure the nitrate uptake by the plant, since a considerable part of the nitrate in the solution appeared to be consumed and converted into organic form by bacteria, which developed in the sugar containing solu-

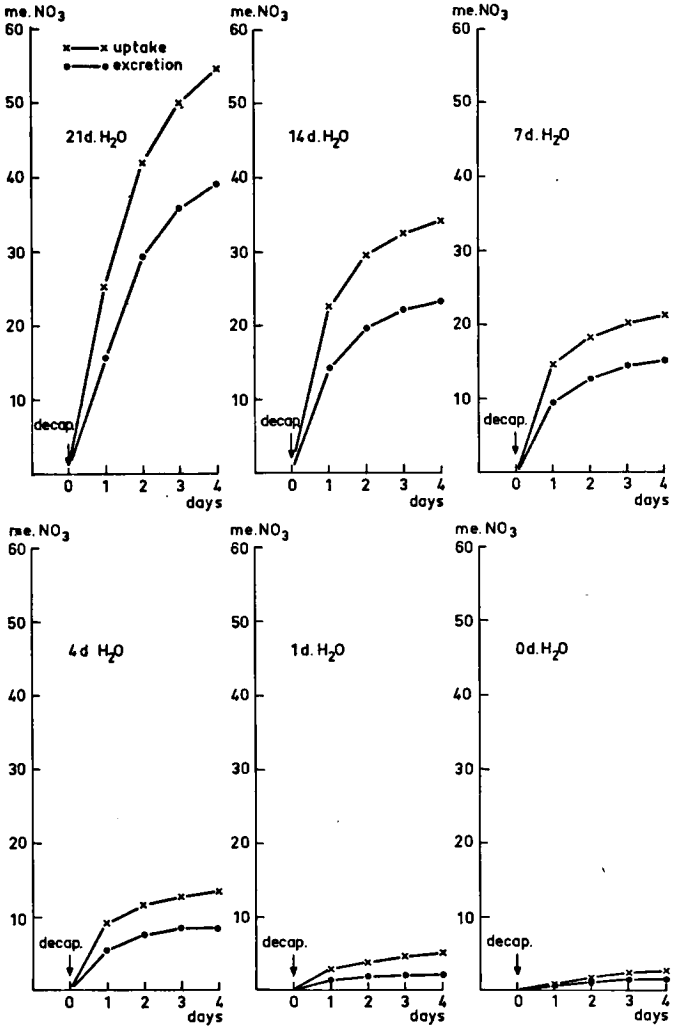

Fig. 4. Accumulated amount of nitrate taken up from the nutrient solution as well as excreted with the bleeding sap from tomato stems with different pretreatment plotted against time (days).

tions. Therefore, the nitrate available to the plants may have been somewhat smaller than in the controls and the mannitol containing solution.

Fig. 5a shows the accumulated amounts of bleeding sap against time. With all plants the rate of bleeding diminished rather sharply between 5 and 10 hours after cutting, thereafter it remained fairly constant. During the whole experiment the rate of bleeding was by far the highest with the plants with glucose in the nutrients solution. The plants with sucrose came next but with

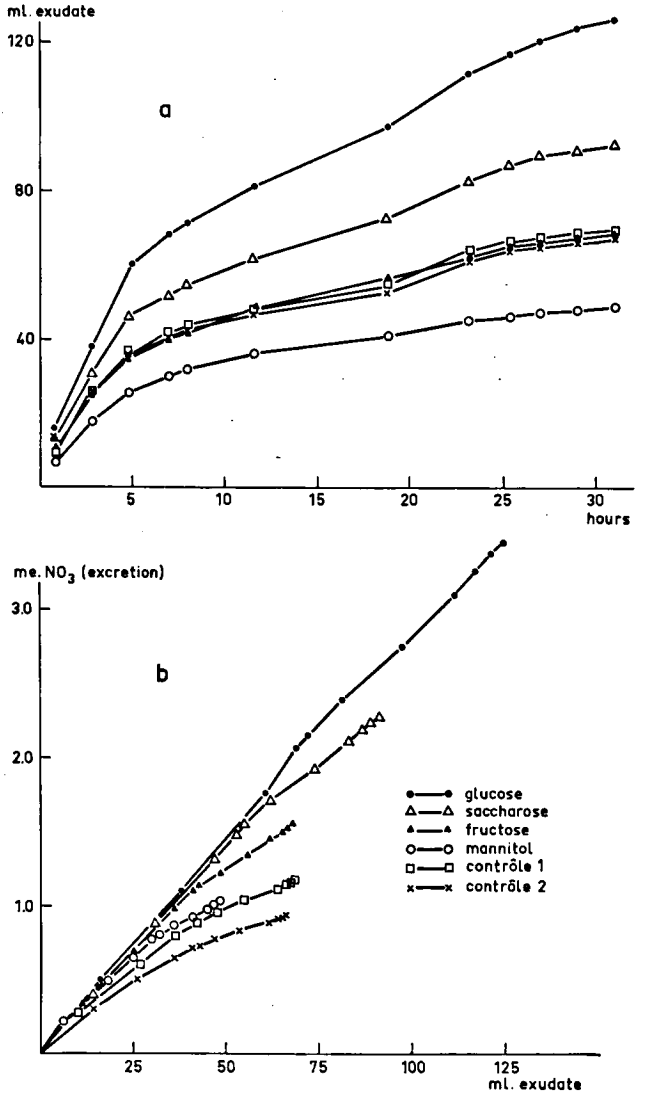

Fig. 5.a. Accumulated amount of nitrate in the bleeding sap of cut tomato plants with different sugars and mannitol added to the nutrient solution at the time of cutting (legend see b).

b. Accumulated amount of nitrate plotted against the accumulated amount of exudate with sugars and mannitol added to the nutrient solution.

much lower rate of bleeding; addition of fructose did not increase the rate of bleeding above that of the controls and addition of mannitol depressed the rate of bleeding, apparently by having only an osmotic effect.

Fig. 5b shows the nitrate concentration in the bleeding sap. With glucose added to the external solution the nitrate concentration remained constant throughout at a value of about 28 me/l; with the other two sugars it was about the same value at the beginning, but there was a distinct decrease, less with sucrose than with fructose. With mannitol the concentration was also about the same at the beginning, but the decrease was greater. Almost the same was found with one control plant. The other control had a lower concentration from the start.

4. DISCUSSION

Just as in the preceding paper (ALBERDA, LOUWERSE & BROUWER 1964) the experiments demonstrate a very large difference between high-nitrate and low-nitrate plants as to their bleeding performance after cutting. It cannot be emphasized strongly enough that in trying to obtain high-nitrate plants, or, in general, high salt plants, care should be taken that the plants never meet any shortage in nitrogen or any other ion prior to the use in experiments. The withdrawal of salt from the medium for one day prior to cutting had already a marked effect on the rate of nitrate uptake and excretion thereafter.

The results of the present experiments make it necessary to alter the picture of the relation between salt and water uptake as it was given earlier (ALBERDA, LOUWERSE & BROUWER 1964). It was then supposed that nitrate ions could be actively pumped into the xylem vessels up to a concentration of about 20–25 me. NO_3/l, since this was the value found in nearly all experiments. Only under conditions of high transpiration could the concentration be lower than the maximum value. In a later paper (LOUWERSE & ALBERDA 1965) nitrate concentrations higher than 25 me/l were found in the bleeding sap and *tables 1, 2,* and *3* show values up to 45 me/l. During short periods even nitrate concentrations of 60 me/l can be found in the bleeding sap. These results make it necessary to revise the hypothesis previously formulated. The underlying principle remains the same: With ARISZ, HELDER & VAN NIE (1951) it is supposed that the active secretion of ions into the vessels causes an osmotic water transport. When high-nitrate plants were cut the rate of excretion of nitrate into the vessels and, consequently, the rate of bleeding diminished very rapidly. Usually the former came practically to a standstill after 24 hours and at that time the rate of bleeding was already very much reduced *(figs. 1, 2, 4)*. When with such high nitrate plants sugars were added to the medium, the rate of nitrate excretion into the vessels was much higher than that of the controls, especially when glucose was added. Since the osmotic value of the medium was enhanced the nitrate concentration in the vessels was also enhanced, apart from any effect on nitrate excretion (*fig. 5*, mannitol). The effect of added sugars makes it highly probable that the reduction in nitrate excretion after cutting is caused

by the interruption of the downward transport of metabolites from the shoot. When the roots were analysed at the end of the experiment the amount of water soluble carbohydrates was neglegible. Thus it seems that with high-nitrate plants the uptake of ions from the medium and the excretion into the xylem vessels is dependent on the carbohydrate supply from the leaves. The same conclusion was reached by BANGE (1956) for the transfer of potassium from the medium to the vessels of maize seedlings. Since apparently the root tissue of high-nitrate plants is "saturated" with nitrate there was not much difference between the amount of nitrate taken in and given off to the vessels *(fig. 1* and *4)*

Low-nitrate plants can be less easily defined than high-nitrate plants since there can be different degrees of the low-salt status as appears from *fig. 4.* When with high-nitrate plants the nutrient solution is changed for tap water nitrate ions will gradually disappear from the root tissue, partly by conversion into organic substances, and partly by excretion into the vessels (tissue secretion, VAN ANDEL 1953). At the same time the carbohydrate content will increase. In these experiments no analyses were carried out immediately after the pretreatment period, but from the sugar analyses at the end of experiments 1, 2 and 3 and from the results with other plant species (ALBERDA 1965) it may be concluded that sugars will accumulate in the roots when the nitrate uptake is hampered. How this affects the uptake and excretion of nitrate into the bleed-

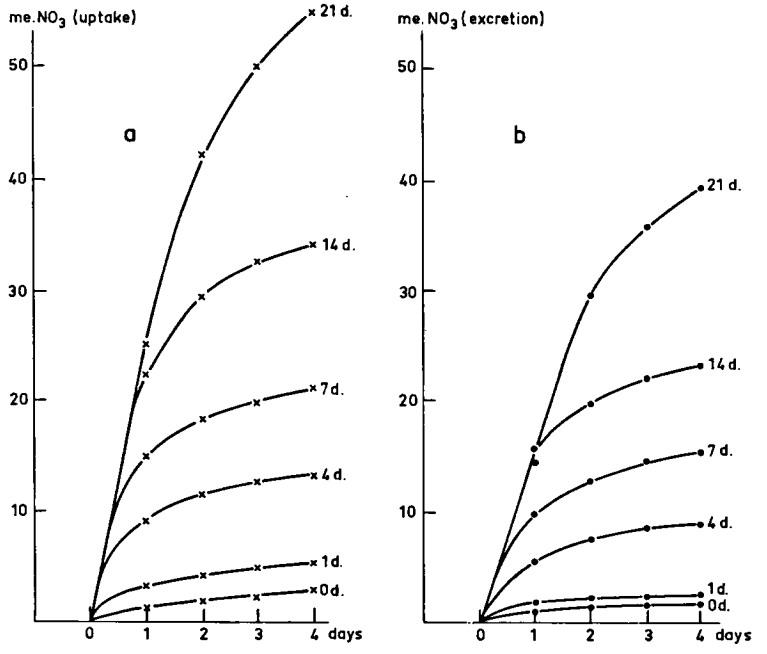

Fig. 6a. Accumulated amount of nitrate taken up from the nutrient solution by cut tomato stems, with different pretreatments (days on top water before decapitation).
b. Accumulated amount of nitrate excreted with the exudate of cut tomato stems.

ing sap of plants cut at the time when nitrate was administred again to the medium may be deferred from *fig. 6*, which is the same as *fig. 4*, but now the intake values are plotted together for all pretreatment periods (a) and the same is done for the excretion values (b). With the 21 days pretreatment the rate of uptake directly after cutting was about 25 me/24 hours and the rate of excretion into the vessels 15 me/24 hours. During the first few hours after cutting the rate of excretion was low *(fig. 1)* since it takes some time before the ions are transported from the medium to the vessels. The rates mentioned could be maintained for about 2 days after which they gradually decreased. With the 14 days pretreatment the initial rates were the same but they diminished already after 1 day. With lower pretreatment times the initial rates of uptake and excretion were only reached for a short time or not at all. Since the root weights were about the same for all pretreatments it lies at hand to ascribe the differences in uptake to the sugar content. When the difference between uptake and excretion is calculated for each 24 hours' period it shows that this difference is considerable only with the longer pretreatment and during a short time after cutting; all plants rapidly reaching the high-nitrate status with small differences between uptake and excretion. Only with the 21 days pretreatment is the difference at the end of the experiment greater than that of the control. The data of *fig. 6* suggests that the diminishing of both the rates of uptake and excretion is caused by the diminishing reserve of metabolites. The longer the pretreatment, the longer the time during which the initial rates could be maintained. During that time other factors than the sugar supply will determine the uptake and excretion. Apparently such a situation only arises after a prolonged treatment on a nitrogen-free medium.

That the root systems, although almost equal in weight, may not be considered completely similar in their behaviour may appear from table IV.

Table 4. Influence of pretreatment on tap water on nitrate uptake, excretion, bleeding and water conductivity of the root system.

days of pretreatment	NO_3-uptake me/24 h	NO_3-excretion me/24 h	bleeding ml/24 h	NO_3-conc. in bleeding sap me/l	k
21	25.2	15.9	372	43.3	9.4
14	22.9	14.5	460	31.6	16.5
7	14.8	9.6	491	18.7	32.8
4	9.3	5.8	413	13.9	40.7
1	3.2	1.7	214	7.9	51.6
0	1.1	1.1	151	7.2	44.3

This table gives the nitrate uptake, nitrate excretion and bleeding values during the first 24 hours after cutting. From these data the nitrate concentration of the bleeding sap was calculated (column 5) and also the water conductivity k (column 6), using the formula of SABININ (1925): $b = k\,(o_x - o_m)$ in which b is

the rate of bleeding, o_x the osmotic value of the xylem sap (for which the nitrate concentration was taken) and o_m the osmotic value of the half strength Hoagland solution. It can be objected that this way of calculating the water conductivity is not correct (see VAN ANDEL 1953), but the k-values obtained here at any rate demonstrate that the properties of the root system change considerably with the pretreatment. This may be due to a change in water conductivity or to a different reabsorption of ions from the xylem fluid or to both. These features complicate the picture if one wants to get an insight into the relation between the uptake of water and salt.

The results of these experiments suggest that in intact high-salt tomato plants it is the rate of transport of metobolites from the shoot that determines the rate of ion uptake by the roots. On the other hand, the relation between water and salt uptake clearly exists, as was recently again demonstrated for high salt castor bean plants by BOWLING & WEATHERLEY (1965). The explanation of this relation as formulated by ALBERDA, LOUWERSE & BROUWER (1965) has to be modified. However, at present the picture is still too complicated to be able to present a better one. Further research on this point is in progress.

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