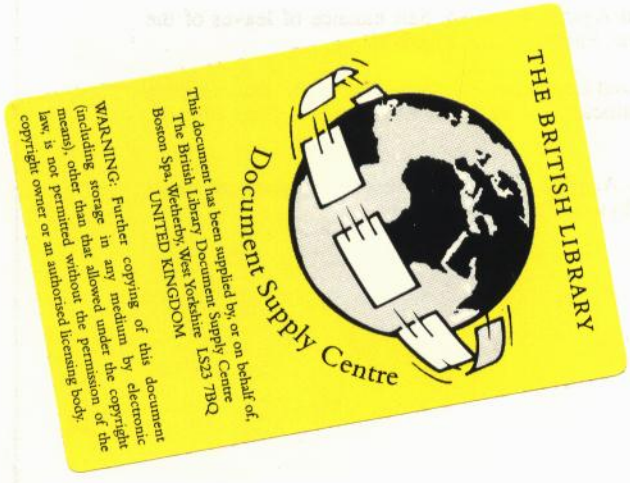

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DAILY VARIATIONS OF THE MINERAL COMPOSITION OF XYLEMIC EXUDATES IN TOMATO

Sylvie Ferrario, Irene Agius, and Alain Morisot

Institut National de la Recherche Agronomique, Station d'Agronomie et de Physiologie Vegetale, 45, boulevard du Cap. BP 2078, 06606 Antibes Cedex - France

ABSTRACT: Tomato plants (*Lycopersicum esculentum* cv. Prisca) were grown on perlite (or rock-wool substrate) in a glasshouse. Xylemic sap was collected hourly for 24 to 48 hours after cutting the stem below the cotyledons, and the mineral composition of these xylemic exudates was determined. The influence of time between excision and collection on the mineral composition of xylemic exudates was studied, then, only the 4 first hours after excision were considered. Daily variations of mineral concentration were observed in exudates. Maximal values were reached during the day for NO_3 and K, and during the night for Ca, Mg, H_2PO_4, and SO_4. Mineral quantities exudated were maximal at midday for all the mineral elements as well as for the exudation rate. No relation with the watering volumes appeared. The daily cycles of mineral composition of xylemic exudates are discussed, pointing out rhythms in uptake or translocation of ions from roots to plant tops.

INTRODUCTION

Mineral absorption by the roots, translocation through the xylemic pathway, then, partitionning between different organs may be important steps for a good nutrient status and a good development and yield of crops and specially tomato (1). Now-a-days, soilless culture methods in glasshouses allow controlled ferti-irrigations integrating changes of nutrient solutions and watering parameters by microprocessing (14). Therefore, a better knowledge of the plant nutrient needs is now required to take a maximum advantage of the technical improvement of culture methods, especially in a day-night cycle. So, we propose to study the

mineral composition of xylemic sap over a 24 h period in tomato plants. Therefore, xylemic exudates were collected and analysed on excised tomato plants.

Only few authors have already reported daily fluctuations of the chemical composition of xylemic sap. Regular variations of K have been observed in phase with exudation rate but not really for Ca and Mg in *Helianthus annuus* (26). Daily cycle of total salt concentration have been reported but out of phase with the exudation rate in cotton (18).

Cyclic variations for K, Ca, and Mg have been observed in the exudates of tomato "Groseille rouge" highly correlated with the exudation rate which periodicity was 12 hours (17). A tendency to a daily variation has been observed only for NO_3 concentration among minerals such as NO_3, H_2PO_4, K, Ca, and Mg in exudates of cucumbers (15). The maximum occured at 3:00 p.m., thus with a small lag in phase with the exudation rate. Cyclic variations of Na, K, and Cl have been mentioned in xylemic sap of *Avicennia marina*, with higher concentrations in the morning and in the evening and lower values before the dawn and in the late afternoon (27).

Therefore, according to these reports cyclic variations have been sometimes observed for some minerals (K, NO_3, Na, and Cl) depending on the plant material and on the experiment. The periodicity of the cycle could be either 24 or 12 hours and maximum value could occur either at midday or at midnight. However no one had reported daily variations simultaneously for NO_3, H_2PO_4, K, Ca, Mg, and SO_4.

In a preceding paper, we already reported a daily variation of the xylemic exudation rate in tomato (7) and the question of a daily pattern in the ion uptake was pointed out, since the exudation is a consequence of ion secretion according to an osmotic phenomenon called root pressure.

MATERIALS AND METHODS

Plant Material and Procedure: Plants of tomato (*Lycopersicum esculentum* cv. Prisca), were grown, as described in (7), in a glasshouse till fruiting for Experiments I and II.

Plants were detopped by cutting the stem below the cotyledons and exudate collection was done as described in (7). One plant was detopped every hour in Experiment I, and the sap was collected every hour over a 24 hour period. In

Figures 2 and 4, each sap result is the mean of sap sample measurements from 4 plants (sap collected on a plant excised 1 hour before the collect for the first one, 2 hours for the second one, 3 hours for the third one and 4 hours for the fourth one). The validity of this procedure is discussed in the first paragraph of results. In the same way, in Figure 3, each sap result is the mean of sap sample measurements from 2 plants

In Experiment II, the nutrient solution which was inside the rockwool was analysed for its mineral composition. An aliquot (5 mL) was sucked up with a syringe needle every hour during the exudate collection period.

Variance analysis (ANOVA - Statview - Macintosh) were used to compare the results.

Analytical Methods: Nitrate concentrations in the exudates were determined by colorimetry procedure (24) after NO_3 reduction by hydrazin and cupric sulfate (25). Potassium concentration was estimated by air-propane flame emission. Phosphate determination in exudates was performed by the colorimetry of the phosphovanadomolybdate complex (21). Calcium and Mg concentrations were estimated in air-acetylene flame in presence of $LaCl_3$ by atomic spectrophotometry. Sulfate concentrations were determined by the turbidimetric method of the Association of Official Analytical Chemists (1984, 14th edition, Method Number 33120). Dilutions of the exudates were made before each mineral determination (1:20 in water for NO_3, H_2PO_4, and SO_4; 1:20 in a 0.5 % HCl solution for K determination and 1:100 in a 0.5% and 1% $LaCl_3$ solution for Ca and Mg determinations).

RESULTS

Influence of Time Between Excision and Collection on the Mineral Composition of Xylemic Exudates: In Experiment I carried out at the fruiting in the glasshouse, the mineral concentrations of exudates collected at 11:00 a.m. and 11:00 p.m. were plotted against the time elapsed between excision and collection as shown in Figure 1A. Just after plant excision, NO_3 and K concentrations were higher in exudates collected at 11:00 a.m. than at 11:00 p.m. These differences were still noticed in exudates collected from plants excised for 12 hours, but NO_3 and K concentrations of the day period decreased regularly after excision, until there was no more difference between the day and night. H_2PO_4 concentration was lower at 11:00 a.m. than at 11:00 p.m. This difference

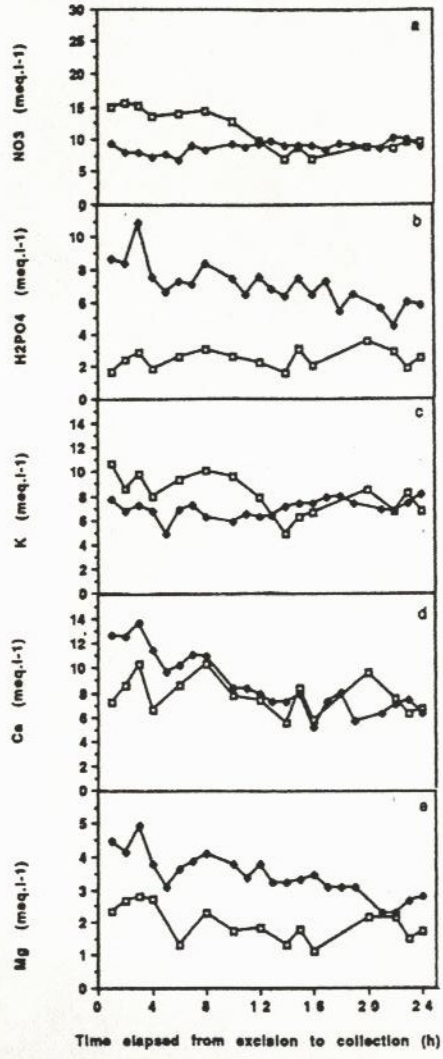


Figure 1 : Evolutions of the mineral composition of exudates versus the duration between excision and collection. Experiment carried out in glasshouse on fruiting plants. Exudate collected at 11.00 a.m. (□) and at 11.00 p.m. (♦).

was still noticed for 36 hours after excision (data not shown). Similar observations with differences noticed between the day and night periods could be made for Ca and Mg, for 6 hours and 24 hours after excision, respectively. Therefore, these data suggested differences between the day and night concentrations of minerals depending on the mineral element and on the time elapsed between excision and collection.

Influence of Collection Time on the Mineral Composition of Xylemic Exudates: In Experiment I, the mineral concentrations of exudates were plotted against the collection time (Figs. 2A and 2B). Considering that the mineral composition of exudates did not vary significantly for the 4 first hours after excision in this experiment, the subsequent results are the means of data from plants excised for 1 to 4 hours (see Materials and Methods).

Daily variations of mineral concentrations were observed (Figs. 2A and 2B). NO_3 and K were significantly more concentrated in the xylemic exudates collected in the day than in the night, whereas Ca, Mg, and H_2PO_4 were significantly more concentrated during the night (ANOVA, $p. < 0.001$).

The highest NO_3 concentration (15 meq/L) was determined in exudates collected between 10:00 a.m. and 12:00 a.m. and the lowest one (6 to 8 meq/L) was observed in samples collected in the night. The amplitude was about 2.6.
A daily variation of K concentration was observed, similar to the NO_3 variation concerning the appearance time of maximal (10 meq/L) and minimal values (6 meq/L), but with a lower amplitude (about 1.7).

The results for Ca concentration exhibited a daily variation as well, with a maximal value (16 meq/L) at the end of the night (6:00 a. m.), and a minimal one (7 meq/L) at midday. The amplitude was about 2.2.

The daily variation for Mg concentration pointed out a maximal value (4.5 meq/L) at the beginning of the night (between 7:00 p.m. and 11:00 p.m.), and a minimal value (2 meq/L) during the day period. The amplitude was about 2.2.

The H_2PO_4 concentration varied daily with a maximal value (10 meq/L) reached at midnight, and a minimal value (2 meq/L) at midday, thus the amplitude was 5. For each mineral element, the concentration in exudates generally appeared higher than in the watering solution.

The total cationic concentration has been calculated in Experiment I as the sum of K, Ca, and Mg concentrations and plotted in Figure 2C. The highest values of total cationic concentration were observed at the end of the night and in the early

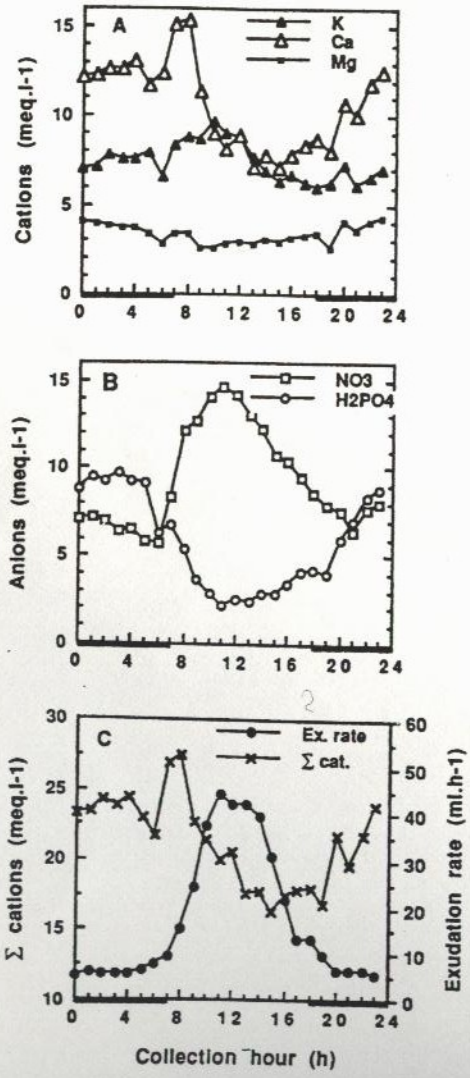


Figure 2 : Variations of K, Ca, Mg (A), NO3, H2PO4 (B) concentrations in exudates, and variations of exudation rate with the sum of cationic concentrations (K+Ca+Mg) (C) versus collection time. Experiment carried out in glass

morning, just before decreasing values in the light period till the next dark period. The ratios NO_3/K, K/Ca, and NO_3/H_2PO_4 were plotted for this experiment (Fig 3), and day-night variations were observed with an increase of NO_3/K, K/Ca, and NO_3/H_2PO_4 in the day.

Finally, considering the volume and the mineral concentration of exudates, the quantities of mineral elements exudated during the day-night cycle were plotted (Fig. 4). A rhythmicity was observed with a maximum for all elements at the middle of the photoperiod and a minimum in the night.

Influence of Watering Volumes: In Experiment II carried out in the glasshouse, two leaching rates (A = 10% to 30% and B = 30% to 40%) were applied by providing to plants the same nutrient solution at the same watering frequency but in 2 different quantities.

Firstly, in this experiment the composition of the nutrient solution bathing the roots inside the rockwool was determined for each leaching rate. It appeared modified in comparison with the watering solution and in function of the mineral element (Table 1). The substrate solutions were more concentrated than the

TABLE 1

Influence of watering volumes on the mineral composition of substrates and exudates for two leaching rates A=10 to 30 % and B=30 to 40 %.

($meq.l^{-1}$)	Nut. solution in watering leaching rates		Nut. solution inside rockwool leaching rates		Exudates (12.00 a. m.) leaching rates	
	A	B	A	B	A	B
K^+	6.2	6.2	4	6.5	11.7	11.2
Ca^{++}	7.4	7.4	21.9	9.3	10.3	8.9
Mg^{++}	2	2	8.4	4.5	4.2	3.5
SO_4^-	3.8	3.8	12.5	5.5	5.2	5.2

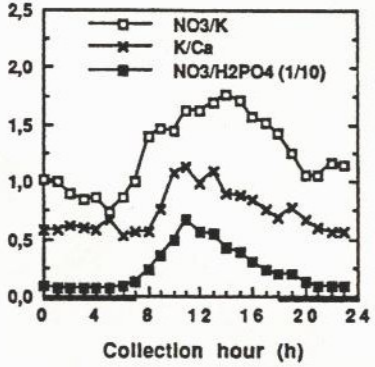
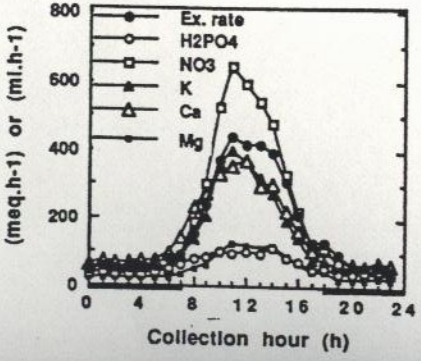


Figure 3 : Variations of NO3/K, K/Ca, and NO3/H2PO4 ratios versus collection time. Experiment carried out in glasshouse on fruiting plants.



watering solution, and the highest concentrated substrate solution was observed for the lowest leaching rate (10-30%), (except for K and H_2PO_4 for the 10-30% leaching rate). Then, no day-night variations were observed for the mineral concentrations of substrate solutions (data not shown).

Secondly, the mineral composition of exudates were determined for the two leaching rates. Daily variations were evidenced again (ANOVA $p < 0.001$). They were very similar to those which were described in Experiment I. Moreover, SO_4 concentration showed a daily rhythm with a maximum (14 meq/L) in the early morning at about 9:00 a.m. and a minimum (5 meq/L) in the night (data not shown).

Finally, no influences of the leaching rates appeared on the mineral concentration of exudates.

DISCUSSION

In these experiments, we tended to show up that mineral translocation in plants vary between the day and night. In this purpose, tomato plants were excised, then xylemic exudates were analysed for their mineral composition. In these conditions, the exudation was only due to root pressure without any influence of transpiration rate, since the upper part of plants have been removed.

In this paper, we observed that the concentration of mineral in exudates was higher than in the nutrient solution. This is a very well known observation which is in agreement with the osmotic hypothesis of root pressure as the main cause of exudation process (16). Absorption of ions by the roots increases the internal concentration of roots, and then a higher water uptake tends to balance this difference of osmotic pressure between xylemic and external media.

Besides, there were differences between each ion exudation. The highest exudation increase during the photophase was observed for NO_3 then for K and Ca and the lowest for Mg and H_2PO_4, while NO_3 and K concentrations increased during the photophase, and Ca, Mg, and H_2PO_4 concentrations decreased. These observations could be related to the circadian variation of exudation rate combined to those of each ionic concentration whose ratio was dependent on the mineral element.

Morever, a comparison between the daily variation of total cationic concentrations in exudates and the exudation rate indicated three phases. The first one occured in the night with a slow increase of cation secretion into the xylem

without any variation of water exudation, then, the second one, till 9:00 a.m., when the increase of cation secretion was faster than water exudation (total ionic concentration increase), and the last one, till the night, when cation secretion decreased before water exudation. Therefore, circadian variations of ion and water exudations seem to be the result of complex interactions among diffusion and active transport as proposed by Fiscus (8).

A solute flux equation was given by Fiscus

$$J_s = C^\circ(1 - \neg)J_v + w(\Pi^\circ - \Pi^i) + J_s^*$$

where C° is the medium concentration, $\neg$ the reflection coefficient, w is the coefficient of solute mobility, Π° and Π^i are the medium and xylem osmotic pressures, J_s^* is the active solute transport and J_v is the volume flux, with

$$J_v = L_p\ [\Delta P - \neg\ (\Pi^\circ - \Pi^i)\ - \Pi^*]$$

where L_p is the hydraulic conductance coefficient, ΔP is the hydrostatic pressure difference between the outside of the root and the xylem and Π^* characterizes the effect of the intermediate compartment.

L_p, $\neg$, J_s^*, w, and Π^* are the transport coefficients studied in *Phaseolus* roots by Fiscus over a 24 hour period and at various hydrostatic pressures applied to the roots of detopped plants resulting in various exudation flow. L_p which did not show diurnal variations, appeared to be of minor importance, although other authors observed diurnal cycling in root resitance to water movement (18,19). Then, according to Fiscus, w, J_s^*, and Π^* accounted for nearly all the diurnal changes in volume flux at low volume fluxes ($\Delta P = 0$) as it was the case in our experiments, since plants exudated freely. Maximal values for w, J_s^*, and Π^* were established by Fiscus during the photophase, but without any distinction between the ionic species (concentration of the exudates was estimated from the electrical conductivity only). w and J_s^* have a positive effect on exudation, while Π^* slows it down.

Active transports in roots are known for NO_3, K, and H_2PO_4 (10,16), but not for Ca (and probably for Mg) (4,12), so J_s^* could be of importance for NO_3, K, and H_2PO_4. Diurnal cycles of uptake have been observed in *Capsicum annuum*

Therefore, we propose that an increase of active transport occured during the photophase for NO_3, K, and H_2PO_4, but in different proportions according to the exudated quantities (NO_3 exudation increased about 10-fold, while H_2PO_4 exudation increased about 2.5-fold). At the same time, exudation rate increased about 8 fold, consequently NO_3 and K concentrations increased and H_2PO_4 concentration decreased. NO_3/H_2PO_4 and NO_3/K showed also diurnal oscillations with maximal values during the light.

Mobilities of NO_3, K, and H_2PO_4 are high (16), so we suppose they do not vary a lot between the day and the night.

On the other hand, temporary stockage of mineral ions has been observed in roots. A diurnal variation in the ability of bean roots to translocate absorbed Rb and H_2PO_4 to the shoots have been reported with a maximum translocation occuring at about midday and a minimum at about midnight, but they have not really studied the composition of exudates (26). Day-night differences in the accumulation and translocation of ^{42}K, ^{86}Rb, ^{82}Br by tobacco plants have been observed (28). A higher accumulation of these ions has been noticed during the night by the roots and a higher transfer to the shoots during the day, but no differences in salt concentration of exudates. A retention of NO_3 has been reported in soybean roots in darkness and its release in the following light period (22,23). Then, we can suppose that Π^* the osmotic pressure of the internal compartment increase in the night for these ions, as an increasing ability to store in the root, slowing the xylemic exudation.

In the case of Ca (and probably Mg), transport in the root is mainly passive at high external concentrations (12) and linked to water flow and cationic exchange capacity of tissues. A higher absorption of Ca in the light and a greater accumulation of Ca in the stem in the dark were observed in tomato (11). Besides, it was proposed that the rate of retention of Ca on the exchange sites is inversely related to the rate of xylem flux (4). Then, w or Π^* could play a role in the exudation process of Ca (and probably Mg). So, during the photoperiod Ca was carried along by exudation flow (Ca exudation increased and the concentration decreased as an effect of dilution) desaturing the exchanges sites.

Ontogenic changes in potassium concentration in xylemic sap of tomato have been already reported (29). A decrease was observed from 12 mM in vegetative

the beginning of dark period, and the ionic selectivity of roots was shown to be modified in relation with the development stage of plants.

Besides, during the night, xylemic sap is not drawn up by transpiration stream and could supply low transpiring organ deprived of xylemic nutrient during the day in particular fruits or meristems. These latter deeply require Ca and Mg which are not provided by phloem, the main translocation way of nutrients towards these organs during the day (6,11).

The influence of the duration between excision and collection on the mineral composition of exudates was observed. Different patterns appeared depending on the mineral element. The mineral composition of exudates was quite steady for 6 hours after excision, and later on a dilution appeared very quickly for NO_3 and K. Such differences could arise the energetic status of roots. Without their upper part, the roots of plants are deprived of substrates coming from the shoot and are obliged to use their stores. The time while ionic concentrations in exudates were quite steady, could be related to the availability of energetic stores of roots. Nevertheless, it is unlikely that a single metabolic mediator is responsible for the variety of changes in ion concentration.

Existence of different types of control in ion uptake by different messages coming from the shoots could be involved. The nature of these messages is still unknown possibly it could be sugars, organic acids, or growth regulators.

Malate could play an important role in the uptake of NO_3 as proposed in (3). According to this model, the synthesis of malate in the plant shoot and its translocation together with K via phloem to the roots could regulate the NO_3 uptake by the roots. In tomato, malate accumulation has been reported (13), resulting from NO_3 assimilation, in particular in plant tops. Then, they estimated the recirculated K to about 20% of the upward flux of K in the xylem stream (2). In this species the relative importance of K recirculation and then malate is not so high, but could play a regulatory role in NO_3 uptake. This hypothesis would be worth to be studied in relation with the daily rhythm of NO_3 and K exudation.

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NITRATE FLUXES IN SQUASH SEEDLINGS MEASURED WITH ^{13}N

J. Wieneke

Institute of Radioagronomy, Forschungszentrum Jülich (KFA),
P.O.Box 1913, D-5170 Jülich, F.R.G.

ABSTRACT: Influx of ^{13}N-labelled NO_3^- was studied in roots of intact squash seedlings *(Cucurbita max. Duch.)* previously grown in nutrient solution without (uninduced) or with NO_3^- (induced). At the uninduced constitutive level NO_3^- influx was saturable (V_{max}=6.2 μmol (g FW)$^{-1}$ h^{-1}; K_m=37.0 μM), but it increased again, probably passively, at high external NO_3^- concentrations. Initial NO_3^- efflux started during induction of the transport system when the influx rate increased and conceivably the NO_3^- concentration in the cytoplasm was raised. Likewise, this tendency was observed after a period of NO_3^- starvation. The kinetic parameters of NO_3^- influx of the substrate-induced high capacity transport system accounted for a V_{max} of 14.0 μmol (g FW)$^{-1}$ h^{-1} and a K_m of 15 μM. Both the uninduced and the induced uptake reacted in a similar pattern to the treatment of inhibitors affecting the protein synthesis (cycloheximide, fluorophenylalanine or puromycin) suggesting a single membrane protein population carrying NO_3^- across the plasmalemma. A rapid onset of a strong inhibition of the NO_3^- influx by the treatment with 1 mM phenyglyoxal was revealed. The influx recovered, but only slowly and was not much enhanced after 5 h of recovery at increasing NO_3^- concentrations (100 to 8000 μM) in the ambient solution.