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Factors affecting absorption and transport of potassium in maize roots

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Abstract

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Before short-term absorption experiments, excised roots of young maize plants were differently treated to alter properties like membrane permeability and concentrations of organic and inorganic compounds. Subsequently, influx and efflux of potassium ions were estimated during the initial and the steady-state phase.
In other experiments, absorption and translocation of K^+ were estimated simultaneously in excised roots of young plants and in decapitated roots of maize plants 5 weeks old.
Absorption, accumulation and upward transport of potassium in maize roots were closely linked. Freshly absorbed potassium was accumulated initially but, with time, internal salt concentration, osmotic pressure and upward xylem transport (exudation) steadily increase. During the steady-state phase, rate of uptake and transport of K^+ to aerial parts were equal and K^+ did not accumulate in maize roots. Freshly absorbed K^+ was immediately transported upwards or exchanged with K^+ already present in the root cells and then temporarily accumulated in root cell vacuoles before being transported longitudinally.

Free descriptors: maize, excised roots, salt deficiency, absorption, translocation, accumulation, potassium ions, bleeding sap, composition.

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Contents

List of abbreviations

A^-	indiffusible or restrained anion
AFS	apparent free space
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
CCCP	carbonyl cyanide m-chlorophenylhydrazone
Ci	citrate
CN	cyanide
DFS	Donnan free space
DNP	2.4-dinitrophenol
DM	dry matter
Fum	fumarate
HA	indiffusible or restrained undissociated weak acid
$K_{chloride}$	K ion with Cl as counterion
$K_{nitrate}$	K ion with NO_3 as counterion
$K_{sulphate}$	K ion with SO_4 counterion
LSD	least significant difference
M	malate
Mo	malonate
S.C.	selectivity coefficient
Succ	succinate
WFS	water free space
WSC	water soluble carbohydrates

Subscripts

c	cytoplasm
i	internal
o	outer or external
v	vacuole, except in J_v, where v means volume
x	xylem
s	salt or solute

Fluxes

ϕ_{oc}	inward plasmalemma flux or flux between outer solution and cytoplasm
ϕ_{co}	outward plasmalemma flux or flux between cytoplasm and outer solution
ϕ_{cv}	inward tonoplast flux or flux between cytoplasm and vacuole
ϕ_{vc}	outward tonoplast flux or flux between vacuole and cytoplasm

In the text ionic species are usually represented by their chemical symbols, omitting charge signs, e.g. K instead of K^+.

1 Introduction

A fundamental problem of plant growth is how inorganic ions enter root cells and then move through the root and up to the shoot. The first step, ion absorption, has been the topic of many studies by a great number of plant physiologists (e.g. Epstein, 1955; Hodges, 1973; Lundegårdh & Burström, 1933; Lycklama, 1963; Pitman, 1975; Wyn Jones, 1975). In most experiments dealing with the uptake or accumulation of salts in the plant root, attention is focused on processes of ion transport at a cellular level, short-distance ion transport. Mechanisms regulating ion fluxes at plasmalemma and tonoplast, the outer and inner membrane of the cytoplasm respectively, have been studied extensively. The distribution of ions between the two cell compartments cytoplasm and vacuole is often used to evaluate the ion absorption mechanism as a whole. In order to study different aspects of the ion absorption mechanism, the external medium (e.g. salt concentration, pH, temperature, O_2-tension), as well as features of the root (permeability and structure of membranes, internal salt status, energy level) are varied during the uptake experiments.

As the plant root consists of different tissues (epidermis, cortex, endodermis, stele), each with its own characteristic cells, ion transport in plant roots will not be restricted to accumulation of salts in the cell compartments cytoplasm and vacuole. It also includes the symplasmatic ion transport from cell to cell and subsequently the upward xylem transport to aerial parts. Therefore, the mechanism of ion absorption or ion transport in plant roots will be complex and dynamic.

As a consequence, ion uptake studies, frequently carried out with unicellular organisms, such as *Nitella* and *Chara* (MacRobbie, 1973; Spanswick & Williams, 1964; Vredenberg, 1971; Barber & Shieh, 1972), provide a poor representation of the mineral relations of plant roots or complete intact plants.

Absorption and radial displacement of ions by the root are only the first steps in salt transport in the plant. Further steps are the longitudinal upward xylem transport, transport of salts from the roots and supply to aerial parts of the plant. Both steps have mostly been investigated separately. Investigators have either been engaged in absorption experiments with excised roots or in exudation experiments with decapitated root systems (Anderson, 1975a; Arisz et al., 1951; Klepper, 1967; Meiri, 1973). For this reason, it seemed relevant to investigate both processes side by side and to find out whether uptake was related to upward salt transport and whether both processes are regulated by identical mechanisms.

In this study, K absorption was studied with excised low salt maize roots to investigate and identify the behaviour of maize roots in relation to K absorption and K accumulation processes. Subsequently, absorption and exudation was studied to analyse simultaneously the short-distance and long-distance transport processes of K in the maize root, and to correlate uptake and upward transport of salts.

2 Literature

This chapter is a short review of literature dealing with a number of select aspects of ion absorption and transport in the plant root(cell). Sections of Chapter 5 and 6 will refer to relevant literature in more detail. Comprehensive reviews on ion uptake and ion transport have been presented by Bowling (1976), Brouwer (1965), Hodges (1973), Fried & Shapiro (1961), Higinbotham (1973), MacRobbie (1971), Pitman (1977).

2.1 ABSORPTION OF IONS IN THE PLANT ROOT(CELLS), A PASSIVE OR ACTIVE PROCESS?

According to Hodges (1973), Lüttge (1973), Nobel (1970), active ion transport is the movement of ions against their electrochemical gradient, whereas transport will be passive if ions are moving down the electrochemical gradient. Possible physical driving forces in salt or ion transport are:

1. concentration gradients of the salts and ions involved,
2. electrical gradients, since the moving particles carry an electrical charge and plant cells show a resting electrical potential of about 100 mV or more, the interior being negative (Clarkson, 1974; Higinbotham et al., 1961; Pitman et al., 1970).

Hence, the passive or active nature of salt transport can be determined by investigating whether the ion fluxes involved obey the Nernst criterion, the Goldman equation, or the Ussing - Teorell criterion (Baker & Hall, 1975; Bowling, 1976; Nobel, 1970), describing passive ion distribution, or whether they deviate from these laws. The origin of the electrical potential across membranes is probably a result of three processes, diffusion, absorption by fixed charges and active electrogenic transport. A diffusion potential can arise from a difference in mobility of ions in a membrane or by differences in the relative permeability of a membrane to various ions. Fixed charges are due to dissociation of organic molecules or complexes being held within the cell envelope. Electrogenic transport is an active transport in which a net charge is transferred across a membrane at the expense of metabolic energy.

Membrane potentials therefore arise as the sum of these three processes. The electrochemical membrane potential is built up by an energy-independent component, the diffusion potential, as well as by an energy-dependent part, an electrogenic component. This means that terms like energy-dependent and energy-independent salt transport are not identical to active and passive salt transport, respectively.

Comprehensive studies of the electrochemical status of ions in plant tissues have been carried out by a number of investigators. Higinbotham et al. (1967) found, in roots of *Pisum sativum*, that all anions (Cl, SO_4, NO_3 and H_2PO_4) were transported and accumulated actively, that is against the electrochemical potential gradient. Transport and accumulation of cations proved to be more variable and unclear. According to

Higinbotham et al. (1967), there is no evidence for active accumulation of Ca and Mg. The behaviour of Na and K is rather complex. According to Etherton (1963) sodium is actively extruded by root cells, whereas others (Shepherd & Bowling, 1973) believe that roots of some plant species accumulate Na actively, dependent on the external and internal sodium concentration of the root cell.

The nature of potassium transport in plant cells seems to be even more variable. Measurements of active accumulation (Pierce & Higinbotham, 1970), passive equilibrium (Higinbotham et al., 1967) and active extrusion (Etherton, 1963) of K in plant roots have been reported in evidence. Jeschke (1970b), on the other hand, found evidence for the existence of a K/Na pump in which active efflux of Na is linked with active K influx.

Altogether, one has to be careful with statements about active or passive movements of ions, because the nature of the transport process depends on plant species, salt and energy status of the root, while data of electrical membrane potentials, measured in root cells of higher plants, should be interpreted with caution.

2.2 ACTIVE ION TRANSPORT AND ENERGY; THE ROLE OF ADENOSINE TRIPHOSPHATASE (ATPase)

Respiration and photosynthesis are generally considered to be the major sources of metabolic energy that drives active ion fluxes in plant cells. According to Lüttge (1975), the energy supply is not specific. Adenosine triphosphate (ATP) seems to drive ion transport, irrespective of the nature of the ATP-providing partial reaction of energy metabolism (e.g. oxidative phosphorylation, non-cyclic photophosphorylation, cyclic photophosphorylation or even glycolysis). As active ion transport must be directly coupled to an energy-releasing reaction and ATP is the energy source for ion transport in roots, attempts have been made to find out whether membranes of plant cells possess ATPase activity just like animal cells. Hall (1969) proved the presence of ATPase in plant cell membranes. Kylin & Gee (1970) and Leonard & Hodges (1973) showed ion-stimulated ATPase activity in isolated membranes of oat roots and in leaves of the mangrove. Moreover, Fischer et al. (1970) have shown that the component of the ATPase, activated by K or Rb, is highly correlated with K and Rb absorption by roots of four plant species. Although some evidence for a connextion between ATPase activity and salt uptake has come from previous work, up till now strict proof is lacking of the presence of an ion-specific ATPase activity in plant cell membranes or, if present, of a link between this enzyme activity and active uptake of related cations or anions.

According to Bowling (1976), active ion transport may be brought about directly by the ATPase acting as a carrier, but active ion transport may also be brought about by carrier systems which are only indirectly connected to the ATPase. The membrane ATPase would have no direct transport role, but would act only by providing energy for active ion transport. This lack of specificity of ATPase for direct cation transport suggests that the ATPase is not primarily a carrier of ions across the membrane, but its main role is to make energy available to specific carrier systems by hydrolysis of ATP (Bowling, 1976).

2.3 RADIAL AND LONGITUDINAL SALT AND WATER TRANSPORT IN ROOTS

2.3.1 Radial transport

Besides absorption, radial transport of ions through the different root tissues needs to be considered. The assumption, made by House & Findlay (1966) and Slatyer (1967) for the osmotic flow of root pressure exudation, that only a single membrane system exists within the root, would seem an oversimplification. A modified and improved model, introduced by Curran & McIntosh (1962) and Ginsburg & Ginzburg (1970) is based on existence of two membranes in series.

In penetrating the root centripetally, the ions pass two tissues, the epidermis and the cortex. Although the epidermis is only one layer of cells, it can fulfil an important role in transport processes: in older roots suberization of the epidermis cells often leads to formation of an identifiable exodermis and a limitation of the transport of water and salts. The cortex occupies about 90% of the root volume in maize plants (Anderson, 1975b). There are two parallel pathways for movement of salt and water across the cortex, one is the extracellular space or apoplasm and the second is the symplasm, the continuation of the cytoplasm of one cell to the next by way of the plasmodesmata.

Salt and water, present in the apoplasm, have not yet passed a biological membrane; both are able to move freely within the apoplasm inwards as far as the endodermis, but also outwards to the outer solution. Diffusion and mass flow will be the driving forces, modified by factors limiting transport rates of salts and water within the apoplasm. After the apoplasm, salts and water have to pass the plasmalemma before being taken up in this cellular cytoplasmic stream. This plasmalemma flux ϕ_{oc} is held to be the rate-limiting factor for cortical transport. In the symplasm, the plasmodesmatal transfer will be the rate-controlling step for the symplasmatic part of transport (Tyree, 1970). According to the work of Arisz (1956), the symplastic transport is sufficiently rapid to account for the majority (up to 90%) of cortical salt transport. For water movement, on the other hand, it is likely that the apoplasm, because of its high hydraulic conductivity, is the preferential pathway across the cortex, rather than the symplasm (Anderson, 1975b).

The aspect of ion exchange has been treated clearly by Hodges & Vaadia (1964). Transport of salts, present in the symplasm, is not simple and straightforward, but is accompanied by a two-way exchange of symplastic and vacuolar salt, expressed by the salt fluxes ϕ_{cv} and ϕ_{vc}. Dependent on the salt status of the root cells and the rate of symplastic salt transport, this exchange mechanism can be predominant or insignificant.

The first real barrier in salt and water transport will be the endodermis. The Casparian bands will block apoplasmatic transport. Solute and water have to enter the cytoplasm of the endodermis cells in order to pass this monolayer along symplasmic pathways.

The stele forms the next tissue in the root cross-section. Before joining the xylem flow, both salt and water have to pass the xylem parenchyma. The way and the nature of this stelar transport are still obscure. Initially, the work of Crafts &

Broyer (1938) assumed an oxygen deficiency within the stele, inducing a leakiness of the parenchyma cells for ions. Ions could then leak out of the parenchyma cells and be transported from one cell to the next ultimately into the xylem vessels. However, more recent work disproves a situation of anaerobiosis and passive transport within the stele. Respiration measurements (Hall et al., 1971) and features of a dual mechanism in isotherms for long-distance ion transport (Läuchli & Epstein, 1971; Läuchli, 1972) prove a symplastic salt transport in the parenchyma cells of the stele. Contrary to this mechanism, Baker (1973) provides evidence that the final passage into the xylem vessels would be more passive.

2.3.2 Longitudinal transport

Subsequent to the transfer into the xylem vessels, water and salts are transported longitudinally; in this way, aerial parts of the plant are supplied with nutrients and water. The anatomic structure of the pathways used for this vertical transport is crucial. Long tubular cells with perforated transverse walls (tracheids) form continuous tubes within the plant through the root and the stem up into the leaves. Protoplasts of these cells, active during the early phases of their formation die after cell differentiation; consequently the functional transport channels are dead and belong to the apoplasmic pathways of salt and water transport. In this way, vertical transport of water and solutes within the xylem vessels is passive.

According to Anderson (1975a), a hydraulic and an osmotic component are involved in longitudinal water flow. Transport of water to the aerial parts of the plants will depend on rate of transpiration by the leaves (hydraulic component) and salt absorption by the roots (osmotic component). Upward salt transport, simultaneous with the water flow, is assumed to be the sum of a convective (mass flow) and diffusive flow. Thus longitudinal transport of water and salts within the xylem vessels is passive.

3 Materials and methods

This chapter describes standard conditions of plant growth and experimental techniques. Modifications in these standard conditions are mentioned in Chapters 4, 5 and 6.

3.1 GROWTH

For uptake experiments, seeds of maize (*Zea mays* L., cv. CIV 2 'Prior') were sown in trays filled with coarse gravel and moistened with demineralized water. After germination, 28 trays were transferred on a container, filled with 120 liters of a $CaSO_4$ solution of $\frac{1}{2}$ mmol l^{-1}. The solution was mixed and aerated by an electric pump (Slangen, 1971). Roots of the plants grown on this $CaSO_4$ solution (low salt roots) were used 10-15 days after germination for the different uptake experiments and also for Transport Experiments 35, 36, 37 and 41. For all other transport experiments, seeds of the same maize variety were germinated in quartz sand and moistened with demineralized water. One week after germination, seedlings were transferred to a continuously aerated nutrient solution with a composition as shown in Table 1. Once a week the nutrient solution was completely replenished; each day pH was adjusted back to 5.0 with a HNO_3 solution 1 mol l^{-1}. Four weeks after germination all plants were transferred from the complete nutrient solution to a $\frac{1}{2}$ mmol l^{-1} $Ca(H_2PO_4)_2$ solution. One week later transport measurements were performed with the excised root systems of these maize plants (exudation experiments).

The low and high salt plants were grown in a glasshouse throughout the year. During October-April, an illuminance of 20 000 lx and a minimum temperature of 20 °C was guaranteed by artificial lighting (HPL lamps) and heating, respectively. During summer, temperatures occasionally reached 30-35 °C. As a result of diurnal and seasonal fluctuations in temperature and light intensity, plants of successive experiments were sometimes different.

3.2 EXPERIMENTAL TECHNIQUE

3.2.1 Uptake experiments

The following types of measurements were performed on plants or plant organs:
1. Influx measurements (a tracer method). Roots were excised and rinsed in demineralized water. Dependent on the aim of the experiment, roots were used for the influx experiment directly or after further treatment. After blotting to remove excess water, portions of 10 g fresh root were placed in cheesecloth 'teabags' (Epstein et al., 1963) in a volume of 500 ml aerated experimental labelled solutions at the appropriate concentration and

Table 1. Composition of the nutrient solutions in mmol l^{-1}. Trace elements (mg l^{-1}): 0.5 B, 0.5 Mn, 0.05 Zn, 0.02 Cu, 0.01 Mo, 0.4 Fe as Fe-EDTA and 0.4 Fe as $FeSO_4$.

	K	½Ca	½Mg
NO_3	2.5	5.0	
H_2PO_4	0.5		
½SO_4			2.0

containing in addition 0.05 mmol l^{-1} $CaCl_2$.

In preliminary uptake experiments (Chapter 4), ^{86}Rb was used either simultaneously with ^{42}K as a tracer for potassium (double labelling), or as a tracer for Rb influx measurements. In uptake and transport experiments, described in Chapters 5 and 6, ^{86}Rb, ^{36}Cl, ^{35}S and ^{22}Na (Radiochemical Centre, Amersham) were used as radioactive tracers for K, Cl, S and Na, respectively. The molar activity at the beginning of the experiment was about 333 MBq mol^{-1} monovalent cation or anion. The temperature of the solution during the experiment was maintained between 20 and 23 °C. All influx experiments were carried out at least in duplicate. When necessary, pH was adjusted by means of acid or base. Experimental time was 1-10 h. The rate of ion influx was measured either by:

-depletion: at appropriate time intervals (t = 0, 15, ..., 600 min), 10 ml aliquots of the well-mixed and labelled experimental solutions were pipetted into counting tubes. At the end of the absorption period, depletion of the ambient solution was calculated from radioactivity of the different samples, or by

-accumulation: the absorption period was terminated by desorption of exchangeable bound ions. The teabag, containing the root tissue, was dropped into a volume of about 200 ml of a cold (4 °C) unlabelled solution containing 10 mmol l^{-1} KCl and 0.05 mmol l^{-1} $CaCl_2$. This treatment was repeated four times in successive fresh aliquots of identical solutions. The five desorption periods took 3 x 5, 15 and 30 min, respectively. Finally, the tissue was rinsed twice with water for a total rinsing time of five minutes. After these treatments, the fresh material was dried at 70 °C for 24 h and weighed. After digestion of the dry root tissue, ambient solution and the digested samples were analysed by liquid scintillation counting.

2. Influx/efflux measurements (a tracer method). Efflux was measured always simultaneously with the influx. For 12 h, two portions I and II of excised roots were allowed to accumulate ions from a labelled or an identical unlabelled salt solution, respectively. Subsequently, after washing the roots for 10 s in demineralized water, the portions I and II were transferred either to fresh identical unlabelled or labelled experimental solutions and efflux and influx were measured during the next 4-10 h. At appropriate times, 10-ml aliquots were pipetted out of the experimental solutions. A release or depletion of label by the roots was measured by counting of radioactivity of the samples.

3. Net uptake measurements (continuous titration method). Net uptake (influx-efflux) of potassium was measured by continuous titration (Breteler, 1973). With an automatic titration equipment (Radiometer, Copenhagen) in combination with an ion-specific K^+ electrode (Philips), concentration of K^+ in the absorption solution was kept constant for 4 h. Portions of 10 g of freshly excised low salt root material were put in 500 ml

of absorption solution at appropriate concentration of K. The temperature of the absorption solution was maintained at 20 °C. On a recorder sheet, the amount of titration solution, a potassium salt solution, that was used to keep K constant was recorded. Net uptake was calculated as the product of titration rate and concentration of the titration solution.

4. Salt accumulation by intact plants (long-term experiments). The uptake techniques, as well as the plant growth conditions, employed in preliminary long term K-Rb uptake experiments with intact plants were different and will be discussed in Chapter 4.

3.2.2 Transport experiments

The following types of measurements were performed on plants or plant organs:

1. Vascular influx and efflux by excised low salt roots. Longitudinal xylem transport of potassium was measured in excised roots of low-salt gypsum plants. As shown in Diagram 1, 10 excised roots were fixed with paraffin in a plastic cup. Only the cut end of the excised roots was placed in the upper compartment (I); the root itself was immersed in the lower compartment (II). Flow of the upper solution to Compartment II was prevented by the paraffin. The volumes of Compartments I and II were 25 and 500 ml, respectively. Both compartments were filled with a 1 mmol l^{-1} KCl solution; only Solution II was aerated continuously during the flux experiments. The temperature of both solutions was 20-22 °C. In vascular efflux experiments only the lower Solution II was labelled with ^{86}Rb. At appropriate times, samples were taken from both compartments. After measurement of radioactivity, potassium influx and vascular efflux of freshly absorbed K were calculated. For K(total) transport, measurements of [K(total)] were carried out in the compartment I samples. In vascular influx experiments, labelling was the reverse. Samples from both compartments were analysed and influx by the cut end of the excised roots was calculated. All transport experiments were in triplicate.

2. Exudation experiments with complete root systems with cut stump. For exudation experiments tops of plants were removed by cutting about 5 cm above the stem base. The roots were rinsed in demineralized water and each plant was placed in 6 l of aerated absorption solution at 20-22 °C. A rubber tube was fastened to the stump.

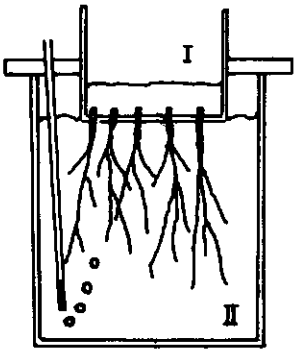

Diagram 1. Experimental arrangement for measurement of vascular influx and efflux of K in excised low-salt maize roots. Excised roots were fixed with paraffin in the plastic upper cup (Compartment I), while roots themselves were immersed in the lower Compartment II. Both compartments are filled with solutions of equal composition, with or without labelling.

Exudates were collected periodically from the rubber tubes with a pipette and stored in a deep-freezer. At the end of the experiment weight and composition of all exudates were measured. Ion influx and subsequent transport of freshly absorbed salts were measured by depletion and by analysis of the bleeding sap. In samples of absorption solution and exudates, collected at appropriate time intervals, radioactivity was measured. For K(total) transport also K(total) concentration in the exudates was analysed. In Experiments 39 and 40, absorption, accumulation and transport isotherms of different ions were measured without radionuclides. After experiment, roots were rinsed three times for 5 min each in demineralized water and dried for 24 h at 70 °C. Subsequently, cations and anions were analysed in the exudates and in dried root as described in Section 3.3. Total salt uptake was assumed to be the sum of salt accumulation plus salt transport. Exudation experiments were at least in triplicate; exudation was mostly for 24 h.

3.3 CHEMICAL ANALYSIS

Radioactivity in samples of absorption solutions and exudates were counted without any further treatment. Analysis of the root samples was done after a digestion of the dried root material in concentrated sulphuric acid and hydrogen peroxide. Counting of radioactivity (β-radiation) was with an automatic Nuclear Chicago Mark I liquid Scintillation counter. For ^{86}Rb, ^{42}K, ^{22}Na and ^{45}Ca, Cerencov radiation was measured. To measure ^{36}Cl and ^{35}S, scintillation solution was added, e.g. a mixture of 1,4- dioxane (800 ml), 2-ethoxy-ethanol (160 ml), naphthalene (48 g) and 2,5-diphenyl - oxazole (9.6 g). Of this solution, 9 ml was mixed with 1 ml sample. As a result of double labelling in Experiments 1 and 2, samples were counted twice. Immediately after finishing the influx experiment, the sum ^{42}K + ^{86}Rb was measured. After ^{42}K decay (one month), ^{86}Rb was counted.

Inorganic constituents in non-radioactive samples of root material, absorption solutions and exudates were measured by the method of Slangen & Hoogendijk (1970). Potassium concentrations in radioactive samples were analysed with an ion-specific K^+-electrode (Philips). In plant material, Rb was estimated by atomic absorption spectrophotometry. Organic constituents like carboxylates and water-soluble carbohydrates were measured by the method of Breteler & Wittich (1973).

3.4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

Data on uptake and translocation experiments are mostly calculated on a rate basis and presented in figures or tables. Further:

- Extremely high absorption rates during the initial phase of most experiments had to be left out.
- Mean rates of uptake and translocation are plotted at the midpoint of each measurement period. The length of the different measurement periods is indicated in most figures by vertical blocks. Measurement periods are equal for the different curves in one figure as well as for curves in combined figures (A,B, ...).
- During periods of fast change in rates, for example initially, drawing the curve

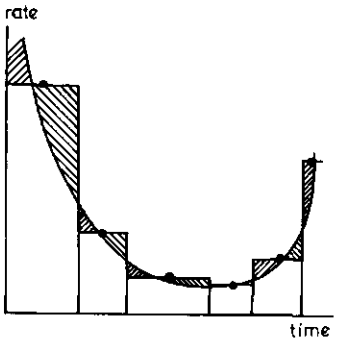

Diagram 2. Graphical representation of the rate of ion absorption versus time. Measurement periods are indicated by vertical blocks. • midpoints of the different measurement periods.

through the midpoints of the measurement periods would be wrong, since areas inside and outside the blocks have to be equal (Diagram 2).

The mathematical method to calculate the rate of uptake and translocation as a differential of relevant incremental curves is not feasible in this work, because of the limited number of data per experiment.

In some experiments, results are checked for statistical significance by the Student's t test (Snedecor & Cochran, 1967). In tables, data that differ significantly from the control data are marked with a single ($P = 0.05$) or a double ($P = 0.01$) asterisk. To check statistical significance of data presented graphically, least significant differences (LSD) (Snedecor & Cochran, 1967) are placed above the curves. These LSD values ($P = 0.05$) are only calculated for influx, efflux, transport during the steady-state phase of the experiment or, if not reached, for the last measuremental period.

4 Preliminary experiments

Because of the fast decay of ^{42}K ($T_{\frac{1}{2}}$ = 12 h), the more stable isotope ^{86}Rb ($T_{\frac{1}{2}}$ = 18 d) is frequently used as a physiological substitute for ^{42}K in potassium absorption and transport experiments. Although literature on this subject is quite extensive (Marschner & Schimansky, 1971; Mesbahul et al., 1971; Schimansky, 1970), there is no clear indication that a potassium-rubidium substitution is fully justified under all circumstances. Because of the negative results of Jeschke (1970a) and West & Pitman (1967), a few exploratory short-term K influx experiments were done to investigate:

- ^{42}K - ^{86}Rb substition with excised maize roots low and high in salt and at different K concentrations of the absorption solution;
- K and Rb influx by excised high and low salt maize roots;
- the K/Rb selectivity in uptake during a long-term experiment with intact maize plants.

4.1 SHORT-TERM K/Rb SUBSTITUTION EXPERIMENTS WITH EXCISED ROOTS

Experiment 1: ^{86}Rb as a tracer for potassium influx measurements. Influx from a 0.1 mmol l^{-1} KCl solution in excised low salt roots, estimated from depletion.

Figure 1 shows that after 15-30 min (initial phase), the rates of potassium influx, measured with ^{42}K and ^{86}Rb do not differ significantly. Obviously, these low salt maize roots do not discriminate between ^{42}K and ^{86}Rb as a tracer for potassium, at least at a potassium concentration of 0.1 mmol l^{-1} in the absorption solution.

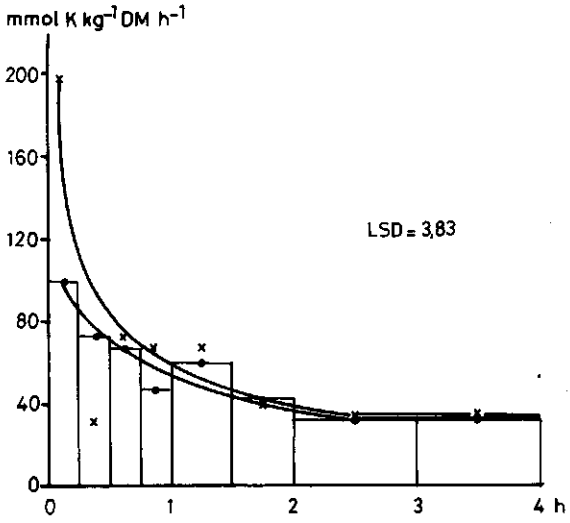

Fig. 1. Experiment 1. Influx of K into excised low-salt maize roots from an absorption solution with KCl at substance concentration 0.1 mmol l^{-1}, estimated with ^{42}K (x) and ^{86}Rb (•) as tracers for K.

To investigate the effect of the external potassium concentration, the next experiment was started.

Experiment 2: ^{86}Rb as a tracer for potassium influx measurements with excised low salt roots. Influx from KCl absorption solutions 0.1, 0.5, 1.0, 5.0 and 10.0 mmol l^{-1}, for 4 h, estimated from accumulation.

Both influx isotherms of the low salt root material (Fig. 2A) show a good agreement in the lower concentration range of the absorption isotherm. At KCl concentrations of 5.0 mmol l^{-1} and more, ^{86}Rb gives significantly lower K influx than does ^{42}K tracer. In Figure 2B, both isotherms are presented for maize roots rich in K. Roots of plants, grown 8 d before the influx experiment on a complete nutrient solution (Table 1) were investigated. Except with the highest external concentration of KCl (10 mmol l^{-1}), preloading of the roots with potassium depressed potassium influx, measured with the tracers ^{42}K and ^{86}Rb. This equal inhibition of both ^{42}K and ^{86}Rb influx with an increased internal cellular concentration indicates an identical behaviour of K and Rb in the plant cell, at least under these conditions.

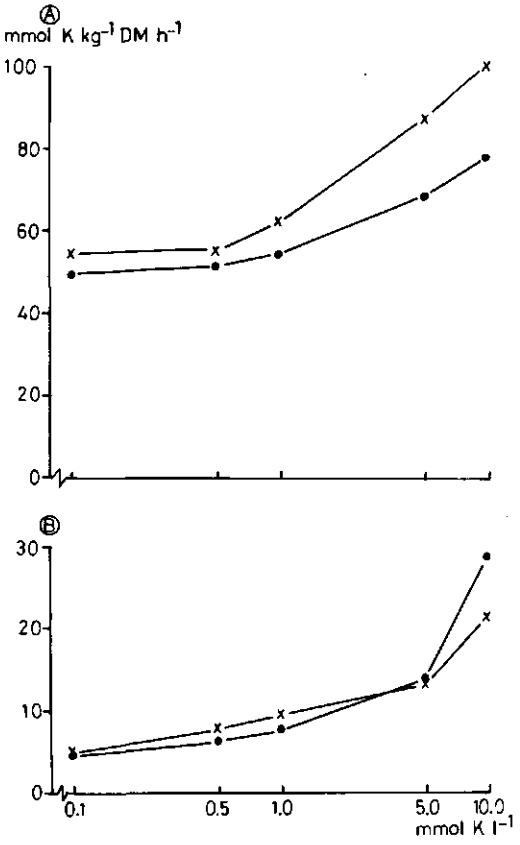

Fig. 2. Experiment 2. Influx of K in excised maize roots at different external concentrations of KCl. A. Low-salt roots. B. Roots loaded with K. Influx of K was estimated with both ^{42}K (x) and ^{86}Rb (•) as tracers.

Experiment 3: Influx of K and Rb in excised roots low and high in salt from a 0.1 mmol l^{-1} KCl and RbCl solution, respectively. K and Rb influx were both measured by depletion with the tracers ^{42}K and ^{86}Rb, respectively.

The time courses of the influx for K and Rb indicate, that
- for roots rich in K, also the Rb influx was reduced significantly (Fig. 3A,B);
- for low salt roots, the rate of Rb absorption dropped significantly after about 2-3 h, whereas K influx reached steady-state after about 1 h (Fig. 3A).

Under equal experimental conditions, potassium absorption from a 0.1 mmol l^{-1} solution of KCl is much higher than the Rb uptake from a RbCl solution of equal concentration. However, the results in Experiment 2 suggest that at extremely high K/Rb ratio in the absorption solution (^{86}Rb only as a tracer), maize root does not discriminate between K and Rb. Obviously, the K/Rb molar selectivity coefficient approaches unity only at high substance ratios of K to Rb in the absorption solution.

4.2 SUBSTITUTION OF Rb FOR K IN LONG-TERM EXPERIMENTS WITH INTACT PLANTS

Selectivity and the role of Rb as a physiological substitute for potassium was investigated in long-term absorption experiments with intact plants. After germination, 5 maize plants were placed on 500 ml of a well aerated nutrient solution which, in addition to K and Rb (as chlorides) contained the following salts in mmol l^{-1}:

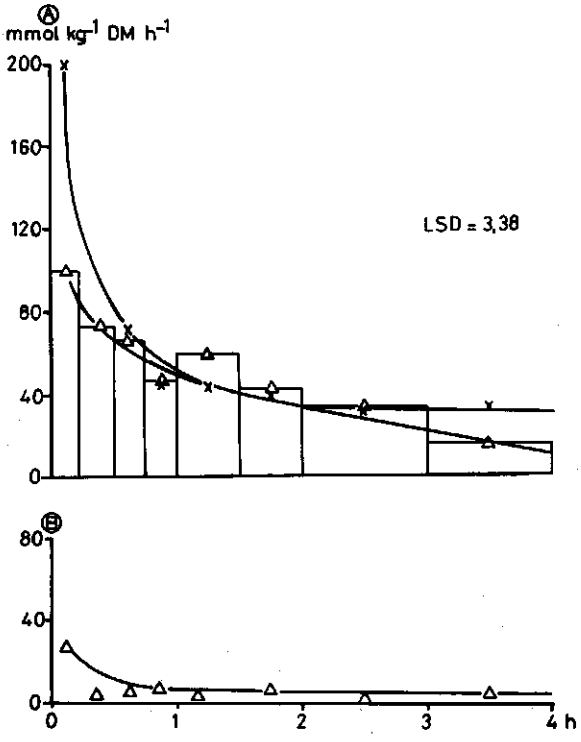

Fig. 3. Experiment 3. A. Influx of K (x) and Rb (Δ) to excised low-salt maize roots from 0.1 mmol l^{-1} solutions of KCl and RbCl, labelled with ^{42}K and ^{86}Rb, respectively. B. Influx of Rb to excised K-preloaded maize roots from 0.1 mmol l^{-1} RbCl absorption solution, labelled with ^{86}Rb.

1 $Ca(NO_3)_2$, 0.5 $Mg(NO_3)_2$, 1 NaH_2PO_4 and Fe and trace elements (Table 1). In these experiments, the concentrations of K and Rb and their ratio were varied. Fresh nutrient solutions were supplied daily. After 12 d, the experiments were terminated. The plants were harvested, and roots and shoots separated. Plant material was dried, weighed and, after digestion, K and Rb were estimated by flame photometry and atomic absorption spectrophotometry, respectively. The selectivity coefficient,

$$S.C._{K/Rb} = \frac{K/Rb \text{ in the plant}}{K/Rb \text{ in the absorption solution}}$$

was used to characterise the selectivity or preference of the maize plant for K and Rb at varying ratios in the external solution. Thus values greater than 1.0 would indicate a preference for K, whereas values smaller than 1.0 would indicate the reverse.

Experiment 4: Selectivity in uptake of K and Rb (accumulation) by intact maize plants for 12 days on a complete nutrient solution with:

a. K and Rb at respective concentrations of 2.0, 0; 1.5, 0.5; 1.0, 1.0; 0.5, 1.5; 0.0, 2.0 mmol l^{-1} (K-substitution series).

b. K and Rb at respective concentrations of 2.0, 0; 2.0, 0.5; 2.0, 1.0; 2.0, 1.5; 2.0, 2.0 mmol l^{-1} (Rb-addition series).

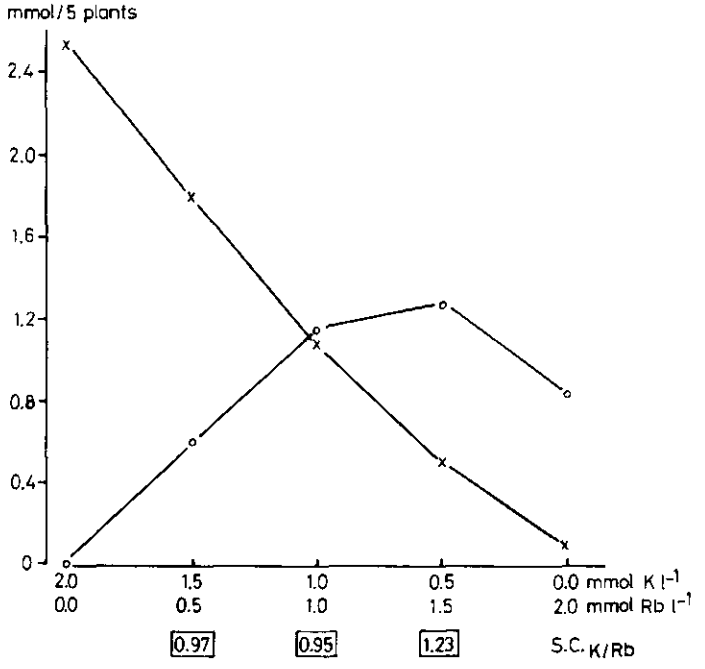

Fig. 4. Experiment 4. Accumulation of K (x) and Rb (o) in intact maize plants (samples of 5 plants), grown for 12 d on complete nutrient solutions with different K and Rb concentrations (K-substitution series).

Accumulation curves for K and Rb in Figure 4 show that a substitution of Rb for K decreased accumulation of K and simultaneously enhanced accumulation of Rb. Within the range K/Rb 2.0/0.0 - 1.0/1.0, the reduction in accumulation of K was compensated by an equal increase in Rb accumulation. The sum [K + Rb] accumulated by the plants is almost constant and the selectivity coefficient does not significantly differ from unity. At substance ratios of 0.5/1.5 or less, plant growth was inhibited (Table 2), and selectivity was changed. As $S.C._{K/Rb}$ within this range became greater than unity the plant prefers K; the reduction in dry matter production at K/Rb ratios < 1 proves that Rb cannot completely take over the role of potassium within the plant. Half the potassium can be substituted without growth reduction or deviation of the $S.C._{K/Rb}$ from unity.

Increasing additions of Rb to a constant K concentration of 2.0 mmol l^{-1} decreased accumulation of K and increased the accumulation of Rb (Fig. 5). However, for all substance ratios, the $S.C._{K/Rb}$ did not differ significantly from unity; also the production of maize dry matter was not significantly affected by additional Rb in the nutrient solution up to a ratio of 1 (Table 2). This Rb addition series also proved that Rb took over some function(s) of potassium in the maize plant. With Rb substitution or addition down to a ratio ≥ 1, the maize plant did not discriminate between K and Rb in its uptake functions, nor show any depression in dry matter production for 12 days.

4.3 CONCLUSIONS

The use of ^{86}Rb as a tracer for ^{42}K in uptake experiments with excised roots of maize is justifiable. Even in long-term experiments, Rb substituted for up to half the K in the nutrient solution. As the concentration of ^{86}Rb in K absorption solutions was always low (only tracers), the ratio was high. Results of influx experiments with excised roots, and double labelling with ^{42}K + ^{86}Rb confirmed this. Only at high external potassium concentrations (5 mmol l^{-1} or higher) was use of ^{86}Rb risky.

According to Hodges (1973), selective ion transport by plants is almost sure to depend on electric field strenght of the ion-binding sites and on shifts in the electric field strength of the sites. When the electric field strength of the negative site is weak, the ion absorption sequence Cs > Rb > K > Na > Li is preferred, and as the elec-

Table 2. Dry matter (DM) production from 5 plants and selectivity coefficient of K over Rb ($S.C._{K/Rb}$) in intact maize plants, grown for 12 d on a series of nutrient solutions. DM production and $S.C._{K/Rb}$ were statistically tested, with DM of the zero-rubidium treatment and $S.C._{K/Rb}$ = 1 as controls, respectively. Experiment 4.

K- substitution series				Rb- addition series			
c(mmol l^{-1})		DM	$S.C._{K/Rb}$	c(mmol l^{-1})		DM	$S.C._{K/Rb}$
K	Rb	(g/5 plants)		K	Rb	(g/5 plants)	
2.0	0.0	1.85		2.0	0.0	1.68	
1.5	0.5	1.92	0.97	2.0	0.5	2.01	1.02
1.0	1.0	1.62	0.95	2.0	1.0	1.70	0.98
0.5	1.5	1.45*	1.23**	2.0	1.5	1.82	0.95
0.0	2.0	0.91**		2.0	2.0	1.59	0.99

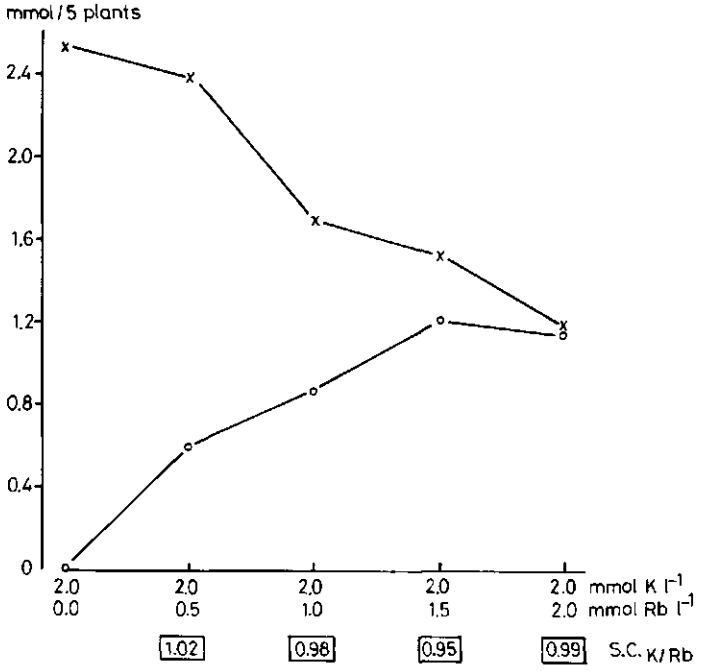

Fig. 5. Experiment 4. Accumulation of K (x) and Rb (o) in intact maize plants (samples of 5 plants), grown for 12 d on complete nutrient solutions with constant K but increasing Rb concentrations (Rb-addition series).

tric field strength of the negative site increases, the specificity of ion binding progressively shifts at high electric field strength to the sequence Li > Na > K > Rb > Cs. Thus, a shift in selectivity with an increase in electrical field, induced by an increase in external ion concentration is in agreement with predictions of Hodges (1973).

5 Potassium uptake in excised roots

5.1 TIME COURSE OF ION ABSORPTION

In the literature, biphasic absorption - time curves have been presented by many investigators. After a rapid uptake of salts during the first period, the initial phase, the rate of absorption becomes constant during a subsequent phase of steady-state uptake.

However, potassium absorption measured by continuous titration (Section 3.2) showed, in addition to these two phases, also a transition phase. It seems to be a step between the phase of initial and the phase of steady state uptake.

Such an uptake - time curve, precisely and continuously recorded, was obtained in Experiment 5.

Experiment 5: Rate of potassium absorption in excised low salt maize roots during absorption for 90 min. Absorption from a solution with KCl at concentration 5 mmol l^{-1} was measured continuously by titration.

Figure 6 shows three phases during the 1.5 hours of absorption. Just as reported by Heller et al. (1973) and Lüttge & Pallaghy (1972), after a first short period of rapid uptake, the rate of potassium uptake was extremely low during a second phase of about 15 min. During the third phase, uptake became linear with time.

The question arises whether this second phase in the three phasic pattern of

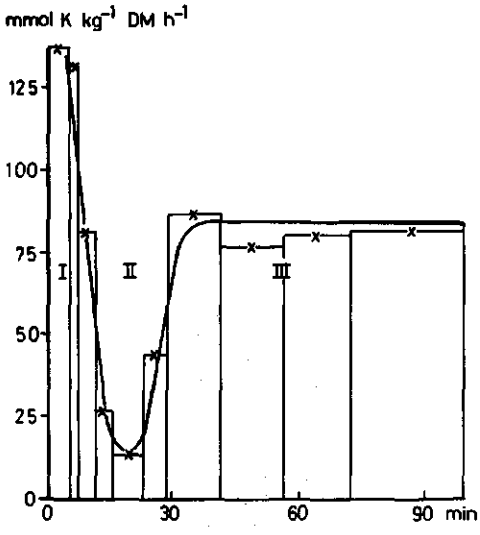

Fig. 6. Experiment 5. Rate of potassium absorption into excised low-salt maize roots during a 90-min absorption period. Absorption solution with KCl at substance concentration 5 mmol l^{-1}; absorption of K was measured by titration.

potassium uptake is a real transitional phase between a process of initial uptake and a subsequent process of steady-state uptake. A transition could imply that at the beginning of absorption only the initial uptake or filling of apparent free space (AFS) exists, whereas after completion of this process, the steady-state phase starts after about 15 min (lag phase). In the three phasic absorption-time curve, the second phase can then be explained as a transition between the two processes of ion uptake.

Experiment 6: Potassium absorption in excised maize roots low in salt from an absorption solution with KCl at concentration 1 mmol l^{-1}. Absorption was calculated after 0, 5, 15, 30, 60, 120 and 240 min by depletion and accumulation. Rubidium-86 was used as a tracer for potassium.

At the end of absorption, the roots were treated in an unlabelled solution of KCl at concentration 10 mmol l^{-1} for 1 h to remove the potassium (^{86}Rb) from the AFS (Fig. 7). The fraction retained by the root-tissue was called Fraction B and equals the potassium accumulated in the root. After about 1 h, the filling of the AFS seemed to be completed and subsequent K absorption only adds to Fraction B.
From the beginning of the absorption period, the process of accumulation perhaps exists and consequently the initial uptake is superimposed on an accumulation. Figure 8 also proves that the rate of potassium accumulation is similar to the rate of K absorption found in Experiment 5 by titration. A phase of rapid accumulation during the first 15-20 min was followed by a short period of little or no accumulation and a third stage of steady-state uptake.

Thus uptake of potassium shows a three-phasic absorption - time curve and not a two-phasic pattern. Ion accumulation starts immediately after the beginning of absorption

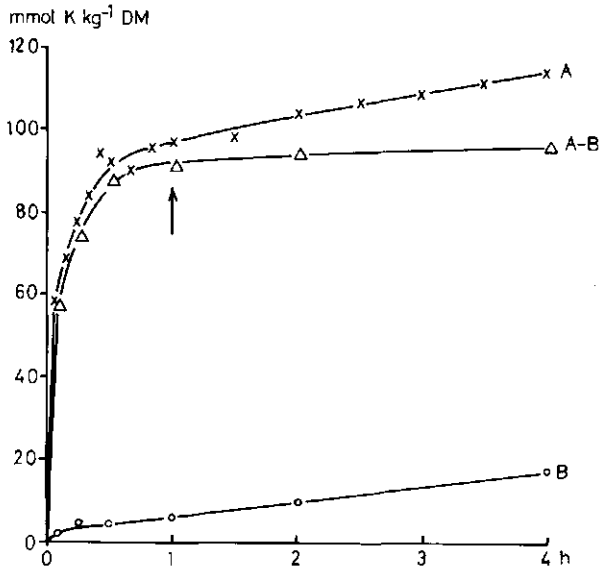

Fig. 7. Experiment 6. Time curves of absorption (A), accumulation (B) and potassium present in the apparent free space (A - B) in excised low-salt maize roots. Absorption and accumulation of K from a 1 mmol l^{-1} KCl absorption solution were calculated from depletion and accumulation, respectively, with ^{86}Rb.

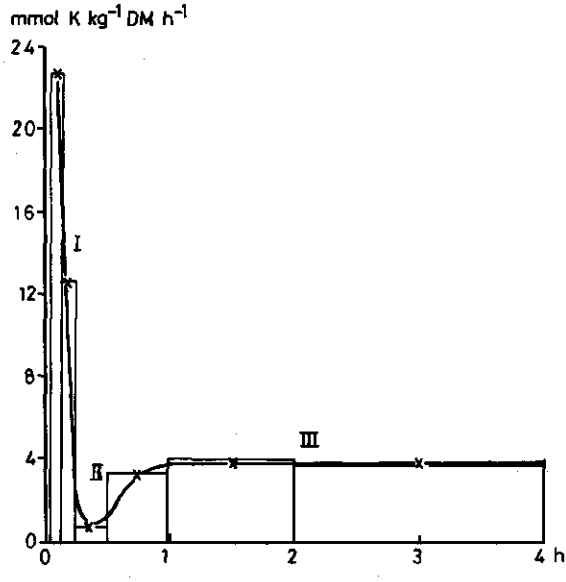

Fig. 8. Experiment 6. Rate of accumulation of K in excised low-salt maize roots over 4 h. Absorption solution with KCl 1 mmol l^{-1}. Accumulation of K was calculated from accumulation of ^{86}Rb.

and proceeds simultaneously with initial uptake. The three-phasic model is shown not only in the depletion curve, but also in curves of accumulation.
This second phase in the three phasic model is not a transitional stage between initial and steady-state uptake.

5.2 FACTORS AFFECTING ION UPTAKE

5.2.1 Effect of pH value of the absorption solution

Soil pH affects growth and ion uptake of plants mainly indirectly by a change in nutrient availability, microbial activity or soil structure. However, the patterns of salt uptake by plants growing on a nutrient solution depend on pH too. Such a direct effect of pH on the absorption of salts has been demonstrated for different cations and anions (Jacobson et al., 1957; Lycklama, 1963; Rains et al., 1964; Tromp, 1962). As mentioned by Rains et al. (1964) this pH effect, consisting of a reduction in cation uptake with decreasing external pH, can be caused either by injury to the root cells or by competition between cations.

There is evidence that H^+ may cause a general derangement of, or damage to, the ion absorption mechanism. One type of injury at low pH could be denaturation of proteins, nucleic acids, phospholipids and other polymers involved in membrane structure and functions. A second could be reduction in calcium uptake by the plant at low pH (Arnon et al., 1942; Pala, 1975). Calcium deficiency in plant cells results in disintegration of cell walls, loss of integrity of cellular membranes and consequently in a changed permeability of cellular membranes for electrolytes (Albrecht, 1968; Waisel, 1962). So hydrogen ion interacts with calcium.

According to the competition mechanism, pH will not affect cellular walls and

membranes; a change in permeability or enhanced leakage of salts will not occur. At low pH of the external solution, the rate of absorption of potassium or cations in general will only be reduced by competition between H^+ and the substrate cations for available carrier sites.

Effects of pH were studied with excised low salt roots during short-term absorption. Special attention was paid to the effect of external pH on initial ion uptake and on subsequent simultaneous influx and efflux of salts in the root.

Experiment 7: Potassium influx in excised maize roots low in salt for 10 h, at a low (2.0), medium (5.0) and high pH (7.6) of the 1.0 mmol K l^{-1} absorption solution. K influx was estimated from depletion with ^{86}Rb.

The influx data of potassium (Fig. 9) confirm the data of Jacobson et al. (1960). In steady-state, the influx of potassium at a pH of 2.0 was significantly less than at pH 5, while a further increase in external pH from 5.0 to 7.6 does not alter potassium influx further. Only at relatively high hydrogen ion concentrations (10^{-2} mmol l^{-1} or more), this ion is involved in cation absorption. At lower concentrations, concentration of H^+ is so low that any further decrease does not affect potassium influx. To investigate whether this pH effect is an injury or a competition effect, a simultaneous influx - efflux experiment was set up. If pH is active by injury of the walls and membranes of root cells, a low pH of the external solution would probably increase efflux (leakage) of cytoplasmic or vacuolar potassium.

Experiment 8: Simultaneous influx and efflux of potassium in excised low salt maize roots. Flux measurements for 10 h by the standard method with ^{86}Rb as tracer. The pH of absorption solutions (1 mmol K l^{-1}) were 7.6, 5.5, and 2.0.

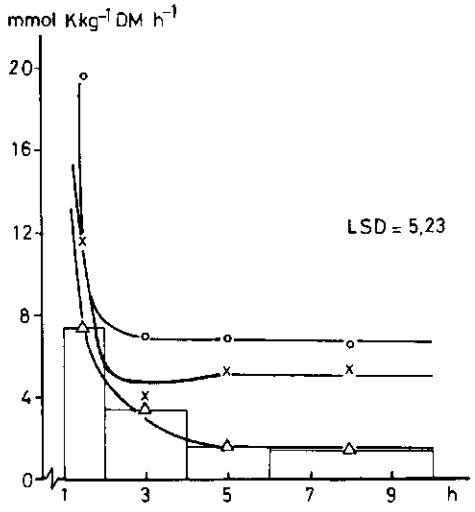

Fig. 9. Experiment 7. Influx of potassium in excised low-salt maize roots over 10 h from a 1.0 mmol l^{-1} K absorption solution, at a low pH (2.0; Δ), medium pH (5.0; x) and high pH (7.6; o).

Figure 10 confirms that substance flux of K in steady-state was halved at pH 2. However, influx curves in Figure 10A in combination with corresponding efflux curves in Figure 10B show that the inhibition in potassium influx at pH 2 was not a result of an enhanced efflux of freshly absorbed potassium. All three efflux curves do not differ significantly over 10 h. Even an extremely low pH of the experimental solution (pH 2) did not change or destroy root cell membranes to such extent that salts already present in these root cells would leak out immediately after the maize roots had been transferred to this extremely acid medium. So during these short-term experiments (10 h), pH effects are not due to membrane injury. Probably the hydrogen ion is only active by competition. Especially at high H^+ (1 mmol l^{-1} and higher), the concentrations of hydrogen ion and substrate cation are almost equal and cation competition is probable. Effects of injury, direct or indirect, probably build up only on the long term.

Data of Experiment 7 (Table 3) show that values for K accumulation in the AFS during the initial phase of ion absorption, obtained by graphical analysis, were higher at high pH of 7.6, than with the medium and low pH of the external solution.

This aspect has been investigated more extensively to check whether pH might regulate steady-state K uptake by a change in initial uptake.

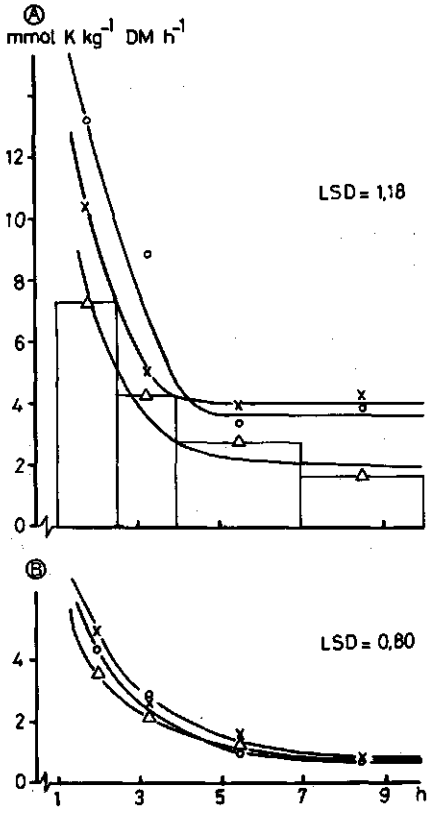

Fig. 10. Experiment 8. Potassium fluxes in excised low-salt maize roots over 10 h from a 1.0 mmol K l^{-1} absorption solution at a low pH (2.0;Δ), medium (5.5;x) and high pH (7.6;o). A. Influx. B. Efflux.

Table 3. Effect of the pH of the external medium on K accumulated initially in the AFS and on K influx during the subsequent steady-state phase. Statistically tested with pH 5.0 as control. Experiment 7.

pH	K accumulated in AFS (mmol kg^{-1} DM)	Steady-state K influx (mmol kg^{-1} DM h^{-1})
2.0	109.8	1.41*
5.0	111.4	5.62
7.6	129.6*	6.57

Experiment 9: Initial uptake of K and Cl in excised roots low in salt from a 2 mmol l^{-1} KCl absorption solution with a low (2.8) and high pH (6.5). Influx of K and Cl were measured by depletion, using ^{86}Rb and ^{36}Cl as tracers. Before the absorption experiment, intact plants were grown for 48 h on a ½ mmol l^{-1} $CaSO_4$ solution with a low and high pH of 2.8 and 6.5, respectively.

Cumulative curves for the initial K and Cl absorption, presented in Figure 11, prove that

- at low pH of the external solution, potassium accumulation in the AFS is only about 40% of that at high pH (Fig. 11C);
- at low pH, initial accumulation of cations is almost equal to that of anions; this contrasts with the ratio at high external pH (Fig. 11A,B);
- accumulation of Cl is almost equal at low and high pH of the external solution (Fig. 11D).

These findings suggest that the initial absorption of cations and anions is a dual process, e.g. a process of pure mass flow of solvent plus solutes into the water free space (WFS) and a second process of salt flux and accumulation into a Donnan free space (DFS), controlled by physicochemical forces. According to Briggs et al. (1961) and Nobel (1970), fixed carboxyl groups at cell wall surfaces or restrained indiffusible anions, such as dissociated organic acids or amino acids within the cytoplasm, are responsible for these physico-chemical forces and the subsequent accumulation of cations and depletion of anions within this DFS. If so, the second component of the initial cation absorption mechanism will be dependent on pH, while anion absorption during the initial phase will be restricted to the mass flow component and thus will be almost independent of pH.

The region containing the charged sites, such as carboxyl groups in the cell wall, is frequently referred to as the Donnan phase. At equilibrium, a Donnan distribution of oppositely charged ions, electrostatically attracted to the immobile charges, occurs between the Donnan phase and the adjacent aqueous one, as described by Nobel (1970) and by Bolt & Bruggenwert (1976) for adsorption of salts onto clay minerals. According to Nobel (1970), Donnan phases also occur in cytoplasm, where immobile charges are often due to proteins and organic acids. These organic compounds are fixed in the sense that they cannot diffuse across either the plasmalemma or the tonoplast.

The concentration of these carboxyl groups, proteins or organic acids in plant cell compartments will not alone be responsible for the total negative charge within the Donnan phase, but also the degree of dissociation of these organic compounds. As both

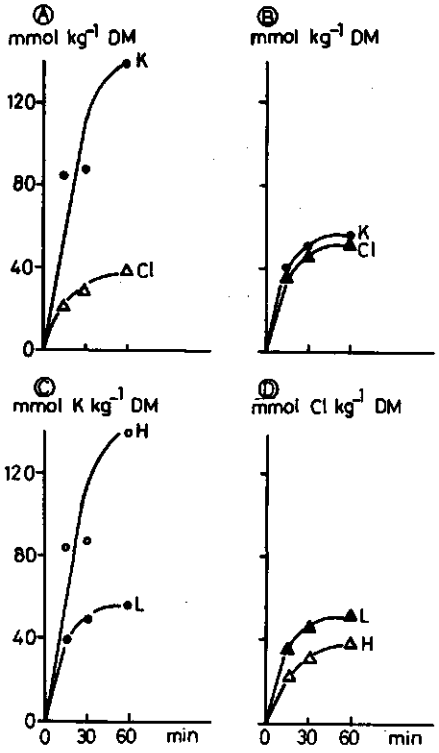

Fig. 11. Experiment 9. Initial absorption of K (o,•) and Cl (Δ,▲) in excised low-salt maize roots from a 2 mmol l^{-1} KCl absorption solution of low pH (2.8) and high pH (6.5), indicated with closed and open symbols, respectively.

p*K* (*K* is dissociation constant) of the organic compound and pH of the Donnan space will determine the degree of dissociation, the whole Donnan system will be characterized by the concentration of the immobile or restrained organic compounds [HA] , the external concentration of inorganic salt, pH and p*K*.

To check whether data gathered in Experiment 9 resemble a Donnan distribution, an ideal Donnan equilibrium was calculated for concentrations of the hypothetical non-diffusible organic compound HA and of the external inorganic salt (KCl); pH of the external medium and p*K* of the hypothetical non-diffusible compounds were varied (Diagram 3).

Under equilibrium conditions

$$[Cl^-_i] + [A^-] = [K^+_i] + [H^+_i] \qquad (1)$$

(electrical neutrality) and

$$\frac{[Cl^-_o]}{[Cl^-_i]} = \frac{[K^+_i]}{[K^+_o]} = \frac{[H^+_i]}{[H^+_o]} \qquad (2)$$

(equal ion ratios; Briggs, 1961). From Equations 1 and 2,

Diagram 3. The scheme of an ideal Donnan model; an external and internal compartment, separated by a membrane or cell wall, indiffusible anions (A^-) and an univalent cation and anion, e.g. KCl.

Concentrations at equilibrium

side o (external solution)		side i (internal solution)
$[K_o^+] = a = 1$ mmol l^{-1}		$[A^-]$
$[H_o^+] = b$		$[HA]$
$[Cl_o^-] = (a + b) = c$		$a = [A^-] + [HA] = 100$ mmol l^{-1}
volume = ∞		$[H_i^+]$
		$[K_i^+]$
		$[Cl_i^-]$
		volume indefinite

phase boundary
(membrane, cell wall)

$$[Cl_i^-] = \frac{[H_o^+]\ [Cl_o^-]}{[H_i^+]} = \frac{bc}{[H_i^+]}$$

and

$$[K_i^+] = \frac{[K_o^+]\ [H_i^+]}{[H_o^+]} = \frac{a\ [H_i^+]}{b} \qquad (3)$$

For a, b and c, see Diagram 3.
Substitution of Equation 3 in 1 gives:

$$bc + [A^-][H_i^+] - \frac{a[H_i^+]^2}{b} - [H_i^+]^2 = 0 \qquad (4)$$

By definition:

$$K = \frac{[A^-]\,[H_i^+]}{[HA]}$$

or

$$K = \frac{[A^-]\ [H_i^+]}{a - [A^-]}$$

or

$$[A^-] = \frac{K\,a}{[H_i^+] + K} \qquad (5)$$

From Equations 4 and 5:

$$[H_i^+]^3 \left(\frac{a}{b} + 1\right) + [H_i^+]^2 \left(\frac{Ka}{b} + K\right) - [H_i^+]\ (bc + Ka) - Kbc = 0$$

This trinomial equation for $[H_i^+]$ has been solved for fixed values of a and $[K_o^+]$ (100 mmol l^{-1} and 1 mmol l^{-1}, respectively). pK values of the hypothetical acid and pH values of the external solution were varied.

Data in Figure 12 show that lowering the pH of the external root medium from 7 to 2 results in a fast decrease of $[A^-]$ at both pK values of the weak acid HA. At a pH of 2, $[A^-]$ is reduced to almost zero. Consequently, an accumulation of cations within the DFS, as shown at high pH, does not occur at this low pH (Fig. 13). Concentration curves for the anion within the DFS show the reverse; only at external pH values << 3, $[Cl_i^-]$ differs from zero, but this is due to the external $[Cl_o^-]$ increase in the model. For relatively high pK values of HA, data gathered in Experiment 9 agree with theoretical calculations as shown in Figure 14. At pK between 4 and 5, the calculated $[A^-]$ and $[K_i^+]$ will be negligible at pH 2 and high at high pH. This cation adsorption at high pH and the absence of anion accumulation at any pH are in agreement with the experimental data of Experiment 9. At low pH, both cation and anion absorption result from mass flow of solvent and solute. At high external pH, supplementary cation absorption or adsorption comes into play, regulated by physico-chemical forces.

The question whether this pH-dependent initial cation absorption regulates the subsequent stationary potassium influx remains unanswered.

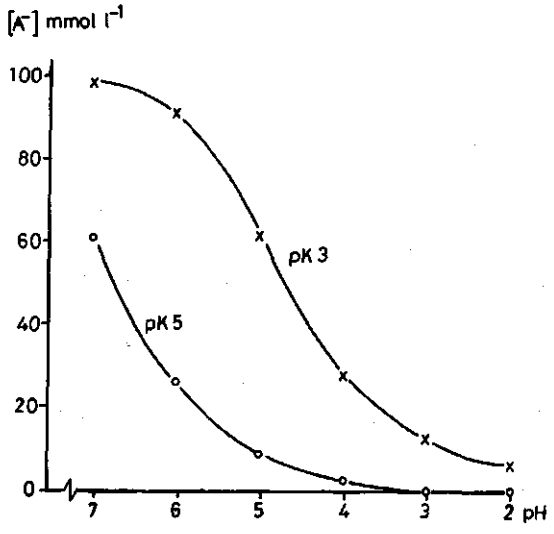

Fig. 12. Diagram 3. Theoretical relation between degree of dissociation (expressed as pK) of the hypothetical indiffusible acid HA and pH of the external solution. Calculations from a Donnan distribution.

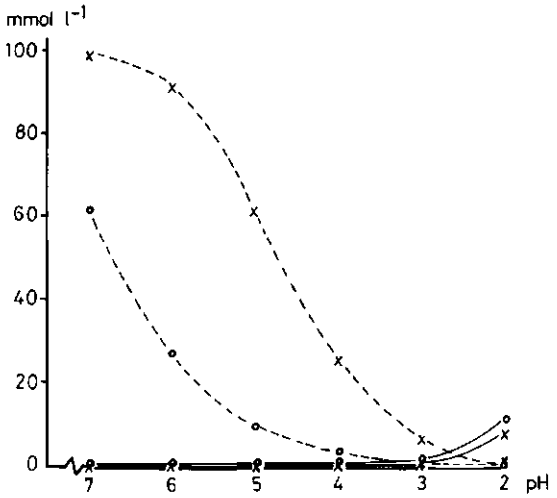

Fig. 13. Diagram 3. Theoretical internal substance concentrations of potassium and chloride at different external pH and 2 degrees of dissociation of the indiffusible acid HA. Calculations from a Donnan distribution. x, pK 3; o, pK 5; broken lines, K; solid lines, Cl.

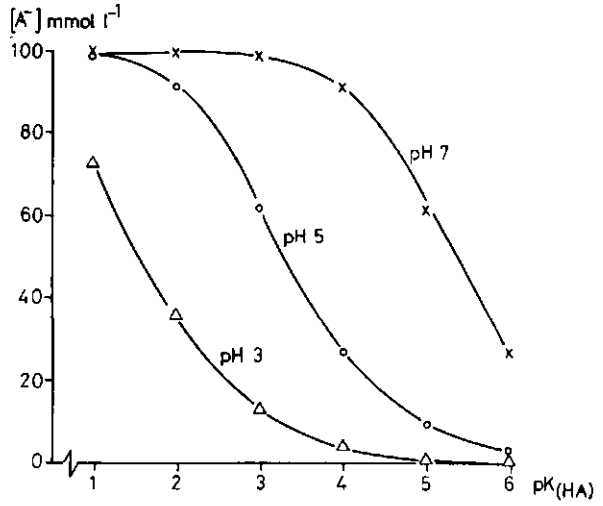

Fig. 14. Diagram 3. Theoretical relation between degree of dissociation and pK of the hypothetical indiffusible acid HA at a low pH (3.0; Δ), medium (5.0; o) and high pH (7.0; x) of the external solution. Calculations from a Donnan distribution.

Remarks

Although data on initial absorption of K and Cl, gathered in Experiment 9, support the existence of a Donnan phase in plant cells,

1. The assumption of the presence of only one acid HA in the Donnan phase will be an over-simplification. Data of several investigators prove the existence of different membrane-bound or cytoplasmic acid compounds, each with its own specific pK value(s). Particularly this mixture of HA compounds, covering a broad range of pK values ensures the presence of negative indiffusible or restrained anions (A^-) over a wide range of external pH. However, the total negative indiffusible or restrained charge will depend on both the amount and quality of the different acids (HA) and the external pH (Fig. 14).

2. Calculations of $[A^-]$, $[K_i^+]$, $[Cl_i^-]$ and $[H_i^+]$ are based on an ideal Donnan distribution

of both cations and anions in plant cells and equilibrium conditions of this Donnan distribution. While accepting the existence of a Donnan phase in plant cells, it still keeps unclear whether real 'equilibrium' will be reached under different external conditions, for instance of pH.
3. In previous calculations the external pH was introduced as an independent variable in the Donnan model, while the composition of the Donnan phase itself will be dependent on or adapted to the external pH. However a strongly buffered and high-electrolyte HA phase is not easily adapted to an unbuffered low-electrolyte absorption solution.
4. At low pH of the external medium, calculated pH of the Donnan phase is low too. Accepting a buffering capacity of a plant cell, this can indicate that
- extremely low pH of the Donnan phase will be reached only at equilibrium and;
- equilibrium is not reached in the short term;
- reaching equilibrium, in the long term, results in extremely low pH of the Donnan phase.
This can support the long-term effect of an extremely low external pH on the ion uptake and growth of plants in general.

5.2.2 Effect of the calcium status and Ca supply to the root

In addition to the nutritional role of calcium for plants (Albrecht, 1968), this cation is involved in the maintenance of the integrity and structure of cell walls and membranes (Steveninck, 1965; Waisel, 1962). According to Marschner & Günther (1964), roots of barley, starving for calcium, show a clear reduction in potassium uptake. Electron micrographs of these calcium-starved root tissues showed cells with a broken tonoplast, a mixture of cytoplasmic and vacuolar sap, and consequently loss of structure of the cell protoplasm. This is in full agreement with findings of Marinos (1962).

In addition, the presence of calcium in the absorption solution was found to promote the uptake of other ions, a phenomenon widely confirmed by Hooymans (1964) and Rains et al. (1964). This effect of calcium supply to roots during absorption experiments is not well understood. Several alternative explanations for the stimulation of potassium absorption add to this confusion. They include blocking of or interfering with the uptake of cations such as H or Li (Jacobson et al. 1960) or increasing the diffusion of K across the outer cell membrane (Waisel, 1962), or a role of a ribonucleoprotein complex containing free SH groups (Tanada, 1962).

To investigate how calcium is involved in the potassium absorption by maize roots, experiments were carried out at different external concentrations of calcium and with roots different in calcium status.

Experiment 10: Potassium influx in excised low salt maize roots from absorption solutions with KCl at concentrations 0.10, 0.25, 0.60, 1.0, 1.5, 2.5, 5.0, 10.0 and 40.0 mmol l^{-1} with and without calcium (½ mmol $CaCl_2$ l^{-1}). Absorption of K was measured over 4 h by accumulation with ^{86}Rb.

The results of this experiment (Fig. 15) demonstrate that potassium influx is differently affected at the various external concentrations of K by 0.5 mmol l^{-1} Ca in

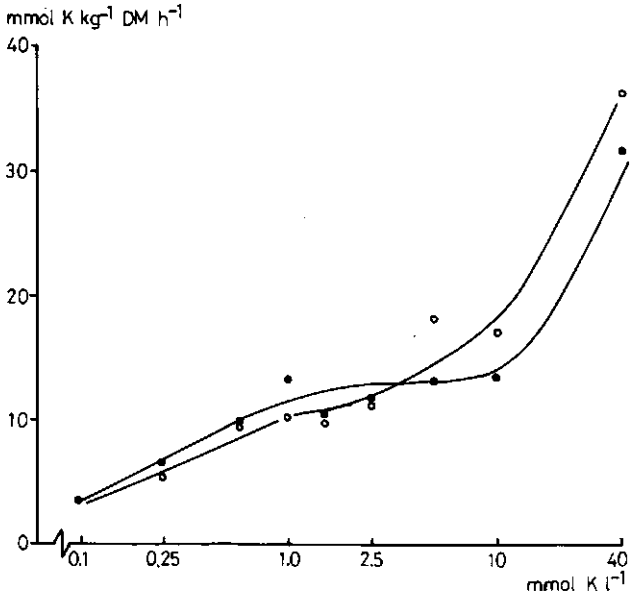

Fig. 15. Experiment 10. Influx of potassium into excised low-salt maize roots with (•) and without (o) $CaCl_2$ at substance concentration 0.5 mmol l^{-1} in the absorption solution. Steady-state influx of K is measured for 4 h.

the absorption solution. Both influx isotherms do not significantly differ for external concentrations of K up to 2.5 mmol l^{-1}. At higher concentrations of K, addition of 0.5 mmol l^{-1} $CaCl_2$ to the absorption solution inhibited influx of K significantly (P = 0.05). This inhibition at high substrate concentrations suggests that calcium comes into play in the concentration range of the isotherm that Epstein (1966) and Cram & Laties (1971) called the low-affinity isotherm or System 2. In view of the postulations by Torii & Laties (1966a), calcium must affect the potassium influx at the second membrane, the tonoplast.

Since a calcium concentration of 0.5 mmol l^{-1} did not significantly affect the potassium influx at an external potassium concentration of 1.0 mmol l^{-1}, in the next experiment the effect of the presence of Ca on the efflux of K was checked too.

Experiment 11: Potassium influx and efflux in excised maize roots low in salt from a 1 mmol l^{-1} KCl solution with and without 0.05 mmol l^{-1} $CaCl_2$. Fluxes were measured by the standard method for 4 h with ^{86}Rb.

Data of both influx and efflux (Table 4; Fig. 16) confirm the absence of any effect of calcium on influx of K; but efflux of K was significantly (P = 0.05) enhanced in the absence of Ca in the experimental solution. In spite of this significant effect of Ca on the efflux of K, the rate of net absorption of K was not affected significantly by Ca. Either Ca inhibited efflux or efflux was stimulated under calcium-free conditions. The latter may indicate that root cells need a constant calcium supply to maintain an optimum function of cellular membranes and walls. Addition of Ca to all absorption solutions proved necessary, as recommended in most of the literature on ion absorption by plant tissues. Calcium can be operative in ion absorption by a stimulated influx as

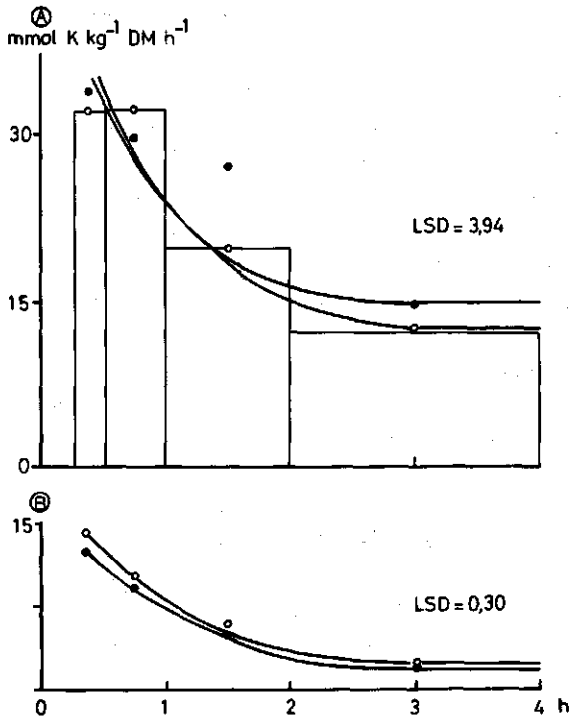

Fig. 16. Experiment 11. Potassium fluxes in excised low-salt maize roots over 4 h with (•) and without (o) $CaCl_2$ at substance concentration 0.5 mmol l^{-1} in the 1 mmol l^{-1} KCl absorption solution. A. Influx. B. Efflux.

Table 4. Effect of the presence or absence of calcium (0.5 mmol l^{-1}) in the absorption solution on influx and efflux of potassium in excised low-salt roots. Absorption solution with 1 mmol l^{-1} KCl. Experiment 11.

Ca - treatment	Steady-state K fluxes (mmol kg^{-1} DM h^{-1})	
	influx	efflux
+ Ca	16.3	2.06
- Ca	13.9	2.38*

well as by an inhibition in efflux.

In how far stimulation of K influx depends on the external concentration of Ca was tested.

Experiment 12: The effect of the external calcium concentration on the potassium influx in excised low salt roots. Influx of K was calculated for 4 h from both depletion and accumulation. Calcium concentrations in the 1 mmol l^{-1} KCl absorption solutions were 0.0, 0.025, 0.05, 0.25, 0.5, 2.5 and 5.0 mmol $CaCl_2$ l^{-1}.

The calcium effects are shown in Figure 17 relative to control without calcium. Statistical analysis proves that calcium concentrations of 0.5, 2.5 and 5.0 mmol l^{-1} enhanced influx significantly ($P = 0.05$), for influx calculated from depletion and from accumulation. Contrary to Hooymans (1964), at external Ca concentrations < 1 mmol l^{-1},

these gypsum roots did not show any significant effect. Although all calcium treatments show a positive effect on the K influx in Figure 17, the highest positive effect was at the highest external concentration of calcium (5 mmol l^{-1}). So, unlike univalent cations, Ca at a high concentration stimulates K influx. Because of the high calcium content of the gypsum roots and the short time of uptake, calcium deficiency is improbable. The hypothesis of a calcium stimulated K influx by way of a calcium stimulated or calcium induced transport carrier or by a substrate calcium complex is more probable.

The essential and irreplaceable role or function(s) of Ca in membranes and walls of different plant cells was demonstrated by several investigators (Bangerth, 1970; Goor, 1968; Steveninck, 1965).

To check the effect of the calcium status of the root on the behaviour, e.g. influx and efflux of potassium, maize plants were grown for different times on a calcium-free medium and then used for flux experiments (Experiment 13).

Experiment 13: (1) Potassium influx in excised roots of plants grown before the influx experiment for 0, 5, 8, 11, 15 and 18 d on a calcium-free solution (demineralized water). Influx measurements by both depletion and accumulation. (2) Potassium desorption or exchange. After the influx period of 4 h, roots were then washed 5 times with fresh aliquots of a 10 mmol l^{-1} unlabelled KCl solution for 5, 5, 5, 15 and 30 min.

With increasing time of starvation of the intact plants, Ca content of roots and shoots decreased (Fig. 18), and steady-state K influx was depressed considerably (Fig. 19). Presumably calcium starvation for at least 5 d significantly reduces influx of K to the root. A relation between the calcium content of the root and K influx in the root, as found in this experiment and presented in Figure 20, may not be quite correct,

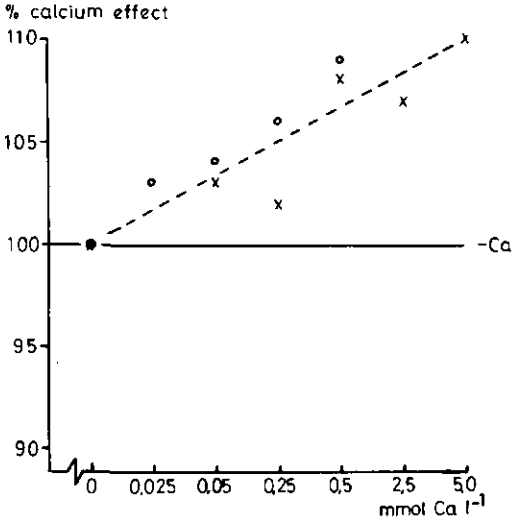

Fig. 17. Experiment 12. Effect of different additions of Ca to the 1 mmol l^{-1} KCl absorption solution on influx of K in excised low-salt maize roots over 4 h. Absorption calculated by depletion (o) and accumulation (x). Minus calcium = 100.

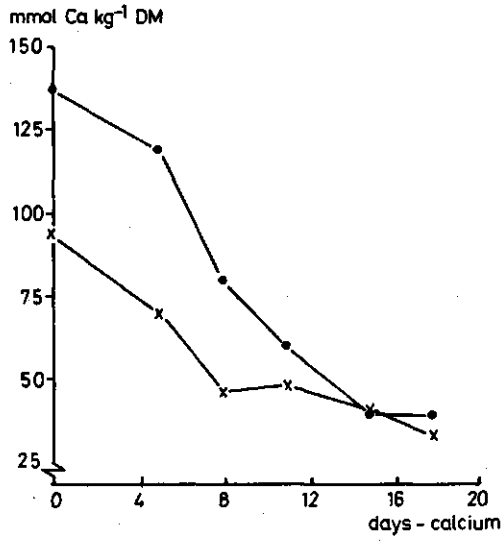

Fig. 18. Experiment 13. Content of calcium in shoots (●) and roots (x) of maize plants grown for different times (0–18 d) on a medium without calcium.

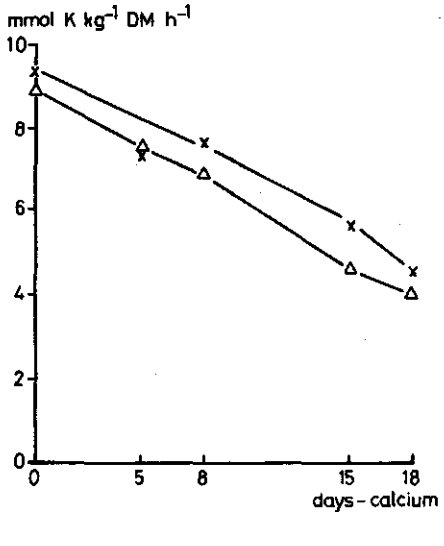

Fig. 19. Experiment 13. Steady-state influx of potassium from a 1 mmol l^{-1} KCl absorption solution in excised low-salt roots of maize grown for different times (0–18 d) before the 4-h absorption experiment on a medium without calcium. Influx was measured by depletion (Δ) and accumulation (x).

because the calcium content used in this figure is a mean value of the whole root tissue. Probably the root tissue includes a Ca-rich part, developed during enrichment, and part formed during starvation that was low in Ca. The latter will have the features of Ca deficiency and be responsible for the deviation or inhibition in influx of K.

Time-curves for influx and desorption of K, presented in Figure 21 for roots grown during periods of 0, 8, and 18 d before influx experiment on a calcium-free solution, show that:

- with increasing time of Ca starvation steady-state influx of K was inhibited significantly;
- with increasing time of starvation, desorption kept almost constant, but the pattern of desorption during exchange for 60 min was different.

Just like influx, the flux (desorption) in roots low in Ca as well as rich in Ca

was fast during the initial phase of the exchange period. The desorption rate of the Ca-rich root material kept higher during the subsequent phase of cytoplasmic efflux. A higher rate of cytoplasmic exchange of Ca-rich roots may indicate that calcium starvation alters the outer cellular membrane, the plasmalemma. An increase in the solute permeability of this membrane may be expressed by fast release or leakage of cytoplasmic solutes, while the efflux of salts by the well-developed cellular membrane with a normal content of Ca is more gradual and higher over a longer time (Macklon & Higinbotham, 1970). Whether calcium starvation also affects structure or permeability of the inner membrane, the tonoplast, is unclear. To get this information, the exchange period should have been continued much longer.

5.2.3 Effect of the internal K status of the root

One would expect, as indeed found by Cram (1973b) and Glass (1975), that influx of ions by roots is negatively correlated with internal concentration of ions. The ion uptake in plants may be regulated by a form of feedback control in which the absorbed ion acts as the regulator of further uptake through its effect upon influx. If so, the dynamics of salt influx must be different for plant roots which have been starved for inorganic ions for varying periods (low salt roots) and for roots which have been grown in solutions rich in mineral ions (high salt roots). In the two-compartment model of plant cells with the plasmalemma and tonoplast placed 'in series' (Cram, 1968; Pitman, 1963), at least two different components of tracer flux into plant tissue will exist: ϕ_{oc}, the inward plasmalemma flux and ϕ_{cv}, a flux between the cytoplasm and vacuole, the tonoplast influx. According to this hypothesis, both fluxes ϕ_{oc} and ϕ_{cv} will depend on the internal cytoplasmic and vacuolar ion concentrations. Consequently, the dynamics of the total flux, a complex flux, will depend on the salt status of the different cell compartments and thus also on the internal salt status of

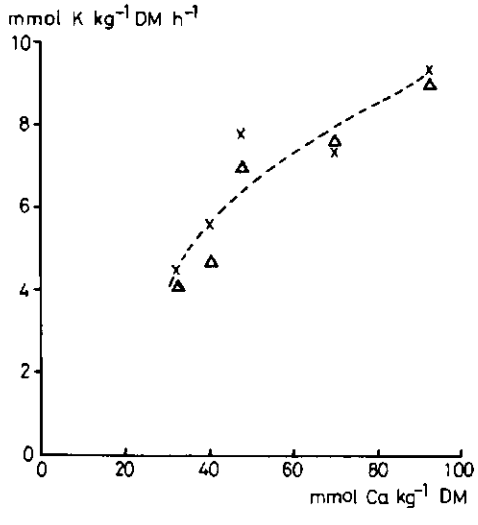

Fig. 20. Experiment 13. Relation between steady-state influx of K over 4 h and content of Ca in excised low-salt maize roots. Absorption solution had KCl at substance concentration 1 mmol l^{-1}. Influx measurement by depletion (Δ) and accumulation (x).

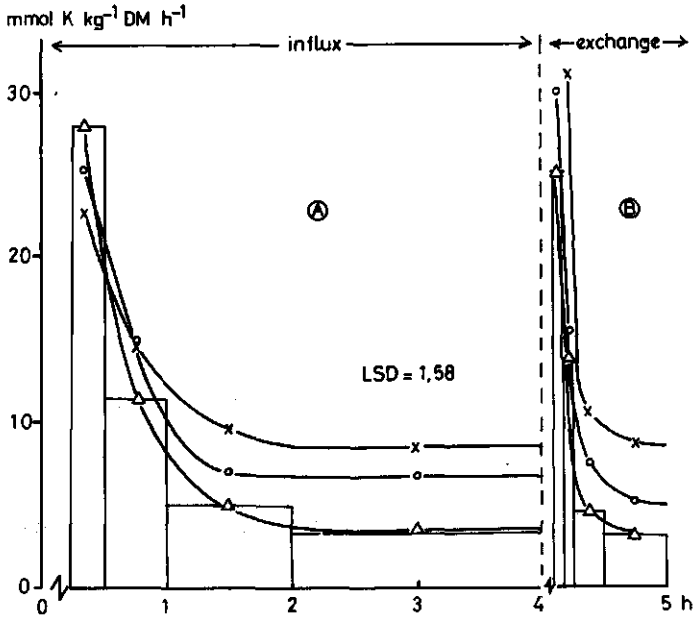

Fig. 21. Experiment 13. A. Influx of K for 4 h in excised low-salt roots of maize grown for 0 d (x), 8 (o) and 18 d (Δ) before the absorption experiment on a medium without calcium. B. Rate of K exchange during a subsequent 1 h period. Exchange medium was a solution 10 mmol l^{-1} unlabelled KCl.

the entire root cell.

To investigate the effect of the internal potassium status of maize roots on the subsequent influx of K, the following experiment was started.

Experiment 14: Potassium influx in excised maize roots in relation to the internal K status of the root. Plants were grown on a complete nutrient solution (Table 1) and transferred 0, 2, 4, 6 and 8 d before the absorption experiment to a fresh identical solution minus potassium. Potassium influx was measured for 4 h by both depletion and accumulation with ^{86}Rb as tracer.

With increasing time of K starvation, the K content in the plant root dropped rapidly, whereas the rate of steady-state influx of K increased almost linearly (Fig. 22). As root cells of plants grown on a potassium-free medium get more and more exhausted and show a low internal concentration of K, $[K_c]/[K_o]$ and $[K_c]/[K_v]$ are leveled down. Especially the latter can be responsible for the enhanced influx of K (Fig. 22).

The relationship between the influx ϕ_{oc} and $[K_i]$, the mean internal cellular potassium concentration, seems to be an exponential one, similar to that observed by Cram (1973b). Plotting ϕ_{oc} against $1/[K_i]$ (Fig. 23) showed that this relationship is linear with a correlation coefficient of 0.98. This supports the suggestion that the influx of K in maize roots is regulated as a negative feedback system. Increasing $[K_i]$ would automatically retard the uptake of K. Whether an additional feedback system is at work, and how the regulator signal is then perceived and translated to control

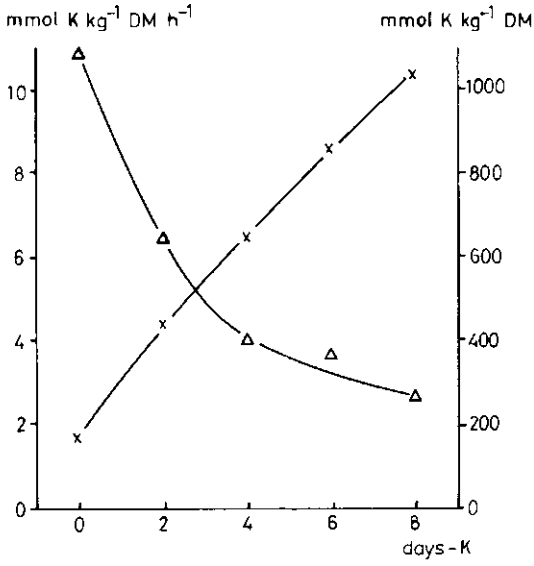

Fig. 22. Experiment 14. Steady-state influx of K over 4 h (x) and substance content (Δ) of K in excised roots of maize plants grown for 0-8 d before the absorption experiment on a medium without potassium. Absorption solution had KCl at substance concentration 1 mmol l^{-1}.

influx is an open question. The place in the cell (membrane, compartment) where the system should be operative is also far from understood.

Another phenomenon observed during this experiment was the effect of the K content of the root on the absorption of K during the initial phase. In Figure 24, influx curves have been presented for both the depletion and accumulation method. These curves show that the fraction of potassium accumulated during the 4 h absorption in the AFS and desorbed during the 1 h exchange was significantly lower at 0 d in the treatment without K than if K had been withheld for 2 or more days. This demonstrates that 2 or more days of K starvation significantly reduce the fraction of K, accumulated or adsorbed in the AFS.

Thus, after K starvation for 2 d or longer, initial and steady-state K influx are increased during a subsequent period of at least 4 h; steady-state K influx seemed to be inversely correlated with internal K concentration.

5.2.4 Effect of a treatment with various inorganic and organic salt solutions

This section treats effects of treatment of low salt roots with different salts on the subsequent salt uptake. In general, a transfer of low salt plants to a high salt medium will increase the salt status of the plant and change the subsequent uptake pattern of ions in general or particular. For example, some sort of a direct coupling or feedback system seemed to exist between K status of the root and influx of K. In the tests here described, salts (inorganic or organic) were used that do not increase the internal cellular salt status per se but allowed the following three phenomena to be studied:

1. Anion effect. As reported by Epstein and Hagen (1952) and Hiatt (1967), the uptake of cations by the low-concentration mechanism (System 1) was indifferent to the nature of

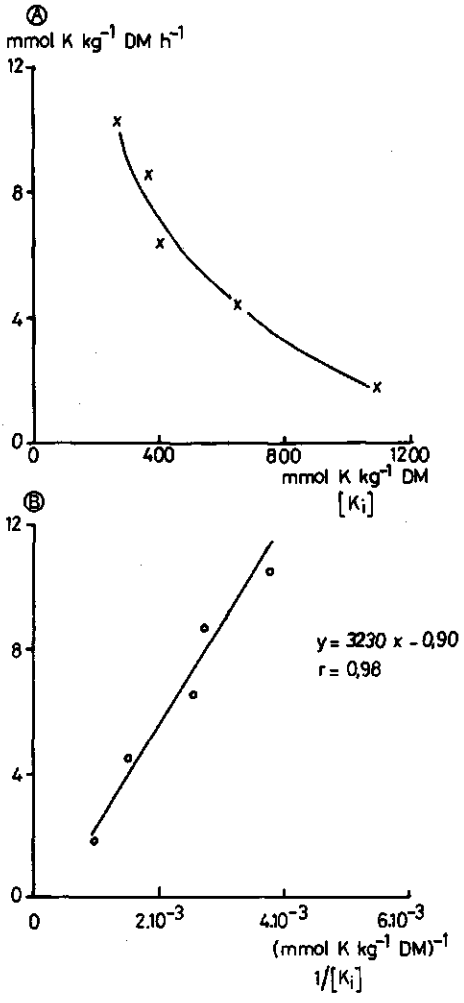

Fig. 23. Experiment 14. Relationship with steady-state influx of K over 4 h. A. Substance content of K in roots ($[K_i]$). B. reciprocal value of K content of roots ($1/[K_i]$).

the anion, while uptake by the high-concentration mechanism (System 2) was markedly influenced by the anion. So at external salt concentrations exceeding 1 mmol l^{-1}, K influx will depend on the identity of the anion. According to Hiatt (1968), an ion entering the root by this mechanism must be accompanied by an ion of opposite charge and consequently uptake of K from high concentrations of K_2SO_4 will be retarded because of the relatively slow rate of SO_4 uptake. Hiatt (1968) suggested diffusion to be the high-concentration mechanism of ion uptake. If so, SO_4 inhibits the absorption rate of the counterion (K) and this cation/anion-interaction is only operative at high external salt concentrations. An inhibited anion influx would then decrease the influx of the cation too, but only at high concentrations. To check this hypothesis, roots were enriched with Ca and different anions. Subsequently, influx of K was measured from solutions containing K with different anions.

2. Carboxylate effect. Evidence has been presented that electrostatic binding of cations by organic anions is involved in ion accumulation by certain unicellular organisms

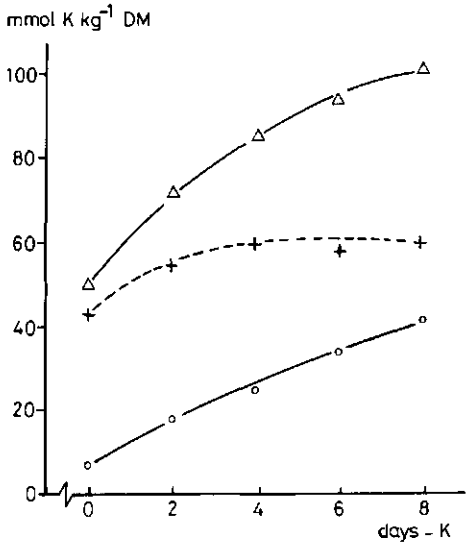

Fig. 24. Experiment 14. Potassium absorption in excised maize roots, estimated by depletion (Δ) and accumulation (o), and potassium present in the apparent free space (+). Before the 4 h absorption experiment, plants were grown for 0-8 d on a medium without potassium. Absorption solution had a substance concentration of KCl 1.0 mmol l^{-1}.

(Legget et al., 1965). Breteler (1975) and DeKock et al. (1973) showed also that rate of ion absorption (NH_4, K, NO_3) by the higher plant can be affected by the internal concentration of organic anions or carboxylates in the root. Intact plants were grown on different calcium salts and different internal concentrations of carboxylate were created by modifying ion uptake and assimilation (Torii & Laties, 1966b). When roots of higher plants absorb cations without concurrent anion absorption, the cations exchange for H^+ from the roots and equivalent quantities of organic acids are sythesized (Hiatt, 1967; Jacobson & Ordin, 1954). Assimilation of nitrate and sulphate is also a process that generates organic acids (Dijkshoorn, 1962; Higinbotham & Pierce, 1974).

3. Amino acid/protein effect. As reported before, NO_3 nutrition of the plant will enhance production of carboxylates by reduction. In the first place, N nutrition in general will result in an increased content of organic N as amides, amino acids and proteins. According to Hiatt (1968), these amino acids and proteins play an important role in ion absorption by plant roots. If Donnan distributions are involved in ion absorption, the total amount of immobile anions in the cytoplasm will be of influence. Proteins could be involved in ion transport in a more specific way. An enhanced protein synthesis probably increases availability of sites binding K or carriers. Apart from this function of proteins as carriers, these organic compounds are involved in the structure of cellular membranes. According to the fluid-mosaic membrane model, postulated by Singer & Nicolson (1972), globular proteins are partially embedded in the membrane, penetrating into the lipid phase from either side, while others are completely buried in it. Types or numbers of specific proteins on each surface of the asymmetric membrane will contribute to the features of the membrane.

In addition to these three specific effects, the changes brought about by the various salt solutions may be both structural and functional. An increase in internal salt concentration will influence structure of the protoplasmic layers, viscosity and hydration of the cytoplasm and hence metabolism.

Experiment 15: Potassium influx in excised maize roots after rearing plants on gypsum and 96 h on a 5 mmol l^{-1} solution of $Ca(NO_3)_2$, $CaCl_2$ or $CaSO_4$. Potassium influx from a 1 mmol l^{-1} solution of KCl, $\frac{1}{2}K_2SO_4$ or KNO_3 was measured over 4 h by depletion with ^{86}Rb as tracer.

The results of Experiment 15 (Fig. 25) indicate that in roots enriched with a certain anion there was no significant decline in influx of K with an anion identical to the one used during enrichment. This is true for all three anions Cl, SO_4 and NO_3. Although plants enriched with $CaCl_2$ and $CaSO_4$ did not significantly differ in uptake of K, irrespective of the nature of the accompanying anion, enrichment with $Ca(NO_3)_2$ significantly increased influx of K for all three anions. Obviously, a reduced anion influx does not negatively affect influx of the cation, at least at a concentration of the absorption solution of 1 mmol l^{-1}.

To check whether plant roots enriched with a certain anion really inhibited influx of the same anion, the next experiment was set up.

Experiment 16: Potassium and chloride influx in excised maize roots after enrichment as in Experiment 15. Influx of K and Cl from a 1 mmol l^{-1} KCl solution was measured over 4 h by depletion with ^{86}Rb and ^{36}Cl as tracers for K and Cl.

Figure 26 proves the influx of Cl to be depressed significantly after treatment of the plant with $CaCl_2$. This indicates that, as expected, enrichment of the root with Cl reduced subsequent Cl influx. This experiment indicates also that treatment with $Ca(NO_3)_2$ increased influx of Cl during the influx experiment as well as influx of K. Probably a treatment of the intact gypsum maize plant by a $Ca(NO_3)_2$ solution for 96 h stimulates the growth of the plant more than treatments with $CaCl_2$ or $CaSO_4$ solutions. The development of new and more active root material during the $Ca(NO_3)_2$ treatment can probably result in a higher uptake capacity of the root for both cations and anions.

Because anion influx is not expected to affect the influx of the cation at external

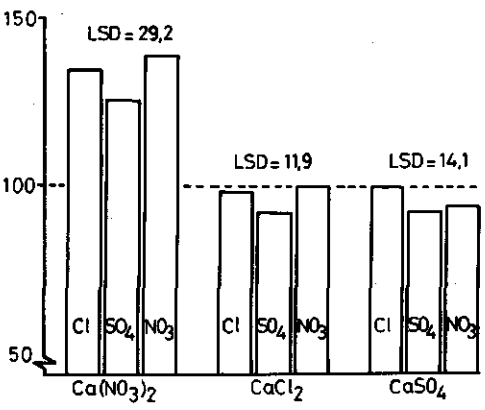

Fig. 25. Experiment 15. Uptake of potassium over 4 h into roots of maize plants after treating intact plants for 96 h on solutions with $Ca(NO_3)_2$, $CaCl_2$ or $CaSO_4$ 5 mmol l^{-1}. Subsequent uptake from a solution of KCl, $\frac{1}{2}K_2SO_4$ and KNO_3 1 mmol l^{-1}. Potassium uptake from solution of KCl by $CaSO_4$ treated roots taken as reference (100).

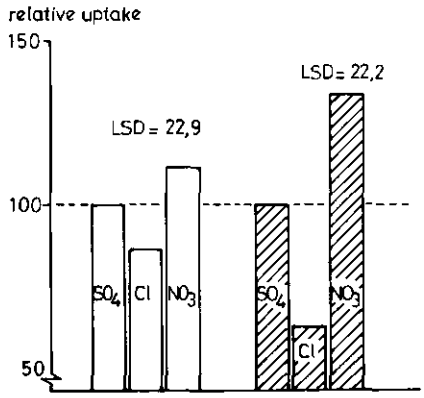

Fig. 26. Experiment 16. Uptake of potassium (□) and chloride (▨) in excised roots of maize plants after treatment of intact plants for 96 h on solutions of $Ca(NO_3)_2$, $CaCl_2$ and $CaSO_4$ 5 mmol l^{-1}. Absorption solution was a solution 1 mmol l^{-1} KCl. Uptake of K and Cl by roots treated with $CaSO_4$ taken as reference (100).

K concentrations below 1 mmol l^{-1} (System 1), potassium influx was measured also at a higher concentration of the absorption solution (System 2).

Experiment 17: Potassium influx in excised maize roots after a treatment as in Experiment 15. Influx of K from a 20 mmol l^{-1} KCl solution was measured over 4 h by accumulation with ^{86}Rb.

Data of K uptake (Table 5), show that:

- At high external salt concentrations, influx of K was markedly affected by the uptake rate of the counterion. An enrichment with Cl (Table 6), depressed influx of Cl, and reduced influx of K.
- A treatment with $Ca(NO_3)_2$ did not enhance influx of K at high external salt concentrations. This is in contrast to the observations with low salt concentrations in the absorption solution.

Experiments 15-17 show that contrary to low external salt concentrations, high external concentrations tend to make cation uptake significantly dependent on the uptake of anion. This confirms the idea of Epstein and Hagen (1952) and Hiatt (1968) of the existence of at least a dual isotherm of cation uptake; an anion-independent one for low external concentrations and an anion-dependent one for high external concentrations of K. The existence of a multiphase model of the uptake isotherm, as postulated by Nissen (1973), can be neither disproved, nor confirmed by these experiments.

In the preceding experiments, the anion effect may be mixed up with both a carboxylate and a protein/amino acid effect. Therefore, in the next experiments not only more, but also pure carboxylate and nitrogen effects are introduced.

Experiment 18: Influx of potassium in excised maize roots after a treatment of the intact gypsum plants for different times on a 5 mmol l^{-1} solution of $CaSO_4$, $Ca(NO_3)_2$, $(NH_4)_2SO_4$ or urea. Influx of K from a 1 mmol l^{-1} solution of KCl was measured over 4 h by depletion with ^{86}Rb.

Table 5. Relative uptake of K in excised maize roots after treatment of intact plants for 96 h on different enrichment solutions. Absorption medium is a 20 mmol l^{-1} KCl solution. Absorption time is 4 h; $CaSO_4$ treatment as control. Experiment 17.

Enrichment solution substance	(mmol l^{-1})	Relative K uptake
$CaCl_2$	5	70**
$Ca(NO_3)_2$	5	98
$CaSO_4$	5	100

Table 6. Content Cl, Na, SO_4 and N_{org} in maize roots after treatment of the intact plants for 96 h on different enrichment solutions. Experiment 17.

Enrichment solution substance	(mmol l^{-1})	Content (mmol kg^{-1} DM)			
		Cl	NO_3	SO_4	N_{org}
$CaCl_2$	5	382	17	48	702
$CaNO_3)_2$	5	54	405	62	1230
$CaSO_4$	5	82	7	104	813

Table 7. Relative K uptake in excised maize roots after treatment of intact plants for 96 h on different enrichment solutions. Absorption medium is 1 mmol l^{-1} KCl solution. Absorption time is 4 h. $CaSO_4$ treatment as control. Experiment 18.

Enrichment solution substance	(mmol l^{-1})	Relative K uptake
$CaSO_4$	5	100
$Ca(NO_3)_2$	5	104
$(NH_4)_2SO_4$	5	88*
Urea	5	85*

Table 7 presents potassium uptake for roots treated on different containing nitrogen solutions with a $CaSO_4$ solution as control. If the stimulating effect of $Ca(NO_3)_2$, as shown in Experiments 15 and 16, is a pure nitrogen effect, and acts by enhancement of internal cellular protein or amino acid content, treatment with N compounds such as NH_4 and urea should stimulate influx of K. However, influx data in Table 7 disprove this assumption. After a treatment of the plants with NH_4 or urea, influx of K was significantly less than with $CaSO_4$ of $Ca(NO_3)_2$. The positive effect of $Ca(NO_3)_2$ cannot be a nitrogen effect in general but a carboxylate effect induced by a considerable assimilation of NO_3 (reduction), or else a positive effect of nitrogen is exceeded by a negative effect of NH_4. Figure 27 demonstrates the effect of the NO_3 and the NH_4 treatment with different times of treatment or incubation. The NO_3 and NH_4 curves differ almost immediately after the beginning of treatment, while after about 150 h no further divergence takes place.

A further resolution of the different effects on influx of K was attempted in the next experiment.

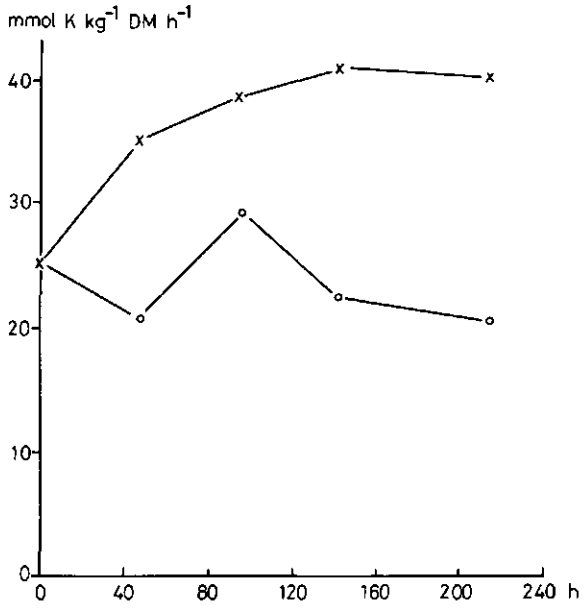

Fig. 27. Experiment 18. Potassium absorption into excised roots of maize plants over 4 h after a treatment of intact plants for different times (0-220 h) on solutions of $Ca(NO_3)_2$ (x) and $(NH_4)_2SO_4$ (o) 5 mmol l^{-1}. Absorption solution was a solution 1 mmol l^{-1} KCl.

Experiment 19: Potassium influx in excised maize roots after treating the intact gypsum plants for 96 h on solutions with substance concentration of glutamic acid 10, aspartic acid 10, succinic acid 10, $CaSO_4$ 5 and $Ca(NO_3)_2$ 5 mmol l^{-1}. The first three solutions were adjusted to pH 5.5 with NaOH; to other solutions, NaCl was added to obtain equal concentrations of Na. Influx of K in excised roots was measured as in Experiment 18.

The results (Table 8) indicate that succinate did not significantly alter influx of K compared with the $CaSO_4$ as control. Similarly, Breteler (1975) and Dijkshoorn (1973) found no positive or even negative effects of succinate on uptake of NH_4 or K by excised maize roots grown on a $CaSO_4$ medium. This experiment confirms the lack of effect of succinate with low salt roots. The reason for this ineffectiveness may be the higher internal content of carbohydrates in these roots than in high salt roots. Treatment with amino acids gives a similar increase in K influx to a treatment with a $Ca(NO_3)_2$ solution. Thus, unlike treatments with succinate, NH_4 or urea, incubation with amino acids stimulated influx. This leads to the following conclusions:

1. The absence of any succinate effect on influx of K proves the positive $Ca(NO_3)_2$ effect not to be the result of an increased carboxylate content.
2. Since treatment with one of the amino acids aspartic acid or glutamic acid affected subsequent K influx positively, it is probable that
- amino acids and proteins affect the uptake capacity of the roots directly;
- nitrate stimulates influx of K by enhanced production of amino acids or proteins.
3. The negative effect of NH_4 or urea on influx of K can be explained by the role of NH_4 as inhibitor on the subsequent influx of K (cation competition). This inhibition must exceed the positive effect of a stimulated amino acid or protein production as suggested

Table 8. Relative K uptake in excised maize roots after treatment of the intact plants for 96 h on different solutions. Solutions with amino acids and organic acids were brought to pH 5.5 with NaOH; to $CaSO_4$ and $Ca(NO_3)_2$ solutions equivalent amounts of sodium were added as NaCl. Absorption solution is a 1.0 mmol l^{-1} KCl solution ($CaSO_4$ treatment as control).

Treatment solution		Relative K uptake
substance	(mmol l^{-1})	
glut. acid	10	116*
aspar. acid	10	114*
succ. acid	10	101
$CaSO_4$	5	100
$Ca(NO_3)_2$	5	119*

before.

4. The fact that the positive effect of NO_3 was found only at low external concentrations of K suggests that this influence is active only at low external concentrations (System 1).

5.2.5 *Surface-active chemicals and ion uptake*

As reported by Kuiper (1967) and Newman & Kramer (1966), the permeability of cellular membranes can be modified by surface-active organic chemicals. According to their findings, permeability of cellular membranes increased for solvent as well as for solutes after treatment of plant tissue with certain concentrations of these chemicals. Since the permeability of a membrane governs uptake of single ions and relative uptake of ions (ion selectivity), it sometimes may be useful to alter the permeability features of a membrane (permeability coefficient *P*) to increase flux of water and salts within the tissue. As demonstrated by Kuiper (1967), organic compounds with a hydrocarbon chain, carbon amides of decenylsuccinic acid, acetylated compounds and certain fluorinated compounds increase the electrolyte permeability of plant roots significantly. However an increase in permeability of plant roots by these organic chemicals, measured by an enhanced electrolyte efflux or leakage, does not necessarily stimulate inward flux of salts. A changed permeability of root systems to water and salts will probably result in a positive net inward salt flux if the chemical does not seriously injure tissue, and only alters the structure or configuration of the membrane. According to Ariens & Simonis (1976), this can be caused by

- changes in permeability of pores (size, polarity)
- changes in permeability of the lipid double-layer. Incorporation of the molecule of the organic chemical into the lipid layer of the membrane or dissolution of hydrophobic or hydrophilic parts of the lipid double layer can probably alter the permeability of the membrane for polar and apolar compounds.

The behaviour of one of the effective acetylated compounds glyceryl triacetate (Kuiper, 1967) will be examined. To check whether this surface-active chemical affects influx or efflux of the different ions and whether it alters the selectivity of the membrane for different ions too, a number of flux experiments were set up.

Experiment 20: Potassium influx in excised maize roots low in salt after a treatment of the excised roots for 12 h in $CaCl_2$ solutions with concentrations of 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} mol l^{-1} glyceryl triacetate (triacetin). Influx of K from a 1 mmol l^{-1} KCl solution was measured for 6 h by depletion and accumulation with ^{86}Rb.

With $CaCl_2$ and triacetin for 12 h, there was a significant effect of the concentration of the surface-active chemical on the increase in electrical conductivity of the incubation solution (Fig. 28). At concentrations of triacetin greater than 10^{-4} mol l^{-1}, conductivity increased markedly, but at concentrations greater than 10^{-2} mol l^{-1}, there was no further increase in conductivity. Influx of K decreased after increasing concentration of triacetin (Fig. 29). Especially K accumulated analogously to conductivity of the incubation solution (Fig. 28). At concentrations exceeding 10^{-4} mol l^{-1}, triacetin reduced accumulation of K drastically down to nearly nil at a concentration of 10^{-2} mol l^{-1}. Influx of K calculated from depletion dropped with increasing concentration of triacetin, but maintained a significantly higher value than indicated by accumulation. Figure 29 corresponds with the time courses of influx presented in Figure 30, which indicates that only the control and treatment at 10^{-5} mol l^{-1} had a normal influx, but higher concentrations of triacetin differed. After rapid initial influx of K, absorption declined very fast and even reversed, so that after incubation of the excised roots in triacetin solutions with concentrations exceeding 10^{-4} mol l^{-1}, roots were 'filled up' with potassium during the initial phase of the subsequent K absorption experiment. However, during the next phase of absorption, no net accumulation but a net efflux or ion excretion occurred. Obviously, after an incubation in concentrated triacetin solutions, membrane permeability changed in such a way that salt could not accumulate. After

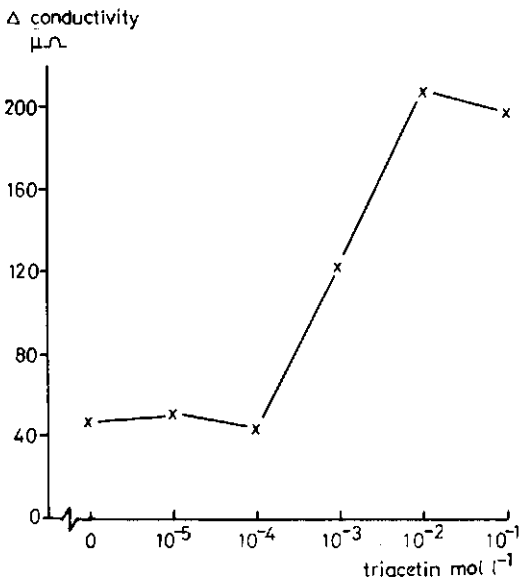

Fig. 28. Experiment 20. Increase in conductivity (ΔμΩ) of the incubation solution after 12 h incubation of excised low-salt maize roots. Incubation solutions were solutions 0.5 mmol l^{-1} $CaCl_2$ with different triacetin concentrations.

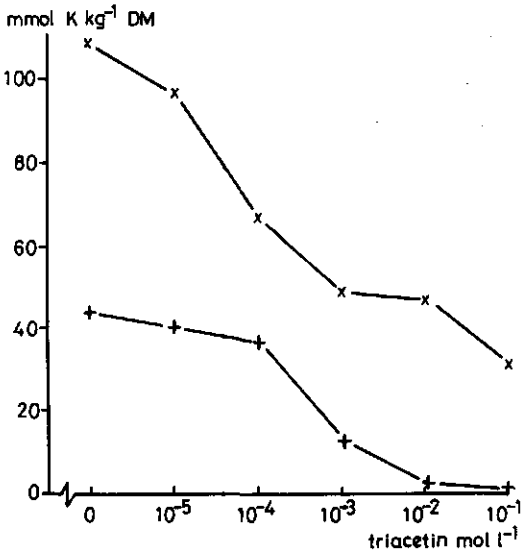

Fig. 29. Experiment 20. Potassium absorption in excised low-salt maize roots after incubation for 12 h in a solution 0.5 mmol l^{-1} $CaCl_2$ with different concentrations of triacetin. Absorption solution had KCl at substance concentration 1 mmol l^{-1}. Absorption was calculated from depletion (x) and accumulation (+).

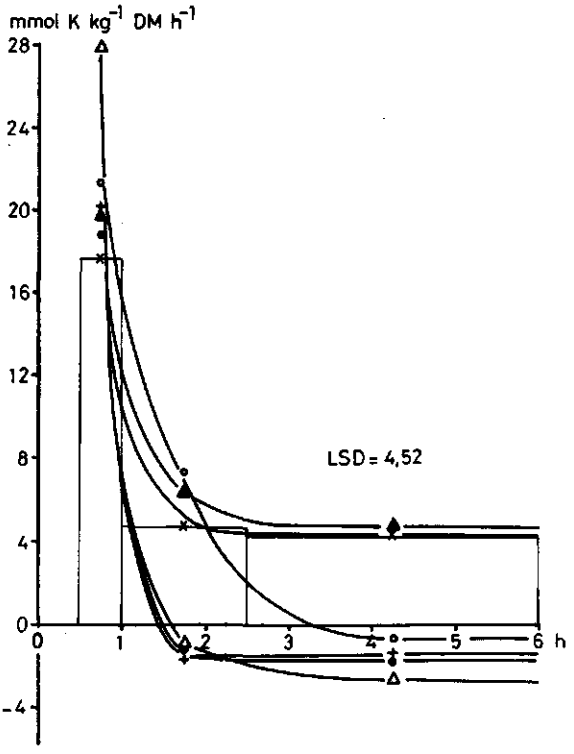

Fig. 30. Experiment 20. Potassium influx in excised low-salt maize roots for 6 h. Before the absorption experiment, intact maize plants were treated on a $CaCl_2$ solution 0.5 mmol l^{-1} with triacetin at substance concentrations 0 (x), 10^{-5} (▲), 10^{-4} (o), 10^{-3} (△), 10^{-2} (●) and 10^{-1} (+) mol l^{-1}. Absorption solution had KCl at 1 mmol l^{-1}.

incubation in triacetin, membrane permeability allowed salts already present in the root to leak out much easier and faster; an enhanced influx of K after treatment with the surface-active chemical is out of the question.

The effect of incubation time was investigated in the next experiment.

Experiment 21: Potassium influx in excised maize roots low in salt after a treatment of the excised roots in a 10^{-2} mol l^{-1} triacetin solution for 0, 1, 3, 5 and 12 h. Influx of K was measured also with untreated roots, but with 10^{-2} mol l^{-1} triacetin in the absorption solution. Influx of K was measured from a 1 mmol l^{-1} KCl solution over 6 h by depletion and accumulation.

Figure 31 confirms the effect of this chemical on the permeability of the root cell membranes. After treatment for 1 h, electrical conductivity of the incubation solution increased significantly over the control in a 0.05 mmol l^{-1} $CaSO_4$ solution. The curves also demonstrated that during 12 h the root cell membranes became more and more permeable to salts.

Increasing incubation time caused potassium influx to fall (Fig. 32), as shown both by depletion and accumulation. Obviously, with increasing time of triacetin treatment, root cell membranes become more permeable; however, this increase in permeability facilitates efflux or excretion of salts but not influx of salts. Consequently, the net uptake or accumulation of salts by the root slows down with increasing time of treatment of roots in a 10^{-2} mol l^{-1} triacetin solution.

Contrary to this picture, the absorption of K by roots without triacetin treatment, but with 10^{-2} mol l^{-1} triacetin added during influx caused a significant increase.

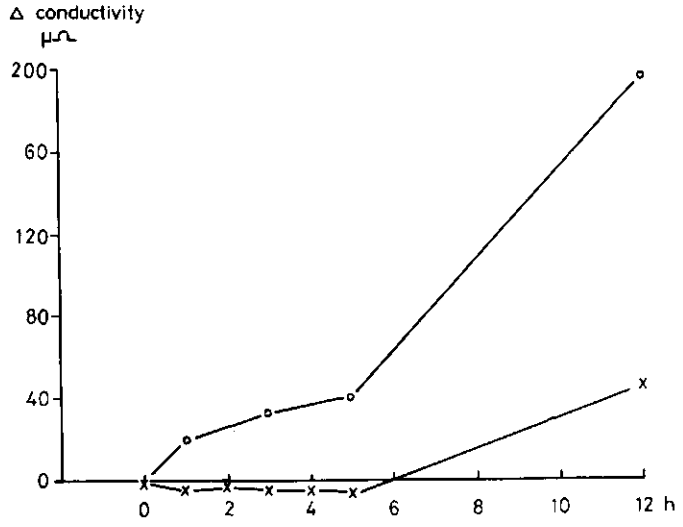

Fig. 31. Experiment 21. Change in conductivity (ΔμΩ) of incubation solutions by excised low-salt maize roots during an incubation period of 12 h. Incubation solutions had $CaCl_2$ at 0.5 mmol l^{-1} with (o) and without (x) triacetin at 10^{-2} mol l^{-1}.

Accumulation of K was roughly doubled (Fig. 32). Probably the surface-active chemical is directly involved in uptake by an increased influx, a decreased efflux or both.

To check whether this triacetin in the absorption solution is operative by influx or efflux, the next experiment was set up.

Experiment 22: Potassium influx and efflux in excised low salt roots over 4 h. Experimental solutions were 1 mmol l^{-1} KCl solutions with and without 10^{-2} mol l^{-1} triacetin. Influx and efflux were estimated by the standard method with ^{86}Rb.

The results of this experiment confirm the suggestion, that the glyceryl triacetate is active by both fluxes. An enhanced influx together with a diminished efflux finally stimulated K absorption (Table 9). This experiment also confirmed that surface-active chemical affects net absorption of K positively only if the chemical is present in the absorption solution. Perhaps the surface-active chemical triacetin is active by a direct coupling of potassium and triacetin molecule to a complex permeating more readily through the membrane than K alone, or perhaps it is built into the lipid double-layer of the root cell membranes. Washing of the roots after triacetin treatment and before flux measurement can remove the organic molecules from the membrane and inhibit the positive triacetin effect or even enhance the permeability or leakage of the cell membrane.

Since permeability of cellular membranes differs for different ions, the effect of a triacetin treatment was investigated also in relation to ion selectivity. If

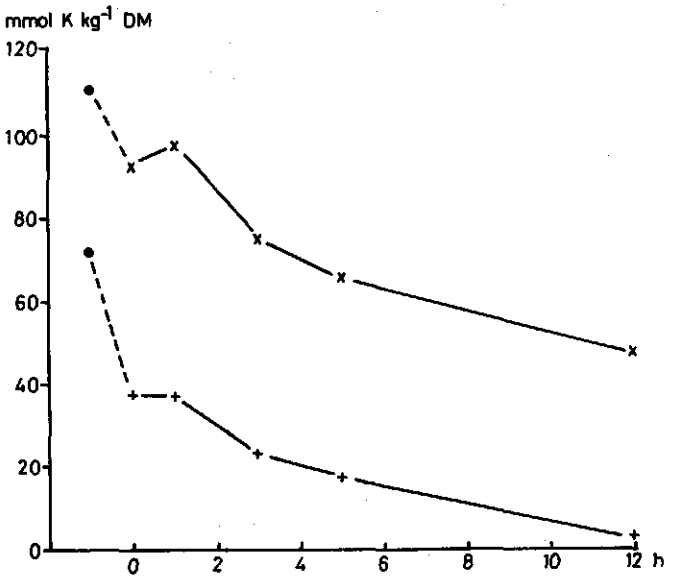

Fig. 32. Experiment 21. Potassium absorption in excised low-salt maize roots after incubation for different times in a solution of 10^{-2} mol l^{-1} triacetin. Absorption during the subsequent 6 h from solutions 1 mmol l^{-1} KCl was estimated from accumulation (+) and depletion (x). Solid circles at the end of the dotted lines represent absorption data of roots not treated with triacetin, but with triacetin at 10^{-2} mol l^{-1} in the absorption solution 1 mmol l^{-1} KCl.

Table 9. Steady-state influx, efflux and net absorption of K in excised maize roots low in salt for 4 h. Absorption medium is a 1 mmol l^{-1} KCl solution without (control) and with 10^{-2} mol l^{-1} triacetin. Experiment 22.

Treatment	Steady-state K fluxes (mmol kg^{-1} DM h^{-1})		
	influx	efflux	net absorption
control	19.19	3.11	16.08
triacetin 10^{-2} mol l^{-1}	30.29**	0.48**	29.81**

triacetin alters the permeability of the root cell membranes, the changes may differ for different ions.

The effect of triacetin on the membrane flux of the ions K, Ca, Na and Cl was studied in the next experiment.

Experiment 23: Influx and efflux of K, Ca, Na and Cl in excised low salt maize roots for 4 h. Absorption solutions were 1 mmol l^{-1} solutions of KCl, $\frac{1}{2}CaCl_2$ and NaCl with and without 10^{-2} mol l^{-1} triacetin. Flux was measured by the standard method with ^{86}Rb, ^{45}Ca, ^{22}Na and ^{36}Cl as tracers for K, Ca, Na and Cl, respectively.

In Figure 33, the influx and efflux data relative to controls (without triacetin) are presented for the four ions. The effect of triacetin on ion flux was totally different for the four ionic species. Only the influx of potassium was stimulated by triacetin, and influx of Ca, Na and Cl was inhibited. Also the effect of triacetin on efflux was totally different. Again, triacetin affects efflux of potassium negatively, whereas efflux of other ions was enhanced by this organic chemical. Consequently addition of this surface-active chemical to the absorption solution stimulated net uptake of K and inhibited net uptake of Ca, Na and Cl.

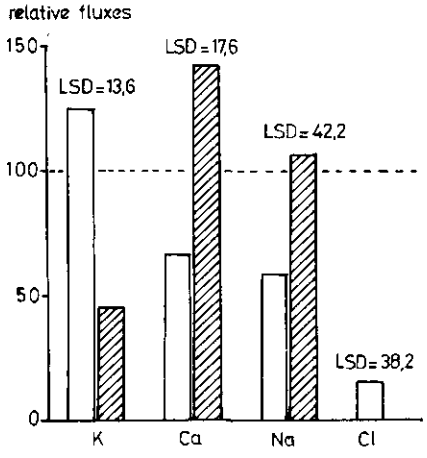

Fig. 33. Experiment 23. Influx (□) and efflux (▨) over 4 h of K, Ca, Na and Cl in excised low-salt maize roots with and without triacetin at 10^{-2} mol l^{-1} in absorption solutions with KCl, $\frac{1}{2}CaCl_2$, NaCl or KCl at 1 mmol l^{-1}. Fluxes for control treatments (minus triacetin) were 100.

5.2.6 Metabolic inhibitors and potassium uptake

Cyanide (CN) and Dinitrophenol (DNP), inhibitor of respiratory electron flow and uncoupler of oxidative phosphorylation, respectively, were used as metabolic inhibitors on potassium fluxes by maize roots. According to Lundegårdh & Burström (1933), Lüttge & Laties (1966) and Robertson (1968) both inhibitors reduce the uptake of ions from the medium and their transfer to the vessels. 2,4-Dinitrophenol was found to uncouple phosphorylation from oxidative respiration and reduce salt uptake, whereas electron transfer through the cytochrome chain was not inhibited and oxygen uptake was increased (Robertson et al., 1951). Perhaps salt uptake is dependent on ATP formation or else both active transport and ATP formation depend on a common process which is inhibited by DNP (Robertson, 1968). CN, on the other side, inhibits or blocks electron transport, at the same time reducing ion uptake and transport.

Although the working mechanism of the two inhibitors is quite well understood, it certainly is not always easy to distinguish between the effect of inhibitors on specific metabolic processes and undesirable or unknown side-effects, such as the alteration of the membrane potential and membrane permeability. Higinbotham et al. (1970) and Pierce & Higinbotham (1970) found a fast decrease in the membrane potential of oat coleoptiles in the presence of cyanide at a concentration of 1 mmol l^{-1}. A similar rapid depolarization of the membrane potential appears to occur in various algae, fungi and bacteria (Slayman, 1974). Studying the membrane permeability, Marschner et al. (1966) reported that K was lost from maize root sections much more under unaerobic conditions than with normal aeration. Electron microscopy indicated that the cytoplasm of the anaerobic roots was deranged and much less dense than in roots receiving air. In general, aerobic metabolism appears to be required to maintain the integrity of cells.

The role was investigated of metabolic inhibitors in influx and simultaneous efflux of potassium in maize roots, in order to understand their effect on the permeability of cellular membranes. First the effect of concentration of the inhibitor DNP was tested.

Experiment 24: Potassium influx in excised low salt maize roots from an absorption solution, containing 1 mmol l^{-1} KCl in addition to 0.0, 10^{-5}, 5.10^{-5}, 10^{-4}, or 10^{-3} mol l^{-1} DNP. The experiment lasted 4 h; ^{86}Rb was used as a tracer for K. (1) K influx was calculated for all DNP concentrations from accumulation. (2) Influx of K was plotted against time for DNP at 10^{-4} mol l^{-1} by the depletion method.

Potassium influx (Fig. 34) demonstrated the DNP effect clearly. With increasing DNP concentration in the absorption solution, K influx dropped fast to a low level. At a DNP concentration of 10^{-4} mol l^{-1}, potassium influx was reduced to about 10% of the control. The absorption curve (Fig. 35) for a 10^{-4} mol l^{-1} DNP concentration, demonstrates that at this concentration the inhibitor starts depressing K influx within 15-30 min after starting the experiment.

The effect of DNP treatment on the potassium isotherm was studied in Experiment 25.

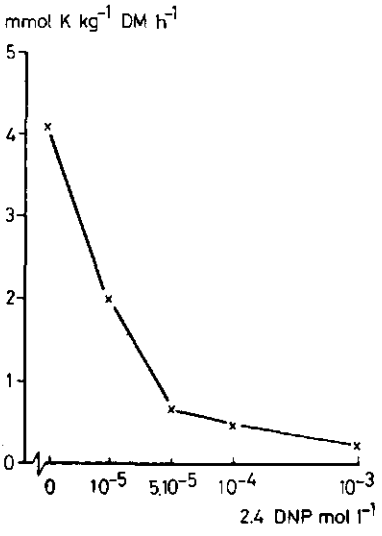

Fig. 34. Experiment 24. Rate of potassium absorption in excised low-salt maize roots from a 1 mmol l^{-1} KCl absorption solution with different concentrations of DNP. Absorption for 4 h.

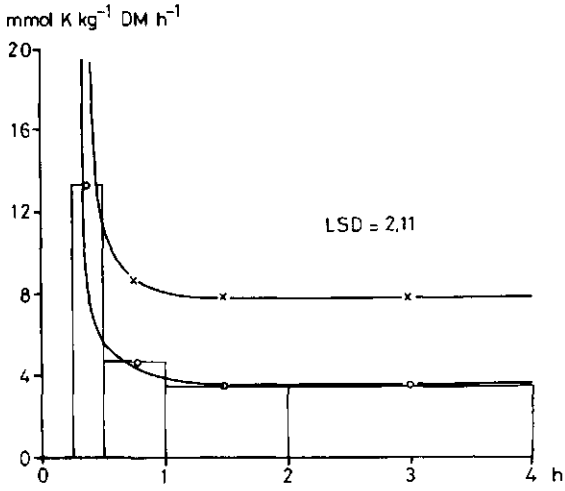

Fig. 35. Experiment 24. Rate of potassium absorption in excised low-salt maize roots for 4 h. Absorption solution is a KCl solution 1 mmol l^{-1} with (o) and without (x) DNP at 10^{-4} mol l^{-1}.

Experiment 25: Potassium influx in excised low salt maize roots from an absorption solution with KCl 0.10, 0.25, 0.60, 1.00, 1.50, 2.50, 5.00, 10.0 and 40.0 mmol l^{-1}, with and without DNP at a concentration of 5.10^{-4} mol l^{-1}. Influx after 4 h was calculated from accumulation with ^{86}Rb as tracer.

- The isotherms for the control (minus DNP) had a dual character (Epstein, 1966; Laties, 1969; Fig. 36);
- Potassium influx was reduced by DNP at all external concentrations of KCl.

Effect of DNP on permeability of root cell membrane was studied in the next.

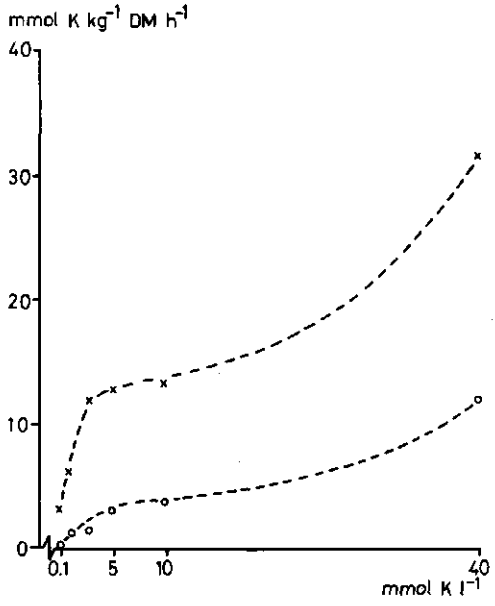

Fig. 36. Experiment 25. Influx over 4 h of potassium into excised low-salt maize roots with (o) and without (x) DNP at 5.10^{-4} mol l^{-1} in the absorption solution.

Experiment 26: Potassium influx in excised low salt roots treated before the influx experiment for 3 h in solutions of $CaCl_2$ at 0.5 mmol l^{-1} and DNP at 0, 10^{-5}, 10^{-4} and 10^{-3} mol l^{-1}. Potassium influx was calculated from depletion and accumulation for 2 h with ^{86}Rb as tracer.

Figure 37 confirms the suggestion that blocked respiration affects permeability of the cell membrane within a relative short time. At concentrations of DNP exceeding 10^{-4} mol l^{-1} the majority of the 'absorbed' potassium is returned during the 5 min exchange after the absorption period. This enhanced release of potassium with increased concentration of DNP of the incubation solution proves that with increasing concentration, DNP becomes more and more active also in affecting membrane permeability. Effects of DNP are not always limited to direct respiration or energy effects. The final effect on ion absorption can be composed of two or more components, for example a metabolic effect and a permeability effect.

In the next experiment the inhibitor CN was tested.

Experiment 27: Simultaneous potassium influx and efflux in excised low salt maize roots from an absorption solution with KCl or KCN at 1 mmol l^{-1}. Flux measurements by the standard method with ^{86}Rb as tracer.

Cyanide at a substance concentration of 1 mmol l^{-1} depresses influx within 15 min of addition (Fig. 38). During the steady-state phase, influx was depressed by a factor 3. So CN inhibits the influx of potassium significantly too. However, the efflux curves of CN look different. Whereas DNP at high concentrations increases the release of accumulated potassium from the root, CN did not affect efflux of potassium at all.

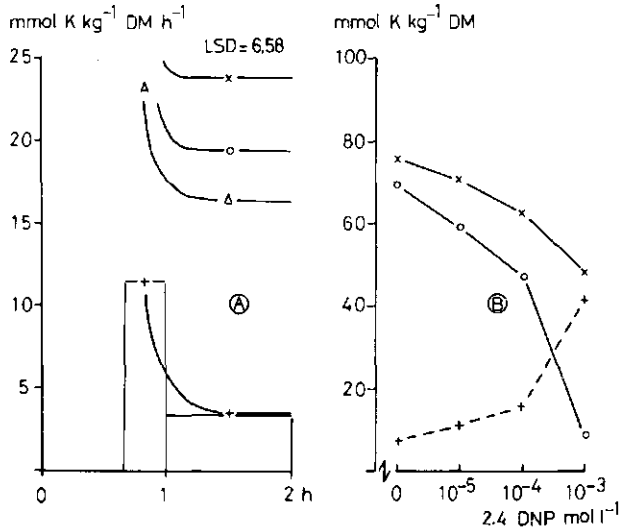

Fig. 37. Experiment 26. Before the absorption experiment, intact plants are grown for 3 h on solutions of 0.5 mmol l^{-1} $CaCl_2$ with DNP at 0 (x), 10^{-5} (o), 10^{-4} (Δ) and 10^{-3} (+) mol l^{-1}. Substance concentration of KCl in absorption solution was 1 mmol l^{-1}. A. Rate of potassium absorption in excised low-salt maize roots for 2 h. B. Potassium absorbed (x) and accumulated (o) in excised low-salt roots after 2 h. (+) represents the fraction of K returned during wash/exchange lasting 5 min after absorption.

Possibly CN concentration was too low to affect membrane permeability. It was impractical to obtain data on influx and efflux at various concentrations of CN, since CN had to be applied as a potassium salt to avoid complications of cation antagonism. A change in concentration of CN would also change the external concentrations of K.

Both DNP and CN reduce K influx significantly at sufficient high concentrations. DNP affects root cell membrane permeability at concentrations exceeding 10^{-4} mol l^{-1}; at this concentration the membrane becomes more permeable (leaky) for potassium. CN does not affect efflux of K at a concentration of 1 mmol l^{-1} or CN does not affect root cell membrane permeability at all, or else a CN concentration of 1 mmol l^{-1} may have been too low.

5.2.7 Effect of glucose supply to the roots and of duration of lighting plants

Since the carbohydrate content of plants generally increases in the light and decreases in the dark (Breteler, 1974), there will be a daily fluctuation in the carbohydrate content of the plant because of the day/night sequence. Moreover, in most plants the sugar content tends to change during the growing season. Michael et al. (1970) found a decrease of the carbohydrate content of roots of sugar-beets with age. Titze (1970) and Zeid & Kühn (1973) found significant variations (mainly increases) in the carbohydrate content of carrots and spring wheat during the growing season. In the literature, a number of papers on the dependence of the ion uptake on the sugar status have been reviewed. According to Hoagland & Broyer (1936), accumulation of bromide by

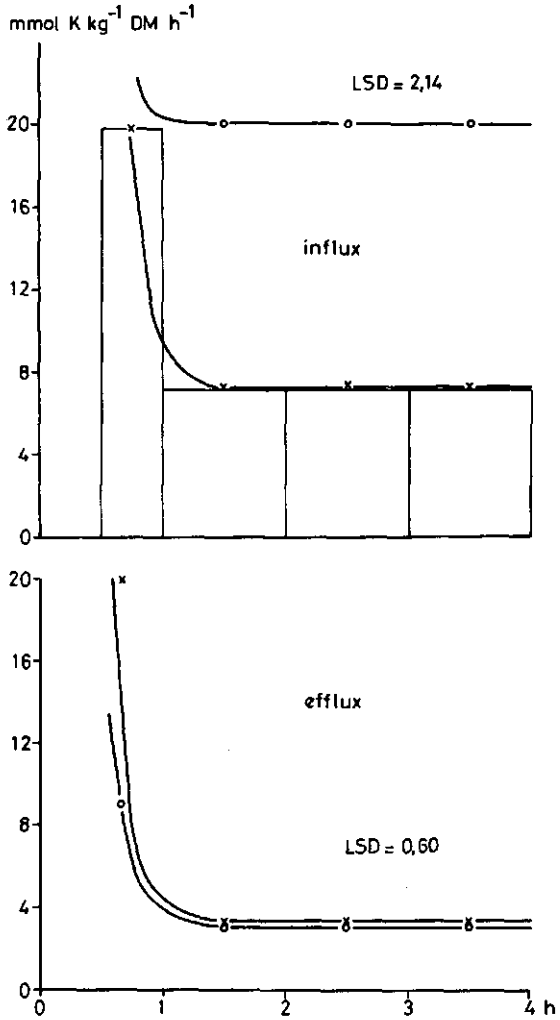

Fig. 38. Experiment 27. Simultaneous fluxes of potassium in excised low-salt maize roots for 4 h. Substance concentration in absorption medium of K was 1 mmol l^{-1} and of CN 1 mmol l^{-1} (x) or zero (o). A. Influx. B. Efflux.

segments of barley roots that were low in sugar could be accelerated by supply of sugar to the roots in the absorption solution. Pitman et al. (1971) even found a close correlation between the endogenous carbohydrate content of roots and the rate of accumulation of chloride. Consequently, both the long-term and diurnal changes in sugar content of a plant (root or shoot) will probably affect ion uptake by the plant.

In the next experiment endogenous sugar content of the excised maize roots was altered by incubation of the roots for a certain time in glucose solutions of different strength.

Experiment 28: Potassium influx in excised low salt maize roots, after incubation of the excised root material for 17 h in solutions of glucose containing 0, 0.5, 1.0, 2.0, 3.0 and 5.0 % (W/V) of glucose. Influx of K from a 1 mmol l^{-1} KCl solution was measured by depletion, using ^{86}Rb as tracer. The experimental time was 4 h.

Incubation in a 0.5-1.0 % (*W/V*) glucose solution tends to give a maximum increase in potassium influx, while with a further increase in strength of the incubation solution, influx curves decline (Fig. 39). At a 5% (*W/V*) glucose concentration, no further stimulation by the glucose treatment occurred. In spite of the high sugar content of these low-salt roots, the results prove that a moderate supply of glucose to the root medium enhanced uptake of potassium. The decrease of the stimulus at 2% (*W/V*) and higher can probably be explained by the high osmotic pressure of the incubation solution, up to 660 kPa at 5% (*W/V*). These high osmotic pressures of the external medium may change the internal cellular structure by dehydration and disturbed metabolism in general. Therefore in further glucose experiments, 1% (*W/V*) glucose solutions were used to get a maximum effect.

In the next experiment, the effect of incubation time on glucose accumulation and influx of K in roots was studied.

Experiment 29: Potassium influx in excised low salt roots after an incubation of excised roots in a 1% (W/V) glucose solution for 0, 2, 4, 6, 8 and 10 h. Potassium influx was measured by depletion over 4 h with ^{86}Rb as tracer. Absorption solution was a 1 mmol l^{-1} KCl solution.

Both the endogenous sugar content of the maize roots and the influx of K increased almost linearly with increasing incubation time up to 6 h (Fig. 40). Incubation for more than 6 h did not increase water soluble carbohydrate (WSC) content any further or even made it decline. Parallel to this, the increase in K influx declined and almost reached equilibrium after an incubation period of 8-10 h. The close relation between increase in endogenous WSC content and influx of K to the root, after an incubation period of 0-6 h, suggests a direct link between sugar content of the organs (root or leaves) and their salt absorption.

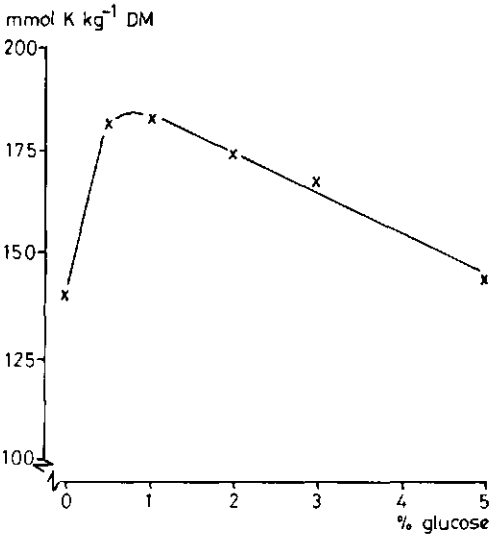

Fig. 39. Experiment 28. Potassium absorption over 4 h in excised low-salt maize roots after a 16 h treatment of excised roots in solutions of different concentrations of glucose. Absorption from a 1 mmol l^{-1} KCl solution.

Measurements during the uptake experiments of the content of water soluble carbohydrates in the absorption solution showed a significant loss or efflux of sugar from the excised roots during the 4 h of uptake (Fig. 41). Although the glucose-enriched roots had a significantly higher efflux of WSC than control roots (without glucose incubation), the latter have a relatively high excretion of sugars either from the cut end of the excised roots (xylem efflux) or from the epidermal cells (radial efflux). This release of sugars by the control roots confirms the high content of carbohydrate (about 10% in dry matter) of this low-salt root material. In spite of this content, a glucose treatment still enhances influx of K. The supply of sugars to the membranes may be a major factor rather than general content of sugar, as suggested by Bowling (1976). This could explain why additional supply of fresh glucose to the roots is still effective in enhancing influx of K.

In the following experiments, potassium uptake of maize plants was studied with dark and light periods.

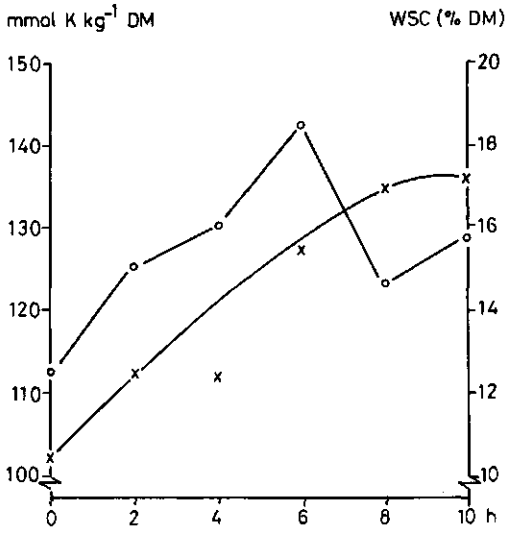

Fig. 40. Experiment 29. Absorption over 4 h of K (x) and content of water soluble carbohydrates (WSC) (o) in excised low-salt maize roots after a treatment of excised roots in a solution of glucose 1% (*W/V*) for different times. Absorption from a solution 1 mmol l^{-1} KCl.

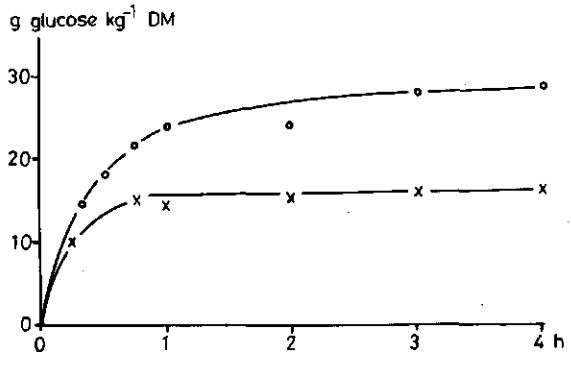

Fig. 41. Experiment 29. Amount of glucose released by excised low-salt maize roots during absorption over 4 h from a solution 1 mmol l^{-1} KCl. Excised roots with (o) and without (x) treatment for 16 h in a solution 1% (*W/V*) glucose.

Experiment 30: Potassium influx in excised low salt roots with and without an incubation in a 1% (W/V) glucose solution for 17 h. The roots were excised from maize plants either grown in continuous darkness, or up till 96 h of light before uptake experiment. Influx of K was calculated from depletion of a 1 mmol l^{-1} KCl solution for 6 h.

Influx of the roots grown in light was significant higher than in ones grown in continuous darkness (Fig. 42A, B). Glucose incubation did not affect influx over 6 h of roots grown in light, whereas influx to roots grown in darkness was significantly enhanced by glucose incubation. Roots grown in light show a steady-state influx after about 2 h; roots grown in dark without glucose do not reach a steady-state of ion uptake.

In continuous darkness, the sugar content of the plants must be low and potassium uptake must be limited, unless stimulated by a glucose incubation. Roots of plants grown in light have a high K uptake, which is not affected by a glucose incubation. This indicates the relatively high content of sugar in the roots.

Shifts in potassium uptake related to the day/night sequence, have been studied in the next experiment.

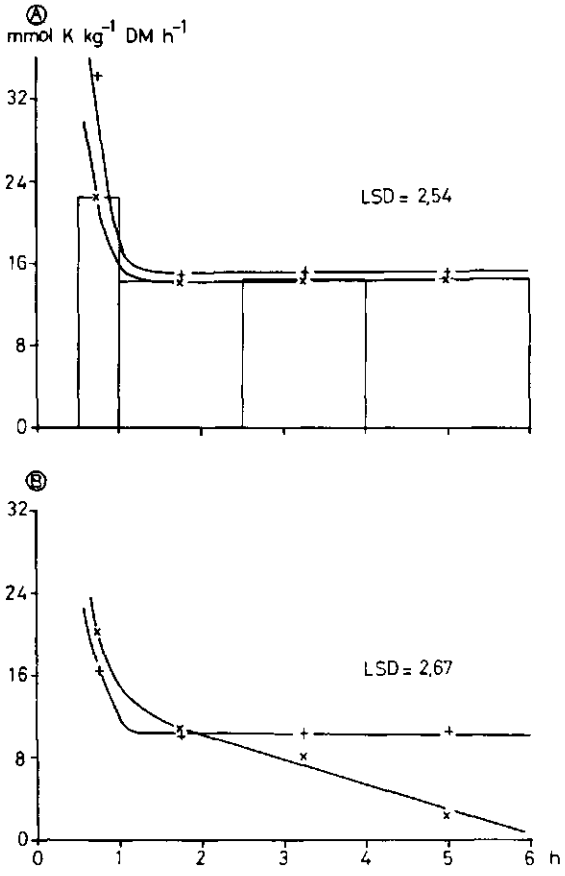

Fig. 42. Experiment 30. Influx of K into excised low-salt maize roots over 6 h with (+) and without (x) previous treatment for 16 h of excised roots in a 1% (W/V) glucose solution. A. Intact plants kept for 9 d in dark and then 4 d in light. B. Intact plants kept for 13 d continuously in dark. Absorption solution was a 1 mmol l^{-1} KCl solution.

Experiment 31: Potassium influx during a 24-h cycle of day and night in intact maize plants grown on a complete nutrient solution (Table 1) for three weeks. Potassium influx from a 1 mmol l^{-1} KCl absorption solution was measured by depletion with ^{86}Rb.

Day/night sequences in potassium absorption are demonstrated very clearly in figure 43A,B. Within 2-3 h of switching off the light, K influx declined significantly (Fig. 43A). At the end of the dark period, influx had almost stopped. After switching on the light again, potassium influx accelerated within 2 h and reached steady-state within 4 to 6 h.

This influx pattern demonstrates most clearly the day/night sequence in K influx and the transition phases after switching on and off the light. At the beginning of the dark period, the plant derives a temporary energy supply from a reserve pool of endogenous sugars; on the other hand, after the start of the light period, it presumably takes some time before sugar production or supply becomes effective for potassium uptake.

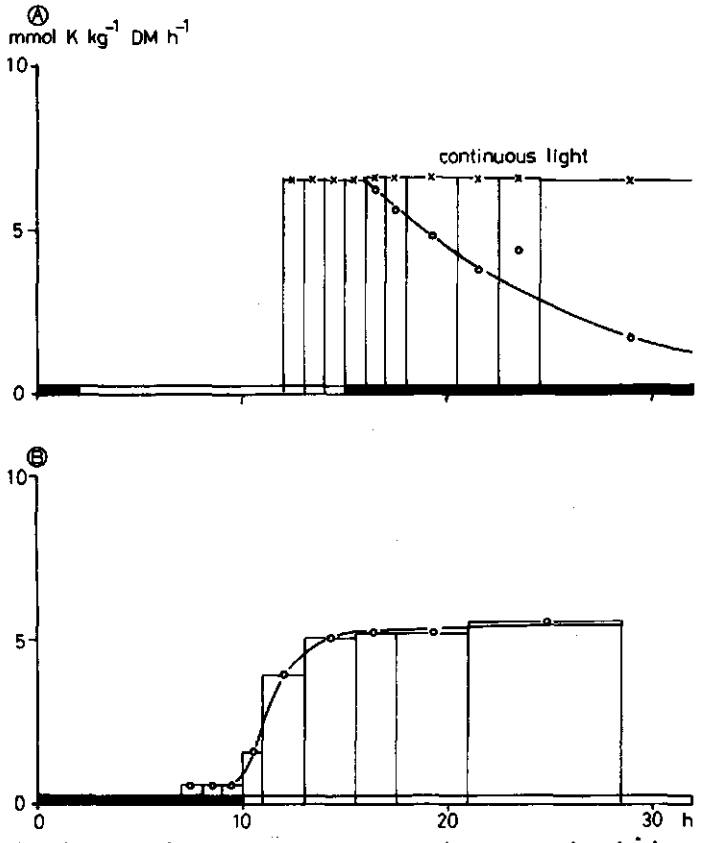

Fig. 43. Experiment 31. Rate of potassium absorption in intact maize plants. A. During transition from light to darkness. B. Transition from darkness to light. Absorption medium is a 1 mmol l^{-1} KCl solution. □ , light; ■ , darkness.

5.2.8 Temperature of the absorption solution and ion uptake

Temperature controls the rate of metabolism. As reported by Jacobson et al.(1957) and Mengel & Herwig (1969), ion fluxes by plant roots depend on ambient temperature. Since temperature regulates many metabolic processes, such as respiration (Mengel & Herwig, 1969), a change in temperature must affect ion absorption by the root. Also permeability of root cell membranes will be affected by different temperature treatments of the root, as has been found by many authors.

To study the effect of temperature on absorption of cations and anions, in particular potassium, the next experiments were set up.

Experiment 32: Absorption of potassium and chloride in excised low salt maize roots from 1 mmol l^{-1} KCl absorption solution at temperatures of 5, 12, 19, 26, 33 and 40 °C. Absorption was measured for 8 h by depletion and accumulation without radioactive tracers.

Net absorption was measured. For a technical reason, no use could be made of radioactive isotopes in the thermostatic baths. Consequently the temperature effect on influx and efflux could not be estimated separately.

Since temperatures of the absorption solutions below 7 °C and 10 °C produced negative net absorption (influx-efflux) of K and Cl (Fig. 44), efflux exceeded influx under these conditions. Probably low temperature inhibited ion influx more than ion efflux. As suggested also by Mengel & Herwig (1969), the efflux of potassium is a less metabolic-linked process than the influx, or efflux might even be a diffusion process. At about 33 °C, net absorption rate of both K and Cl shows an optimum, while with a further increase in temperature, it drops drastically (Fig. 44). Temperature affects absorption of cations and anions in a different way. While at temperatures below 15-19 °C, chloride absorption (which may be mainly diffusion after a period of chloride starvation) exceeds potassium absorption, within the range 19-33 °C, potassium is absorbed preferentially to chloride. This change in K/Cl absorption with different external temperature can be significant, both directly by a changed ion uptake, but also indirectly by difference in OH^-/H^+ efflux by the root, resulting in wide differences in external pH.

Temperature effects on influx and subsequent release of K during a rinse and exchange period were studied in the next experiment.

Experiment 33: Influx of K in excised low salt maize roots from a 1 mmol l^{-1} KCl absorption solution at 5 and 22 °C. After influx for 5 h, roots were bathed five times, each time in fresh aliquots of cold (4 °C) water followed by 10 mmol l^{-1} KCl solution, for 1 h per treatment. Influx was measured by depletion with ^{86}Rb as tracer.

With a decrease in external temperature from 22 °C to 5 °C, potassium influx during the steady-state phase declined from 8.09 to 3.23 mmol kg^{-1} DM h^{-1}. If we assume this stationary influx to have been going on from the start of the influx experiment, there will accumulate during 5 h 40.45 and 16.15 mmol K kg^{-1} DM for the high and the low

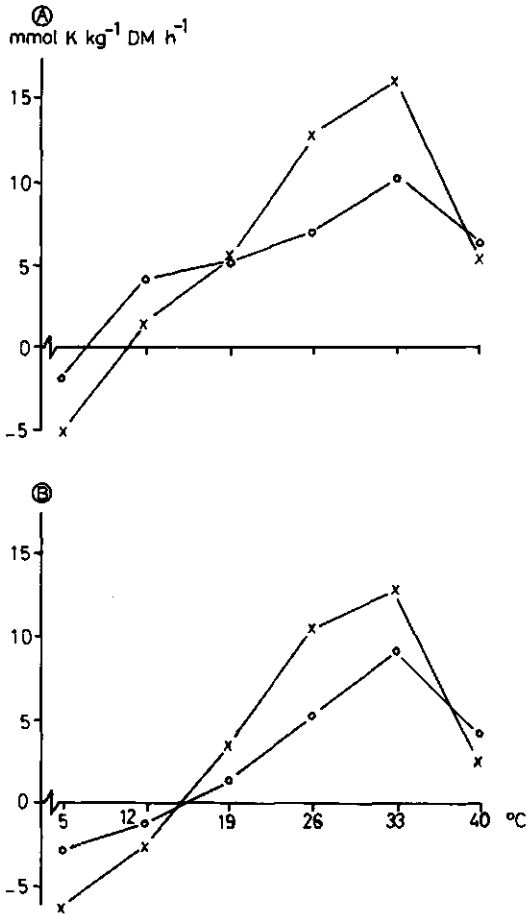

Fig. 44. Experiment 32. Rate of net absorption over 8 h of potassium (x) and chloride (o) in excised low-salt maize roots from 1 mmol l^{-1} KCl absorption solutions at different temperatures. A. Absorption calculated from depletion. B. Absorption calculated from accumulation.

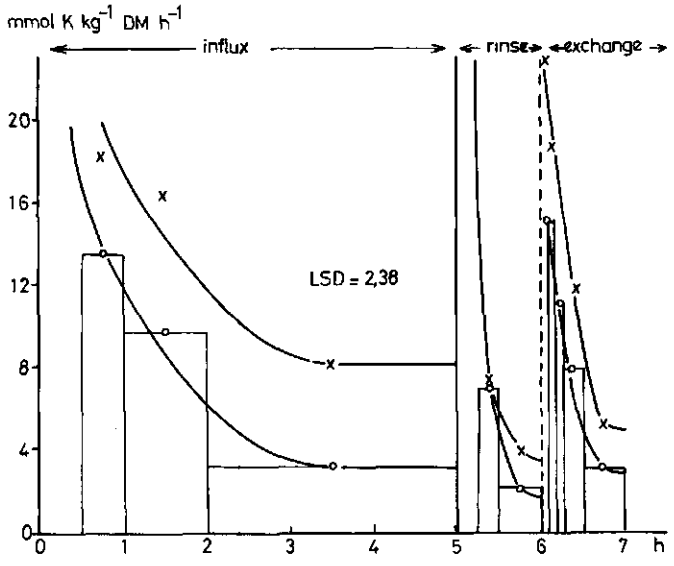

Fig. 45. Experiment 33. Influx of K into excised low-salt maize roots from a solution 1 mmol l^{-1} KCL at 22 (x) and 5 °C (o). After absorption for 5 h, rate of K release was measured during a rinse and exchange period of 1 h each in water and in a solution of KCl at 10 mmol l^{-1}.

temperature, respectively. The amounts of K accumulated, calculated to remain after the 1 h rinse and 1 h exchange period, were 49.71 and 14.49 mmol kg^{-1} DM, respectively. These data and the shape of the release curves (Fig. 45), indicate that:

- potassium accumulated at low temperature is retained completely within the plant root cells during the rinse and exchange period;
- after influx at a high temperature (22 °C), potassium release during the subsequent 1 h exchange may not have been complete.

The first observation proves that the influx of potassium was inhibited significantly at a low temperature, but also that the fraction absorbed during the steady-state phase accumulated actively, either into the cytoplasm or into the vacuole or into both.

6 Potassium transport through excised roots

Ion transport in roots will be subdivided in this report as follows:
- ion accumulation in the root tissue (short-distance transport);
- centripetal transport to the xylem and subsequent upward or xylem transport to the shoot (long-distance transport).

Chapter 5 discussed one stage of the ion transport: accumulation of ions in root cells.

This chapter will present results of experiments dealing with both long-distance centripetal transport of ions to the xylem vessels and subsequent upward movement to the leaves in the xylem. The latter has been studied in experiments on exudation of excised roots, a method used before by many other investigators (Anderson & Collins, 1969; Cooil, 1974; Klepper, 1967; Läuchli et al., 1971; Läuchli & Epstein, 1971; Meiri, 1973; Wallace et al., 1967). Xylem exudation from roots is chiefly water. The general opinion is that this water is driven through the root from the external solution to the xylem vessels by root pressure, i.e. by the osmotic pressure difference between the root medium and the xylem fluid.

The transpiration component is eliminated in decapitated root systems. Therefore, the equation for water movement across the root to the xylem, described by Slatyer (1967) as:

$$J_v = L_p \, (\Delta P - \sigma n R T \Delta c_s) + \Phi_o \qquad (6)$$

can be modified or reduced. The result is the one normally used to describe root pressure exudation of excised root systems (House & Findlay, 1966):

$$J_v = L_p \, (-\sigma n R T \Delta c_s) + \Phi_o \qquad (7)$$

where J_v is the volume flux of water into the root,
L_p is the hydraulic conductivity of the root,
ΔP is the transpiration tension,
σ is the reflexion coefficient of the effective osmotic membrane in the root,
n is the sum of the cation and anion valencies of the completely dissociated salt,
R is the universal gas constant,
T is the absolute temperature,
Δc_s is the substance concentration difference of solute between xylem sap and the external solution,
Φ_o is a non-osmotic (active) water flux.

This simplified model with one osmotic barrier (membrane) assumes the volume flux J_v of decapitated plants to be the sum of an osmotic component ($-L_p \sigma n R T \Delta c_s$) and an active component Φ_o. Consequently the longitudinal or upward salt flux J_s will be equal to the product of J_v and c_i, or:

$$J_s = J_v c_i \quad (8)$$

where c_i is the substance concentration of ion i in xylem sap. Under steady-state conditions, if no synthesis of new adsorption sites takes places in the root:
- J_s will equal the flux of total solute or salt absorption across the membrane;
- ion absorption will equal xylem transport as no net ion accumulation in the root will occur any more.

Whereas all uptake experiments, described in Chapter 5, were with single excised roots of young low salt maize plants, the exudation experiments described in the next sections were performed with excised branched root systems of maize plants 5 weeks old as well as excised young root material as used in previous absorption experiments. The latter allowed accumulation and transport data (Chapters 5 and 6) to be compared and interpreted better. Excised complete root systems with cut stumps were used to collect more exudate. In this way, more measurements (volume of exudate, chemical composition and osmotic pressure) could be done to check the validity of Equation 7.

The salt concentration of the xylem sap, collected by the exudation method, is consistently higher than of the real xylem sap of intact transpiring plants. Therefore, the osmotic pressure component in decapitated root systems is overestimated significantly. Yet, one may assume that the nature of most transport processes has not been disturbed too far to do some investigations on the absorption - accumulation - transport mechanism with decapitated maize root systems. Moreover, taking account that for 24 h the excised plant roots do not suffer from lack of carbohydrates and consequently the uptake of salts was not different for excised and attached roots, the rate of upward salt transport will also be equal for intact plants and excised root systems under equilibrium conditions. However upward water flux J_v and concentrations of xylem salt will be different for intact transpiring plants and excised root systems.

6.1 TRANSPORT AND ACCUMULATION WITH TIME

This section will review results of experiments on the chain of uptake, accumulation and xylem transport of potassium ions by decapitated maize roots, with particular emphasis on the interaction and time course of the three processes. A root immersed in a salt solution can absorb and accumulate ions. Mechanisms of initial and stationary ion uptake from the external salt solution have been dealt with in detail in Chapter 5.

As the cortex of the young maize root occupies about 90% of the root volume (Anderson, 1975b), the majority of ions taken up will be absorbed and temporarily stored in cortex cells before being transported radially inward to supply the cells of the stele and the xylem vessels, which eventually supply the aerial parts of the plants. There are two parallel pathways for movement across the cortex towards the stele, one through the extracellular space or apoplasm and the other through the symplasm, from the cytoplasm of one cell to the next by way of the plasmodesmata (Anderson, 1975b). This implies that the ion supply to the xylem stream is not a straightforward passage from the external solution to the xylem stream by one of the two pathways, the more so since a significant desorption/absorption or exchange of ions between the symplasmic through-put to the

xylem and the vacuoles of the cortical cells will occur. According to this exchange mechanism, freshly absorbed ions, present in the symplasmic stream, can either be accumulated temporarily in the vacuole with or without an exchange against equal ions already present in the vacuole, or else be transported directly (without any exchange) or indirectly (after one or more exchange steps) to the xylem vessels, after passing the endodermis and the stele.

Thus after the initial phase of ion absorption across the plasmalemma, the radial and subsequent upward transport of salts can be delayed, dependent on the intensity of symplasmic/vacuolar salt exchange.

In the following experiments, exudation trials, lasting 60 h with their features such as absorption, accumulation and upward transport of salt were studied both under non-equilibrium and equilibrium conditions, to find out more about salt transport over short and long distances within the root.

Experiment 34: Uptake, accumulation and xylem transport of potassium in decapitated root systems of maize plants. The experiment lasted 60 h in 10 mmol l^{-1} *solutions of* KNO_3, KCl *and* $\frac{1}{2}K_2SO_4$. *Rubidium-86 was used as a tracer for potassium.*

About 24 h after beginning of the experiment, there is a significant decline in the rate of K absorption (A), in the exudation rate (B) and in the K concentration of the xylem sap (C) (Fig. 46). So 24 h after decapitation of the maize plants, the carbohydrate pool of the roots becomes exhausted and root activity declines. Consequently, after experimental times exceeding about 24 h, steady-state does not exist more. Therefore, the uptake, accumulation and transport processes have to be studied with this material for no more than 24 h, as in the following figures. Figure 47 demonstrates that

1. absorption of K was initially high, but reached stationary conditions after about 5 h (I);
2. the bulk of the freshly absorbed potassium accumulated in the root cortex cells during the first 6-10 h, but as the experiment proceeds, cortex cells seemed to become saturated and consequently the rate of K accumulation declined rapidly with time (III);
3. simultaneously with and complementarily to this decline in K accumulation, the rate of upward K transport increased exponentially during the first 10 to 15 h (II);
4. after about 15 h, net accumulation ceased and thereafter the rates of absorption and xylem transport were almost equal and equilibrium or steady-state was reached.

Figure 48A,B,C likewise shows that after about 16 h, steady-state was reached. As soon as the potassium concentration of the xylem sap c_K is constant, Δc_K will be constant too and according to the Equations 7 and 8 both the volume flux of exudation J_v and the substance flux of potassium in xylem J_K must be constant with time too. Figure 48A,C indeed shows a constant flow of water and salt (K) after an adjustment period of about 16 h. Figure 48A,B also proves that initially both c_K and J_v were low, but increased simultaneously. Finally, this also confirms that within 24 h, mechanisms of absorption, accumulation and xylem transport of salts move towards steady-state by:

1. a constant rate of ion uptake;
2. a rate of salt accumulation that is initially high and results in a low rate of xylem

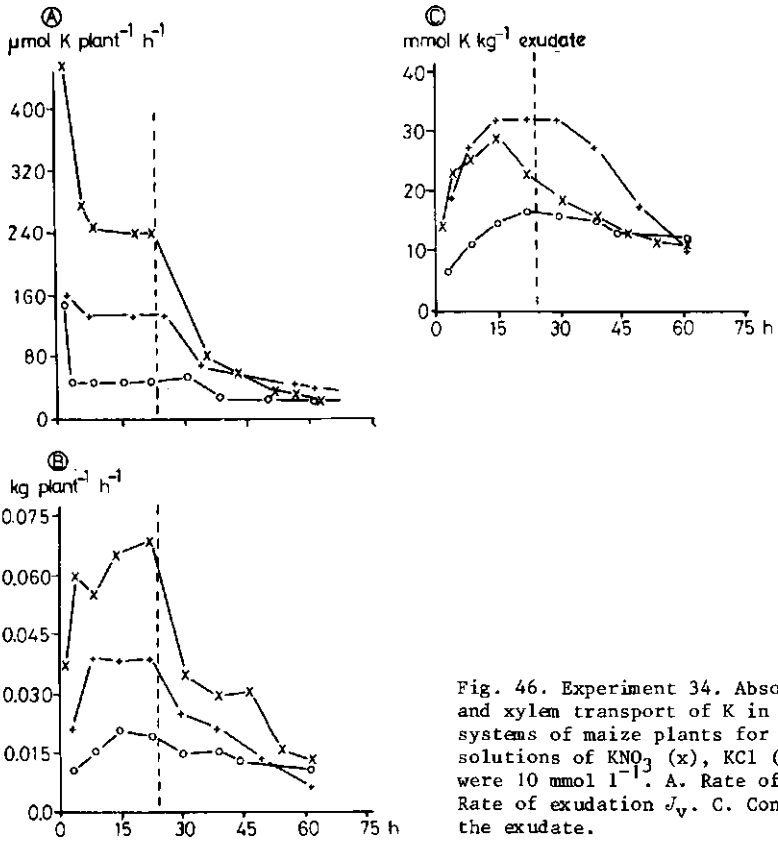

Fig. 46. Experiment 34. Absorption, accumulation and xylem transport of K in decapitated root systems of maize plants for 60 h. Experimental solutions of KNO_3 (x), KCl (+) or $\frac{1}{2}K_2SO_4$ (o) were 10 mmol l^{-1}. A. Rate of K absorption. B. Rate of exudation J_v. C. Concentration of K in the exudate.

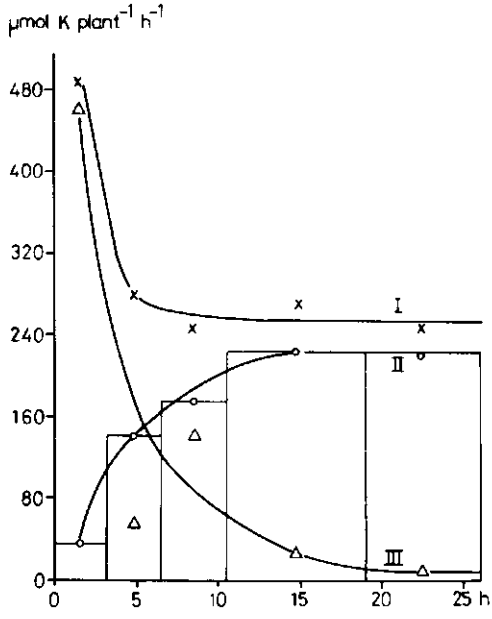

Fig. 47. Experiment 34. Rate of absorption (I), xylem transport (II) and accumulation (III) of potassium in decapitated root systems of maize plants for 25 h. Absorption medium is a 10 mmol l^{-1} KNO_3 solution.

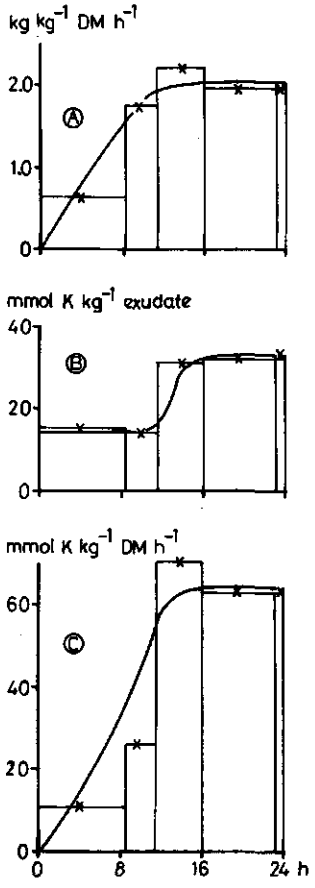

Fig. 48. Experiment 34. Absorption, accumulation and xylem transport over 24 h of K in decapitated root systems of maize plants 5 weeks old. Absorption medium is a 10 mmol l^{-1} KNO_3 solution. A. Rate of exudation J_v. B. concentration of K in the exudate. C. Upward transport of K in xylem sap J_K.

transport, but in a rising concentration of potassium in the symplastic and xylem sap; 3. this loading with potassium will be accompanied by an enhanced rate of exudation and salt transport on the one hand and a decline in K load on the other hand. This enhancement in K transport will continue until c_K and J_K are constant and, moreover, J_K is equal to the rate of K absorption by the root. At this point, steady-state is reached.

6.2 EFFECT OF K STATUS OF THE ROOT

During radial salt transport, symplastic ions can be absorbed or exchanged against the same kind of ions in the vacuole. Because vacuoles may be considered as large reservoirs that accumulate ions or are depleted according to concentration gradients with the cytoplasm, the exchange mechanism of symplastic or cytoplasmic potassium with vacuolar potassium can result in:

- net accumulation or depletion of potassium in the vacuole. In both cases, the concentrations both in the vacuole and in the symplasm/cytoplasm will change and no constant salt transport will be achieved.
- absence of any net salt transport between symplasm and vacuole; in spite of exchange

of vacuolar and symplastic salts, the salt concentration in both compartments keeps constant. Freshly absorbed potassium will be exchanged against potassium already present in the vacuole and consequently potassium from the vacuole will be transported, while freshly absorbed potassium will be accumulated temporarily in the vacuole before being transported radially to the xylem vessels.

Because vacuolar and symplastic salt concentration will regulate and control both fluxes ϕ_{cv} and ϕ_{vc} (Diagram 4), the salt status of both compartments will also regulate the symplastic or radial salt transport. The effect of loading the root cells with potassium on subsequent radial and upward transport of this ionic species was studied in the next experiment. To eliminate the radial desorption of potassium during upward transport in the cut stem of old excised root systems and to make transport data comparable with uptake data of Chapter 5, excised low salt roots of young maize plants were used in the experiment.

Experiment 35: Influx, and rate of accumulation and upward transport of potassium in excised low-salt maize roots after treatment of the intact plants for 24 h in solutions containing 0.5 mmol l^{-1} $CaSO_4$ (control), 1 mmol l^{-1} KCl or 10 mmol l^{-1} KCl. Absorption from a 1 mmol l^{-1} KCl solution was measured for 10 h; Rubidium-86 was used as a tracer for K.

Figure 49A,B indicates that low-salt root material can accumulate all freshly absorbed potassium for the first 6 h. So the bulk of the ions are diverted from the radial symplastic salt transport stream *R* after passing the plasmalemma and are accumulated in the vacuole (ϕ_{cv}). The opposite flux ϕ_{vc} will then be low or even negligible. Afterwards, upward transport of potassium through xylem starts, showing roughly an exponential increase until 10 h. The question whether gross exchange fluxes ϕ_{cv} and ϕ_{vc} proceed during the period cannot be answered by this experiment because potassium,

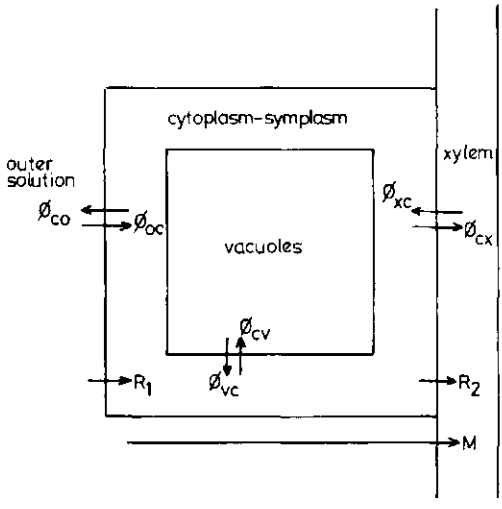

Diagram 4. Scheme of the different transfers in the root (after Pitman, 1971). Subscript o = outer solution; c = cytoplasm-symplasm; v = vacuole; x = xylem. *M* = direct symplastic transfer. R_1 and R_2 are net transfers.

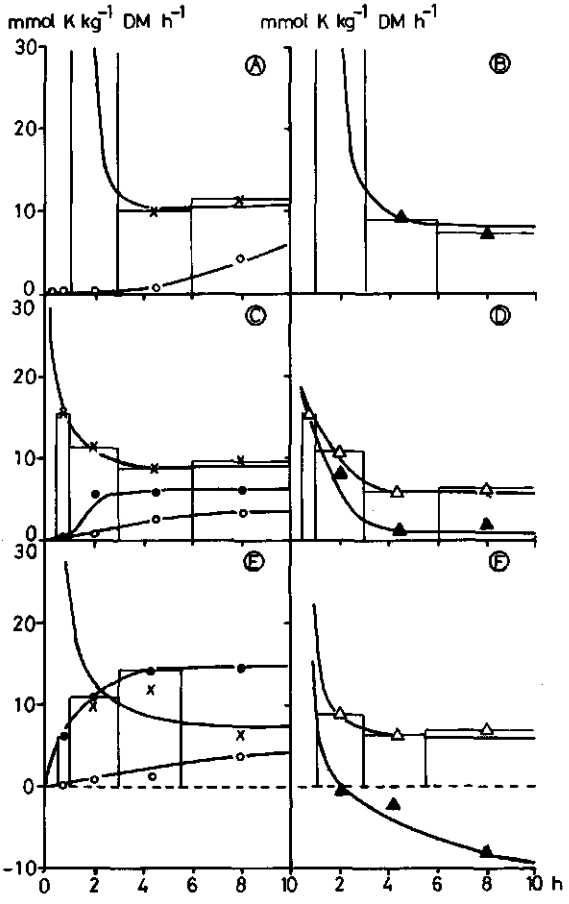

Fig. 49. Experiment 35. Influx (x), upward xylem transport (o,•) and accumulation (Δ,▲) of potassium in excised maize roots for 10 h, after 24 h treatment of intact plants on: A,B. a 0.5 mmol l^{-1} $CaSO_4$ solution (control); C,D. a 1 mmol l^{-1} KCl solution; E,F. a 10 mmol l^{-1} KCl solution. Open and solid symbols represent K and K(total), respectively. Absorption medium is a 1 mmol l^{-1} KCl solution.

accumulated in the vacuole during the initial loading period will be labelled with ^{86}Rb. Therefore freshly absorbed, labelled potassium moving all the way with the symplastic stream is not distinct from labelled potassium desorbed by the vacuole (ϕ_{vc}) and subsequently transported upward by the xylem stream.

Effects on K influx of loading roots for 24 h in an unlabelled solution of KCl, equal to that used during the subsequent absorption-transport experiment, are presented in Figure 49C,D. Figure 49C proves that these roots preloaded with KCl support an upward xylem flux of potassium, which can be measured immediately after excising the roots. This upward flow consisted of both labelled and unlabelled potassium. Potassium that has been absorbed and stored during loading, as it is exchanged gradually against freshly absorbed (labelled) potassium, then moves radially and longitudinally to the cut end of the excised roots. A fraction of the freshly absorbed potassium is not exchanged against vacuolar potassium, but is transported directly to the xylem vessels by the radial symplastic flow of salt.

So unlabelled and freshly absorbed labelled potassium are transported upwards (Fig. 49C), and the fraction of labelled potassium in total accumulation of K increases at the expense of unlabelled potassium, already present (Fig. 49D). Figure 49C,D also indicates that steady-state seems to be disturbed for about 2-3 h, but is restored thereafter.

Loading of roots for 24 h in a solution of KCl 10 mmol l^{-1} yields transport-accumulation curves, deviating from those of Figure 49C,D as follows:

1. Loading in the relatively high external concentration of K raises the internal cellular concentration of K to a higher level than results after transfer to a low external solution of K. Therefore the symplastic (cytoplasmic) K concentration decreases, because of a retarded plasmalemma influx ϕ_{oc}. Next, this lowered cytoplasmic concentration makes the concentration gradient with the vacuole steeper and consequently the ion flux ϕ_{vc} will be enhanced. The latter will result in a faster release from the vacuole of unlabelled potassium, which will then be transported together with the non-exchanged part of the freshly absorbed labelled potassium to the xylem vessels and subsequently to the cut end of the excised roots. Figure 49E illustrates that the majority of the potassium transported upwards is unlabelled potassium, stored in the vacuole before the absorption experiment.

2.After transfer of high-salt roots to a low-salt (K) medium the total xylem transport of potassium exceeds the absorption of fresh potassium by the root. Consequently, net accumulation rate (Fig. 49F) turns negative, while the accumulation rate of freshly absorbed potassium is maintained with positive values.

Symplastic labelled potassium could be isotopically diluted by exchange with unlabelled K already present in the cortical cell vacuoles. This non-physiological process could lead to misinterpretations of transport and exchange if based on nothing but label. One could easily overestimate or underestimate exchange and transport rates ϕ_{cv}, ϕ_{vc} and R (Diagram 4).

According to this and the previous experiment, the time (Δt) between the beginning of the experiment and the first release of potassium from the xylem vessels at the cut end of the excised roots depends on the internal cellular potassium status of the roots. This time lag Δt is also important in interpretation of absorption or influx experiments (Chapter 5). Criticism that exudation may interfere with uptake data derived from partition of salts between excised root material and a bathing solution will be justified if time of absorption exceeds Δt. Absorbed salts may then be partly excreted again into the absorption medium by the excised xylem vessels, that are in open connexion with the external medium. Consequently, absorption data measured from depletion or accumulation will be underestimated. A better understanding about the value of Δt is then desirable.

Experiment 35 indicates very clearly that excised low-salt roots, as used in the uptake experiments (Chapter 5) do not start upward xylem transport before the 6th to 8th hour. Only after loading of the low-salt roots for 24 h in a 1.0 or 10.0 mmol l^{-1} KCl solution, an upward xylem flux of potassium can be measured immediately after cutting off the aerial part of the plant. However, Figure 49C and E indicates that the main part of the upward potassium flux through the xylem consists of a fraction of unlabelled potassium accumulated in the cells (vacuole) during loading, and xylem transport of freshly absorbed labelled potassium does not really get under way until about 4-6 h after starting the experiment. So although there is a vascular efflux of potassium from the high-potassium roots, this efflux consists of unlabelled K during the first 4-6 h. Consequently, the use of excised root material, of low and high-salt status, will be justified in uptake experiments of maximal 6 h and with the use of isotopes. Beyond this period, vascular efflux

of freshly absorbed potassium will be too great and absorption measurements by the depletion or by the accumulation method will be incorrect (too small).

In a few further experiments, special attention was paid to the vascular efflux (upward xylem transport) and vascular influx, both in the potassium absorption. Upward xylem flux, both of low and high salt excised roots, was blocked by sealing the cut end of the root with paraffin.

Experiment 36: Potassium absorption (accumulation) in excised young maize roots with and without sealing the cut end of the roots with paraffin. Before the absorption experiment intact plants low in salt were grown for 24 h on solutions of $CaSO_4$ 0.05 mmol l^{-1} (control), or KCl 1 mmol l^{-1} (K-loading). Absorption from a 1 mmol l^{-1} KCl solution was measured from depletion and accumulation, for 10 h with ^{86}Rb as tracer.

The results (Fig. 50) show that:
- sealed low salt roots (control) accumulate significantly less potassium than the unsealed ones;
- there is no difference in potassium accumulation between sealed and unsealed excised roots after loading the roots with potassium.

Blockage of the vascular flux of potassium and water would enhance K accumulation, especially with the high-salt roots (high vascular efflux). Yet, the experiment proves exactly the reverse. Probably, a relatively high vascular influx of potassium can be responsible for apparent extra K accumulation by the 'open roots' (Diagram 5). That this apparent K accumulation occurs only in low-salt root material supports the postulate, since influx of potassium by the 'open' or cut xylem vessels and the subsequent radial salt transport backward to the stelar and cortical cells, low in K, will be much higher in K-starved than in K-loaded root material.

Experiment 37: Vascular influx and efflux of potassium in excised low-salt maize roots, measured by the standard method, for 10 h. The 1 mmol l^{-1} KCl absorption solutions were labelled with ^{86}Rb as a tracer for K.

Initially, there was a fast vascular influx of potassium by the 'open' xylem vessels (Fig. 51). However, after about 30 min the amount of labelled K, entering by this cut end, started to decline. After 3 h, all potassium that entered the root by this way had been 'pumped out' and from then on a net vascular efflux of potassium started. This potassium had been absorbed by the root cells (epidermis and cortex) and subsequently transported radially and longitudinally to the cut end. Although a net vascular influx existed temporarily, the stimulated K accumulation with excised 'open' roots, as found in Experiment 36, cannot be explained fully by this phenomenon of vascular influx. From the data, it is hard to say whether sealing of the cut end blocks or inhibits special processes related to salt and water accumulation or transport in excised low-salt roots, or the reverse, that these processes are enhanced by an 'open' cut end.

Enhanced osmotic pressure of vacuolar sap during steady-state salt accumulation might increase the water absorption and water content of the root cells continuously, because no

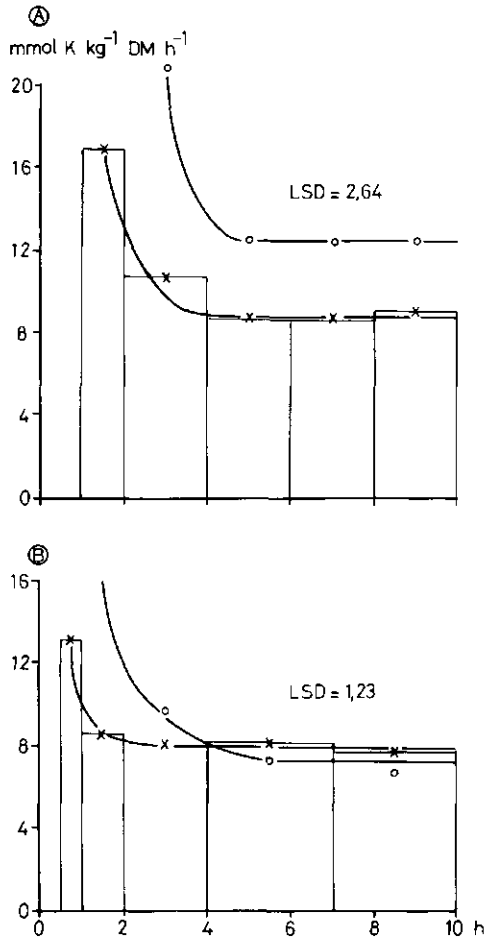

Fig. 50. Experiment 36. Influx of K into excised roots for 10 h. The cut end of excised roots was open (o) or sealed with paraffin (x). Absorption medium is a 1 mmol l^{-1} KCl solution. A. Low-salt roots. B. High-salt roots.

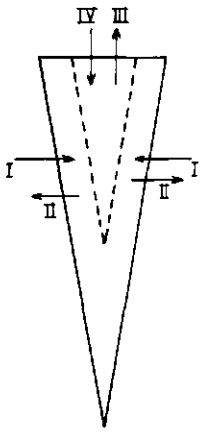

Diagram 5. Scheme of different transfers in excised roots, bathed completely in the absorption solution. Radial influx (I); radial efflux (II); vascular efflux (III) and vascular influx (IV).

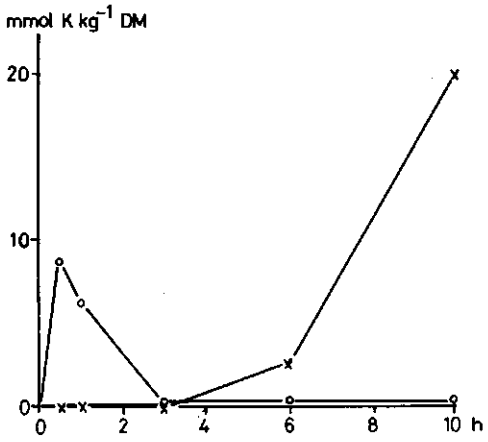

Fig. 51. Experiment 37. Vascular influx (o) and efflux (x) of potassium in excised low-salt maize roots for 10 h (cumulative). Absorption medium is a 1 mmol l^{-1} KCl solution.

water/salt vascular efflux is possible after sealing the cut end. This could result in excessive turgor pressure and in structural changes in cellular compounds and compartments.

6.3 EFFECT OF THE ANION

At low concentration, the uptake of K into plant cells is unaffected by the anion supplied, but beyond a concentration of 1 mmol l^{-1} effects are great (Epstein, 1966; Lüttge & Laties, 1966). Because counterion effects on radial and longitudinal transport of K are less clear, this section compares maize roots for their patterns of absorption, accumulation and upward transport of K^+ supplied at relatively high concentrations with NO_3, Cl and SO_4 as counterion.

Absorption and transport of potassium in combination with NO_3, Cl and SO_4 as counterion, indicated as $K_{nitrate}$, $K_{cloride}$ and $K_{sulphate}$, were examined in Experiment 34. Figure 46 indicates that absorption and xylem transport of potassium decrease in the order NO_3 >> Cl >> SO_4. Another anion effect on the transport of potassium can be seen in the rate of translocation of freshly absorbed potassium from the root to the upper part of the plant. Table 10 indicates that after 6 h K in exudate is entirely from an external labelled solution for KNO_3, while only half is from an external solution of KCl and K_2SO_4. So $K_{nitrate}$, in contrast to $K_{cloride}$ and $K_{sulphate}$, is transported more directly from

Table 10. Relationships of anion source to the proportion (%) of K from the labelled medium (*K) to K(total) in the exudate (100.c (*K)/c(K(total)). Experiment 34.

t (h)	c (KNO_3) (10 mmol l^{-1})	t (h)	c (KCl) (10 mmol l^{-1})	t (h)	c (K_2SO_4) (5 mmol l^{-1})
0	0	0	0	0	0
3.17	68.6	5.75	56.8	6.50	54.4
6.50	100.4	9.75	67.4	1[illegible].00	89.2
10.50	121.1	18.50	77.2	19.25	98.2
19.00	113.0	25.25	75.0	26.00	64.5
26.00	108.6				

the outer solution through apoplasm and symplasm into the xylem stream, being less mixed or exchanged with a pool of potassium already present in the cortical cell vacuoles.

Plants supplied with K_2SO_4 clearly reach steady-state at much lower Δc_s or $\Delta\Pi$ than plants supplied with KNO_3 or KCl, but the reason is still obscure. According to Cooil (1974), sulphate seems not to accumulate in the cells and to be transported to the xylem slowly, suggesting limited penetration at the plasmalemma. Consequently, transport of K exceeds that of SO_4 and endogenous anions are required to balance excess cation transport from K_2SO_4.

Therefore, in the next experiment, absorption and transport rates were measured of both cations and anions by plants supplied with KCl and K_2SO_4. Exudates were analysed for organic anions and inorganic cations and anions. Osmotic pressure of external solutions (Π_o) and exudates (Π_i) was measured to find $\Delta\Pi$.

Experiment 38: Absorption and xylem transport of potassium and its counterion in decapitated root systems of maize plants 5 weeks old. Experimental solutions were 10 mmol l^{-1} solutions of KCl and $\frac{1}{2}K_2SO_4$. Rubidium-86, ^{36}Cl and ^{35}S were used as tracers for K, Cl and SO_4 respectively during this 24 h experiment.

This experiment confirms earlier results. Once steady-state is reached, about 12 h after the beginning of the experiment, almost equivalent amounts of K and Cl are absorbed

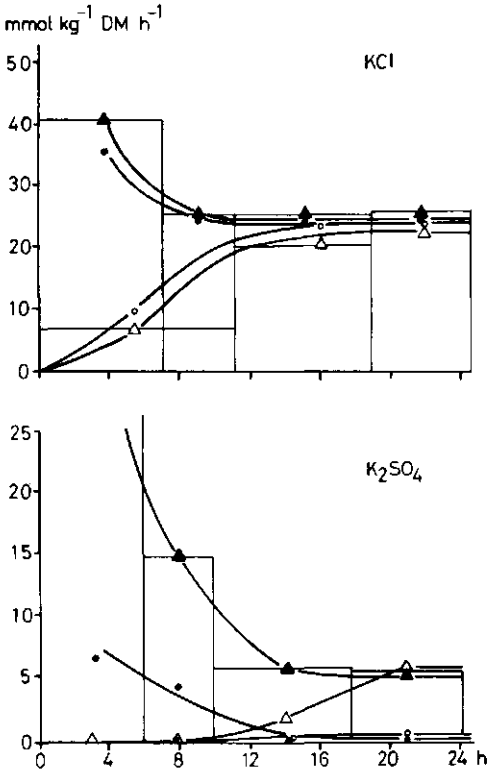

Fig. 52. Experiment 38. Absorption and upward xylem transport of potassium (Δ,▲) and its counterions (o,●) over 24 h in decapitated root systems of maize plants, from 10 mmol l^{-1} solutions of KCl and $\frac{1}{2}K_2SO_4$. Solid and open symbols represent absorption and transport, respectively.

and translocated to the cut end of the root system (Fig. 52). With SO_4 as counterion absorption and transport of K are much less than with the KCl treatment, but also the amount of absorbed and transported anion (SO_4) is much less than that of the positive ion K^+. As a result, a difference in charge has to be compensated to maintain electrical neutrality within the root tissue.

Table 11 presents data of the composition of the exudates collected from root systems, supplied with KCl and $\frac{1}{2}K_2SO_4$, each at external equivalent concentrations of 0.5 and 20.0 mmol l^{-1}. Exudates were collected 18-24 h after starting the experiment (steady-state phase). At both external concentrations, K and Cl in the KCl exudates are almost equally represented; consequently, the organic anion concentration in these exudates is minimal. The K_2SO_4 exudate is characterized by an excess of K over SO_4. This excess in positive charge is compensated by organic anions, mainly of malic acid (about 85%). These anions move together with SO_4 in the xylem stream and neutralize the xylem sap electrically. Measurements of the osmotic pressure of the exudates confirm the big difference between KCl and K_2SO_4 exudates, collected in steady-state. The low osmotic pressure of the K_2SO_4 exudates, combined with the relatively high content of organic anions, proves that at low or high external salt concentrations the counterion SO_4 retards the upward potassium/water transport significantly.

Transport isotherms of K with Cl or SO_4 as counterion were determined in the next experiment.

Table 11. Composition of exudates, collected from maize roots 18-24 h after decapitating and transferring the plants to absorption solutions of KCl and $\frac{1}{2}K_2SO_4$, each at substance concentrations of 0.5 and 20 mmol l^{-1}. Fum = fumarate, Succ = succinate, Mo = malonate, O = oxalate, M = malate, Ci = citrate.

Components in exudates	c (KCl) (mmol l^{-1})		c ($\frac{1}{2}K_2SO_4$) (mmol l^{-1})	
I organic (mmol l^{-1}, titratable)	0.5	20	0.5	20
Fum	0.01	0.09	0.20	0.00
Succ	0.19	0.14	0.41	1.01
Mo	0.05	1.16	0.00	1.53
O	0.16	0.06	0.00	0.08
M	1.53	0.30	4.89	14.42
Ci	0.75	0.40	0.30	0.00
Σ carboxylates	2.73	2.15	5.81	16.77
II inorganic (mmol l^{-1})				
K	26.50	45.24	13.25	24.25
Cl	25.00	42.00		
SO_4			2.12	3.62
(cations - inorg. anions)	1.50	3.24	11.12	20.63
III osmotic equivalent (mmol l^{-1})				
exudate (Π_i)	50.25	82.50	21.00	39.25
ext. medium (Π_o)	1.00	37.00	1.00	25.00
$\Delta\Pi = (\Pi_i - \Pi_o)^o$	49.25	45.50	20.00	14.25

Experiment 39: Upward xylem transport of potassium in decapitated root systems of maize plants 5 weeks old. Rate of K transport was measured during steady-state phase, 18-24 h after transferring the root systems to solutions containing 0.1, 0.5, 1.0, 5.0 or 10.0 mmol KCl or $\frac{1}{2}K_2SO_4$ l^{-1}. Radioactive tracers were omitted.

Transport isotherms significantly deviated from uptake isotherms on two important points (Fig. 53):

1. Quite different from absorption isotherms with excised roots (Fig. 15), the transport isotherms did not show a dual pattern, either with Cl or with SO_4 as counterion. Increasing the external concentration of potasssium raised the rate of stationary upward K transport continuously and gradually, instead of stepwise (two or more steps) as found for uptake isotherms by Nissen (1973).
2. Whereas absorption of the cation was insensitive to the anion, supplied with it in solution at equivalent concentrations below 1 mmol l^{-1}, the present transport isotherms prove that the upward K transport was anion dependent over the whole range from low to high external concentrations of salt.

Both observations indicate that the kinetics of xylem transport differs from uptake kinetics. This phenomenon needs explanation, because in steady-state absorption and subsequent xylem transport are not only interdependent but even have values close together. Consequently, under these conditions absorption and transport should show similar kinetics. The lack of the dual character of the transport isotherm supports the suggestion by Pitman (1970) and Pitman et al. (1968) that the dual absorption isotherm (System 1 and 2) may be due to artefacts of excised low-salt roots. In these experiments, xylem transport of potassium has been measured in steady-state, 16-24 h after starting the experiment. On the other hand, most absorption or accumulation experiments are during the first 4-6 h, mainly

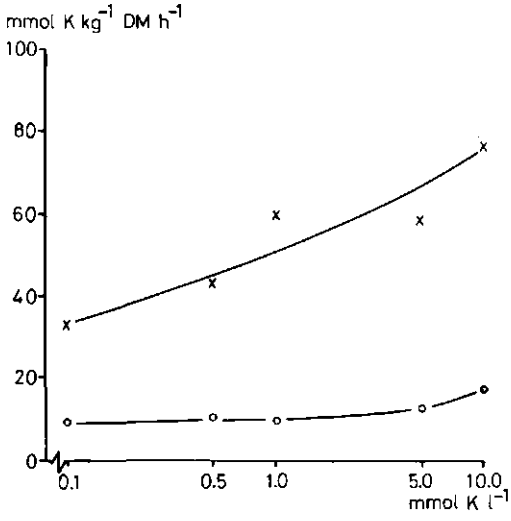

Fig. 53. Experiment 39. Steady-state transport in xylem of potassium in decapitated root systems of maize plants from absorption solutions with different substance concentrations of KCl (x) and K_2SO_4 (o). Absorption was measured 18-24 h after transfer.

a period characterized or dominated by ion accumulation (ϕ_{oc}, ϕ_{cv}), without a significant radial or vascular flux. Probably, only under these non-equilibrium conditions will the fluxes ϕ_{oc} and ϕ_{cv} show a dual uptake isotherm, while later, after reaching real steady-state conditions, both fluxes are reduced drastically and consequently dual characteristics do not exist any more. In fact, this means that only one of the Systems 1 and 2 partakes in upward K transport. In Chapter 7 it will be discussed which system this is.

6.4 TRANSPORT AND ACCUMULATION OF K AS AFFECTED BY THE EXTERNAL K CONCENTRATION

This part gives more details about the consecutive processes of absorption, accumulation and transport of potassium and their response to the external concentration of K and the kind of counterion.

Root systems were transferred to exudation media of different strength and for 24 h the xylem transport by the cut ends of the root systems was estimated periodically from samples accumulated by continuous collection. At the end of the experiment, total absorption could be calculated as the sum of ion accumulation plus ion transport. In the experiments, special attention was paid to the relationship between the external potassium concentration and the time course of potassium transport. According to external K concentration, a drastic change in concentration gradient will develop immediately after the transfer of the low-salt roots to the different exudation media. Ion absorption and ion accumulation (ϕ_{oc} and ϕ_{cv}) will be dependent on or be regulated by the external concentration (Chapter 5). However, how exudation and xylem transport change with time and with external salt concentration is little described in the literature (House & Findlay, 1966; Meiri, 1973).

In the next experiment, potassium transport isotherms were made with Cl, NO_3 and SO_4 as counterion. In this way, special information was obtained on the role of the anion in all three processes during an experimental period that was sufficiently long to reach steady-state. Under these conditions no net accumulation occurs, and absorption and xylem transport of potassium proceed steadily and at equal rates (Section 6.2).

Experiment 40: Absorption, accumulation and xylem transport of potassium in decapitated root systems of maize plants 5 weeks old with Cl, SO_4 and NO_3 as counterion. Absorption media were solutions of KCl, $\frac{1}{2}K_2SO_4$ and KNO_3 0, 0.1, 0.5, 1.0, 5.0 and 10.0 mmol l^{-1}. The experiment lasted 24 h, without use of radioactive isotopes.

As a part of the NO_3 was probably converted into organic nitrogen after absorption and total NO_3 uptake was assumed to be the sum of NO_3 accumulated and NO_3 transported, the values plotted in the nitrate absorption isotherm (I) will probably be underestimated. All three potassium absorption isotherms were similar (Fig. 54); only at external concentrations of K exceeding 5 mmol l^{-1} all three quantities tended to increase. Thus, just like the transport isotherms, presented in Figure 53, none of these three showed a dual character. This confirms the idea (Section 6.3) that during 'long-term' experiments absorption and accumulation isotherms lose their dual character. Because K isotherms with SO_4 and Cl as counterion had about the same shape as the $K_{nitrate}$ one and the transport

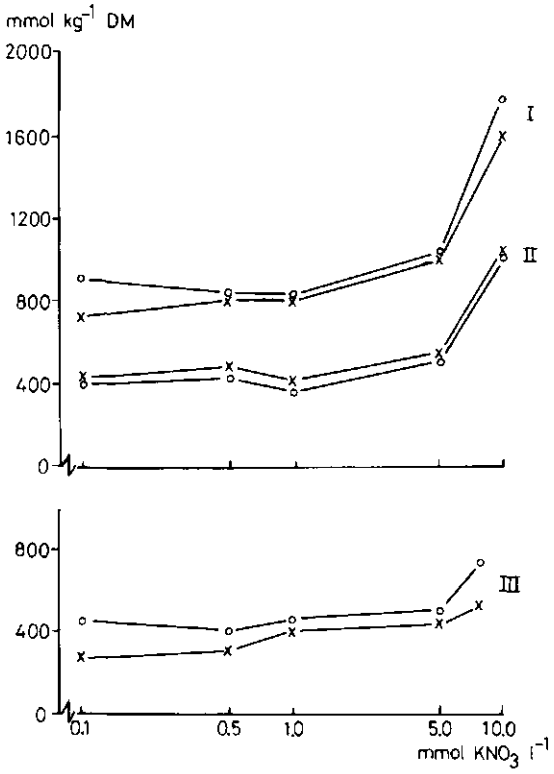

Fig. 54. Experiment 40. Absorption (I), transport in xylem (II) and accumulation (III) over 24 h of potassium (x) and NO_3 (o) in decapitated root systems of maize plants 5 weeks old. Absorption solutions had different concentrations of KNO_3.

isotherms for $K_{chloride}$ and $K_{sulphate}$ have been presented already in Figure 53, the accumulation and absorption isotherms for these two combinations are omitted here.

Figure 55A shows the K concentrations in the different KNO_3 exudates against time. All these concentration curves show a similar pattern. The potassium concentration of the xylem sap increased continuously for about 16 h and then became almost constant. The concentration was higher with higher external concentration. Over the 16 hours, a concentration gradient had built up; consequently, the osmotic equivalent difference $\Delta\Pi$, the exudation rate J_v and K transport in xylem J_K accelerated simultaneously. Figure 55B illustrates that after about 12-16 h the rate of xylem transport of potassium was stationary, but was higher with higher external K concentration.

As shown in Equation 6, the exudation rate J_v depends on the difference in osmotic equivalent between the external medium and the xylem exudate. Although osmotic pressure was not measured in this experiment, osmotic pressure of xylem sap and external medium in similar experiments was measured by Munting (1977). Values prove that for KNO_3 and KCl treatments the osmotic equivalent does not significantly differ from $n.c$, where n is the sum of the cation and anion valences of the completely dissociated salt and c is the concentration of solutes (mmol l^{-1}). This can be true for the chemical composition of these exudates (Table 11). By contrast, the K_2SO_4 exudate contains, apart from anorganic potassium and sulphate ions, a considerable amount of dissociated organic anions as a compensation for the excess of cations (K). Therefore, for this exudate $n.c \neq \Pi$, but

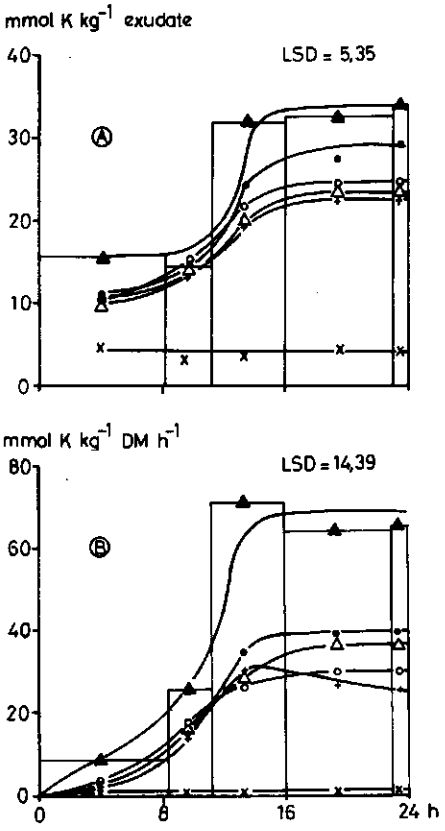

Fig. 55. Experiment 40. Decapitated root systems kept for 24 h in solutions with substance concentrations of KNO_3 0 (x), 0.1 (o), 0.5 (Δ), 1.0 (+), 5.0 (•) and 10.0 (▲) mmol l^{-1}. A. Concentrations of K in exudates. B. Upward transport of potassium in xylem.

$\Pi \approx 2\ c_K$, where c_K is the potassium concentration in the exudate.

The potassium concentrations observed in the exudates in steady-state are presented for different external K concentrations and three different anions in Figure 56A. The following features should be noted:

- The potassium concentrations of the KCl and KNO_3 exudates were almost equal and significantly higher than those of the K_2SO_4 exudates. This was valid for all external K concentrations.
- a 100-fold increase in external K concentration resulted in less than a twofold increase in K exudate concentration. The concentration ratio $[K_{exudate}] / [K_{medium}]$ for low K medium concentrations is extremely high (130-260), whereas this ratio is relatively low (1.7-3.7) for high external K concentrations.

These differences in accumulation ratio of low and high external salt concentrations result in a noteworthy phenomenon of exudation as measured by decapitated root systems (Fig. 56B). In steady-state, the osmotic equivalent difference $\Delta\Pi = n\Delta c$ is not significantly affected by the external concentration, at least within the concentration range 0.1 - 10 mmol l^{-1}. Secondly, if K_2SO_4 is supplied to root systems, $\Delta\Pi$ is much lower than for roots supplied with KCl or KNO_3. This means that decapitated root systems reach steady-state, characterized by a certain $\Delta\Pi$ that is almost independent of the external

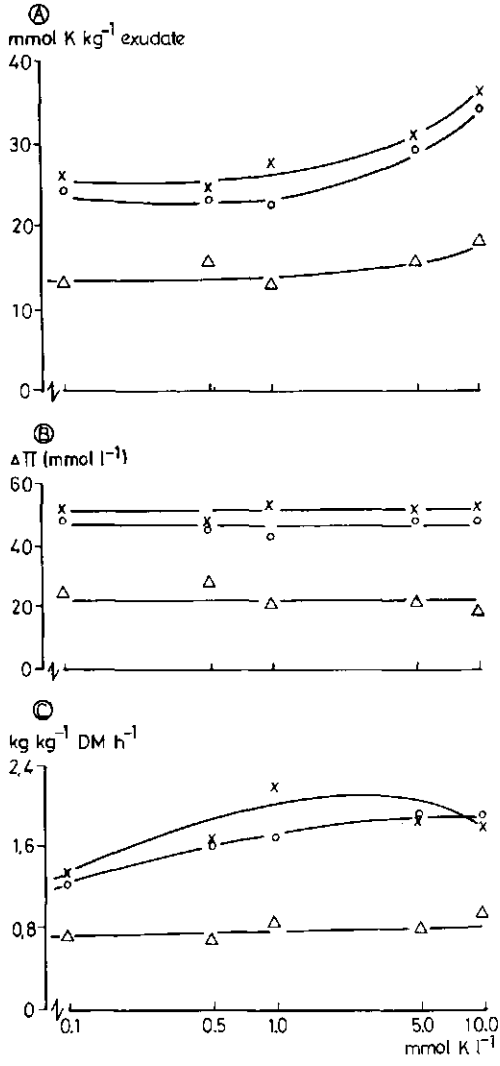

Fig. 56. Experiment 40. Exudation experiment with decapitated root systems transferred to absorption solutions with different concentrations of KNO_3 (x), KCl (o) and $\frac{1}{2}K_2SO_4$ (Δ). A. Substance concentration of K in exudate in steady-state. B. Osmotic equivalent difference (ΔΠ) in steady-state. C. Exudation rate J_v during steady-state.

salt concentration. Although ΔΠ is constant for all the tested external salt concentrations, it is markedly lower for K_2SO_4 plants than for plants supplied with KCl or KNO_3.

How and why plants reach constant ΔΠ for totally different external concentrations for any kind of salt, but reach equilibrium with K_2SO_4 at significantly lower ΔΠ values is still unclear. The exudation rates (Fig. 56C) differ from expected values, at least for the KCl and KNO_3 treatments . If $J_v = L_p\ (-\sigma RTn\Delta c_s) + \Phi_o$ (Equation 7), and Δc_s as well as L_p and σ are constant with different external concentrations, J_v should be constant too. However, both KCl and KNO_3 treatments do not show a constant J_v with varying K concentration of the medium. Perhaps one or both membrane characteristics L_p or σ will vary with varying internal concentration. However, the model with a single osmotic barrier as used for the Flux Equation 6 could also be too simple.

6.5 EFFECT OF GLUCOSE, CN AND DNP ON ABSORPTION AND XYLEM TRANSPORT OF POTASSIUM

Accumulation of ions in the xylem sap strongly suggests that this polar or radial transport of ions is an active process. The electrical potential difference between the exudation sap and the external solution, measured by Bowling et al. (1966), indicates that anions move into the xylem against the electrochemical gradient, also indicating active transport. Cations on the other hand move with the electrical potential gradient, except potassium which is close to equilibrium. Consequently, the centripetal flow of ions appears to consist of an active transport of anions accompanied by cations to maintain electrical neutrality.

Although the concentration and the potential gradients suggest an active polar transport of ions between the external medium and the xylem vessels through symplasm or apoplasm, the mechanism of ion transport during the intermediate steps (e.g. during transport through apoplasm, symplasm or the final secretion into the xylem) is still poorly know and confused. Much criticism has been directed in recent literature against the theory of Crafts & Broyer (1938) that the radial transport of ions can be split up in three subsequent stages:

1. active absorption of ions at the epidermis;
2. transport through the symplasm down a concentration gradient to the stele;
3. leakage of ions from the stelar cells into dead xylem vessels.

Because of the existence of a free space, water and salts can freely penetrate into the root as far as the endodermis through the cell walls. Consequently, it is not the surface area of the root that is the first stage of salt uptake, but the total volume of the cortical cell walls. Thus, active absorption will not be restricted to the epidermis as mentioned under Point 1, but will take place by the outer epidermis cells as well as by the cortex cells as the salts pass the cortex by way of the apoplasm.

According to recent measurements of internal cellular ion activities by use of selective electrodes, Point 2 has also been criticized strongly. According to Dunlop & Bowling (1971), no significant radial trend exists in potassium activity across the maize root. Thus, no concentration gradient exists from the epidermis to the stele and consequently diffusion as the driving force for radial symplasmic transport is doubtful too.

Whether stelar transport and the subsequent final step of salt transport into the xylem vessels is passive is hard to say. That isolated stelar cells have the ability to accumulate ions just like the cortical cells (Dunlop & Bowling, 1971) does not prove the existence of an active stelar ion transport. Also inhibitors like CCCP, DNP and CN have been used to study the transfer of ions from the root tissue to the xylem in maize roots. However these chemicals inhibit absorption and accumulation of ions by the root too (Chapter 5). Consequently, inhibition of ion transfer into the xylem vessels can be an indirect effect of these compounds by their inhibition of ion absorption. There is no evidence whether the site of action of these inhibitors is at the stele or at the cortex.

In the next experiments absorption and xylem transport of ions are studied simultaneously with time. After addition of glucose or one of the inhibitors DNP or CN, the rates of absorption and xylem transport were measured carefully. A difference in time or intensity of the response of absorption and transport would indicate whether stelar

transport of ions is active.

Experiment 41: Absorption and xylem transport of potassium in excised low-salt maize roots with and without an addition of glucose (1% W/V) or DNP (10^{-3} mol l^{-1}) to the 1 mmol l^{-1} KCl absorption solution 24 h after starting the experiment. Total experimental time was 50 h; absorption and transport of potassium were measured with ^{86}Rb as tracer by depletion and cup-technique, respectively (Section 3.2.2).

Figure 57 shows that DNP started to inhibit both absorption and upward transport within 2 h. The rate of K absorption declined very rapidly and even turned negative after the 2 h, whereas the control treatment (without DNP) showed almost steady-state K absorption. Parallel to the inhibition of K absorption, the transport curve for K(total) shows a similar shape. About 2 h after DNP treatment started, K transport was blocked completely and did not recover.

After addition of glucose to the root medium, absorption and transport curves were much different from those with DNP (Fig. 58). The rate of absorption of the control declined with time, probably by exhaustion of endogenous carbohydrates, but addition of glucose kept K absorption almost stationary for 50 h. However, the time curve of the K(total) transport showed a remarkable phenomenon. Immediately after addition of glucose to the external medium, K(total) transport dropped to zero for about 4 h, while subsequent to this 'dead period' K(total) transport started again and accelerated to a high level.

One line of reasoning might be, that the active component of this transport might be

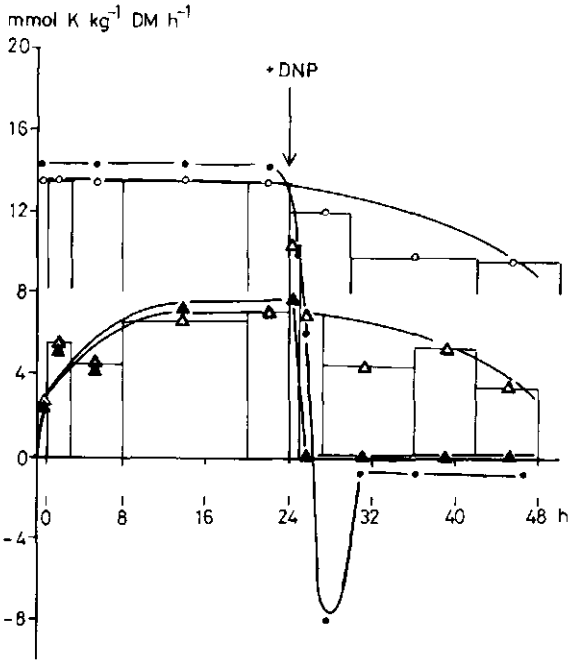

Fig. 57. Experiment 41. Absorption (o,●) and upward xylem transport (Δ,▲) of potassium in excised low-salt roots over 48 h. Open and filled symbols represent control (without DNP) and with DNP at substance concentration 10^{-3} mol l^{-1}. After 24 h, DNP was added to the absorption solution with KCl at 1 mmol l^{-1}.

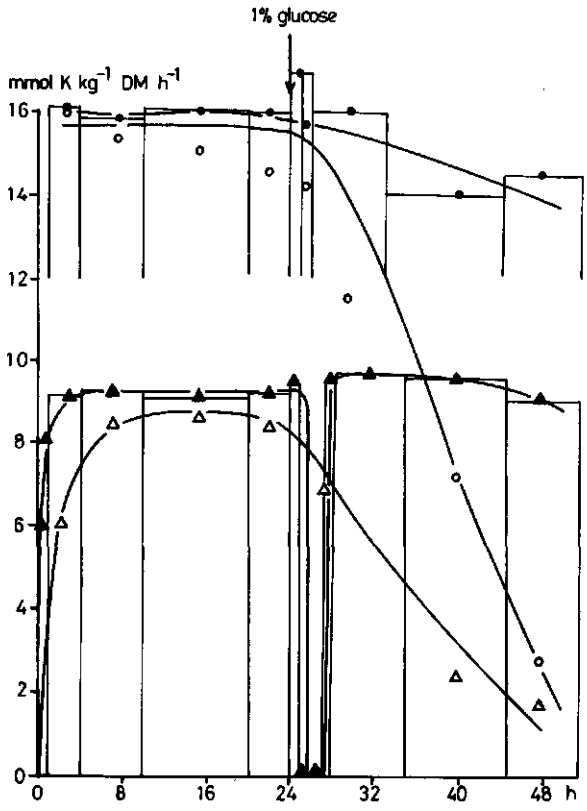

Fig. 58. Experiment 41. Absorption (o,●) and upward transport in xylem (Δ,▲) of potassium in excised low-salt roots for 48 h. Open and filled symbols represent control (without glucose) and with 1% (*W/V*) glucose. After 24 h, glucose was added to the absorption solution of KCl at substance concentration 1 mmol l^{-1}.

the transfer of ions into the xylem vessels rather than generation of a water flux, which could be a secondary osmotic effect. If so, factors that temporarily inhibit flow of water and matter, but allow ion transfer, might produce a store of potassium in the vessels, leading to a more concentrated exudate once mass flow resumes. Figure 58 provides only slight indications that this might be the case.

In the next experiment, the rate of exudation and the concentration of K in exudate were studied against time, with and without addition of an inhibitor and glucose.

Experiment 42: Potassium concentration of exudate and rate of exudation of decapitated root systems of maize plants 5 weeks old, transferred to a 10 mmol l^{-1} KCl solution. The three treatments (1) control (no addition), (2) 1% (W/V) glucose and (3) 1 mmol l^{-1} KCN were started 12 h later and the experiment continued 64 h. Rubidium-86 was used as a tracer for potassium.

Exudation curves (Fig. 59A) for all three treatments illustrate that CN depresses the rate of exudation significantly and almost immediately after addition of inhibitor. Glucose immediately almost halts exudation for about 5 h. After this 'dead period', exudation restarts and soon reaches steady rate.

After addition of glucose, concentration of K in xylem seemed to decrease somewhat

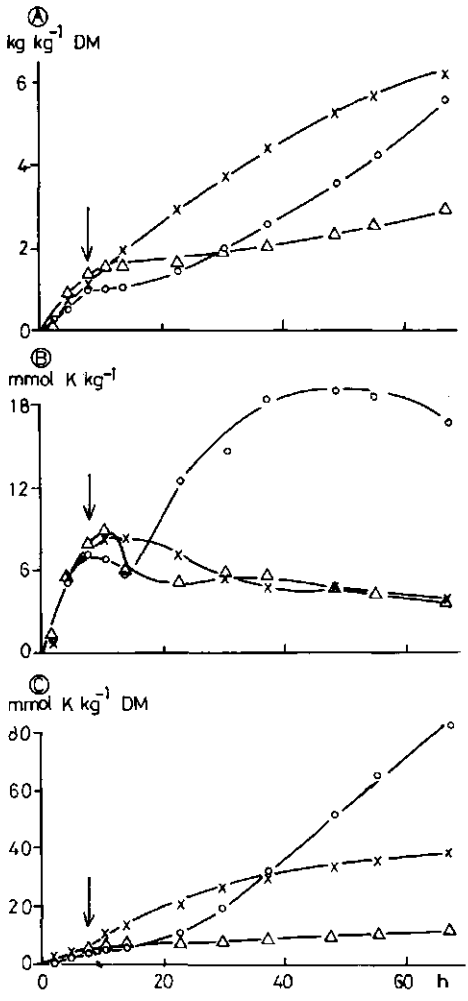

Fig. 59. Experiment 42. Transport of water and potassium in decapitated root systems for 64 h. Absorption solution was a solution of KCl at 10 mmol l^{-1}. (x) control; (o) and (Δ) mean additions of 1% (*W/V*) glucose and 1 mmol l^{-1} KCN to the absorption solution, respectively. A. Exudation (cumulative). B. Concentration of K in exudates. C. Upward transport of K in xylem (cumulative).

for 5 h, and then increased to a high value (Fig. 59B). On the other hand, concentrations in xylem of the CN treatment was less for about 24 h than in the control. Afterwards they were almost equal, but decreased gradually with time. The lack of an increase in concentration in xylem during the 5 h after addition of glucose could be interpreted as indication that stelar transport and final transfer of K into the xylem vessels is passive. Active transfer or active absorption of K into the xylem vessels with simultaneous depression in the rate of exudation (Fig. 59A) would at first sight result in increased K concentration of the exudate but this was not observed. However, the slight and short-lived depression of the K concentration in the exudate could well be caused by continuance or increase of absorption of salts into the cytoplasm after addition of glucose, so that potassium would be withdrawn from the xylem as well as from the medium. This will result in an enhanced accumulation of salts into the vacuoles of cortex cells. However, after some time the osmotic equivalent of the root tissues would increase, by

uptake of glucose and accumulation of salt, to an osmotic pressure exceeding that of the external solution. Absorption, lateral transport and exudation of water would then restart. As a result of the increased internal symplastic concentration of salt in cortex cells, the exudate will be rather rich in K. About 18 h after glucose addition to the 10 mmol l^{-1} absorption solution, new steady rates in xylem transport of K were reached (Fig. 59C).

The results of Experiments 41 and 42 do not justify any statement whether stelar transport is active or passive.

6.6 EFFECT OF pH OF THE EXTERNAL MEDIUM ON pH OF EXUDATE

The external pH seems to affect the uptake of cations and anions significantly (Section 5.2.1.), propably by competition between H^+ and cations on the one hand and a competition between OH^--HCO_3^- and anions on the other.

If the competition between H^+ and K^+ in ion uptake be accepted, one would expect the cellular concentration of H^+ to be affected by this mechanism too. Theoretical calculations about Donnan distributions (Section 5.2.1), indicate that external $[H^+]$ affects the internal $[H^+]$. The relation would be influenced by $[A^-]$, (the internal activity of non-diffusible organic anions), the nature of these organic anions (p*K*), and equivalent substance concentration and nature of the external inorganic salts. The activity and nature of the organic ions, such as organic acids, amino acids and proteins would regulate and buffer the internal cellular pH or $[H_i^+]$.

According to measurements by Bowling (1974), a linear relationship exists between external pH and pH of the vacuolar sap of root cells of *Helianthus annuus*. Within the range of external pH 4-8, vacuolar pH ranges between 4.5 - 6.5. This means that low and high values will be buffered, so that the internal vacuolar pH ranges between about 5 and 6.5. Measurements of vacuolar pH across the root of *Helianthus annuus*, by Bowling (1974), prove that vacuolar pH of all root tissues rose from 5.5 at the epidermis to 6.5 at the protoxylem.

In the next experiment xylem sap was collected from maize root systems grown on absorption solutions varying in pH between 3.8 and 6.8 and its pH was measured.

Experiment 43: Maize root systems were grown on absorption solutions with 10 mmol l^{-1} KCl, KNO_3 and $\frac{1}{2}K_2SO_4$. After the plants were decapitated (18, 42, 66 and 90 h after transferring the intact plants to the different solutions), exudates were collected for 10 h. Low, medium and high external pH were achieved by the different uptake patterns in the absorption solutions of K_2SO_4, KCl and KNO_3.

In agreement with Hiatt's (1967) and Hiatt's (1968) findings, pH of the K_2SO_4, KNO_3 and KCl solutions, respectively, tended to decrease, to increase or to remain almost constant with time (Fig. 60). Consequently, after some time, pH of the external medium varied from nearly 3.5 to 6.8 for the different absorption solutions.

Measurements of the pH of the exudates, collected at different times and for different solutions, illustrate that pH of the xylem sap ranged only between 5.2 and 5.8, while pH

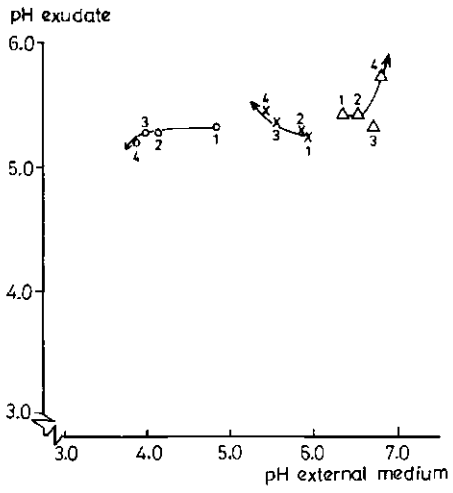

Fig. 60. Experiment 43. pH of exudates at different pH of absorption solution. Different pH of external medium were achieved 18 (1), 42 (2), 66 (3) and 90 (4) h after transferring plants on 10 mmol l^{-1} solutions of $\frac{1}{2}K_2SO_4$ (o), KCl (x) and KNO_3 (Δ).

of the external medium ranged between 3.8 and 6.8. As Bowling (1976) found, either a plant root can exclude H^+ from the root, or the root or plant cells have a more general buffering capacity.

With a K_2SO_4 supply to the plant root and the resulting low external pH, cation absorption will exceed anion absorption and excess of internal positive charge will be compensated by organic acids, as shown in Table 11. These organic acids, mostly weak acids, will buffer pH of the cell sap and the xylem sap markedly. As a result of KNO_3 nutrition, with excess uptake of anion over cation and high external pH, an internal buffering capacity will develop by reduction of a fraction of the absorbed NO_3 in the root and production of amino and other acids. Consequently, both organic acids and amino acids will mostly and generally be the main internal buffering compounds. They will be able to maintain internal pH of cell sap (vacuole and cytoplasm sap) as well as xylem sap within certain limits.

7 Discussion

Our data for uptake of potassium by the root have been presented largely separately from data on subsequent transport within the root tissue, and without any further discussion. Attention will now be paid to basic ideas about uptake and transport of ions through maize roots, in an attempt also to find links between absorption and subsequent transport across the root tissue of both solvent and solute.

7.1 ACCUMULATION OF POTASSIUM IN THE ROOT

Except in a few experiments, all experiments were with low-salt roots. The advantages of using roots of young plants grown on a gypsum medium are threefold, e.g.
- avoidance of isotopic exchange during absorption experiments with tracers;
- absence of release of potassium already present in the root before the beginning of the absorption experiment;
- a relatively high rate of ion uptake.

But these low-salt roots have a chemical composition quite different from roots cultivated on a complete nutrient medium. The high content of carbohydrate and the low content of salt in these $CaSO_4$ roots make comparison with results obtained with high salt roots difficult (Pitman et al., 1971).

As to the uptake technique employed, one must distinguish between the two rather different methods, that have been called 'uptake by depletion' and 'uptake by accumulation'. In short-term experiments, data from the two methods are divergent. In such experiments, the contribution of the initial uptake in the total uptake can be very high (Section 5.1). Absorption data obtained by the accumulation method include only part of the solutes. It is the part absorbed during the steady-state phase, because salts present in the AFS are removed by a rinse and exchange treatment of the roots (Epstein et al., 1963). In most experiments, the depletion method was chosen, because the time course of the absorption, obtained very easily by this method, gives information about both the initial and stationary phase of ion uptake, whereas data obtained by the accumulation method are limited to the rate of stationary ion uptake. However, in some experiments, with too small depletions (concentrated absorption solutions) or with conditions interfering with the β-radiation counting technique (such as coloured solutions), the accumulation method was used.

On the use of ^{86}Rb as a tracer for potassium in plant physiology, opinions are divided. West & Pitman (1967) report a different behaviour of ^{42}K and ^{86}Rb in short-term absorption experiments with the marine algae *Ulva* and *Chaetomorpha*. However results of preliminary experiments (Chapter 4), and investigations by Maas and Legget (1968), Marschner & Schimansky (1971), Mesbahul et al. (1971) and Schimansky (1970) prove the

suitability of ^{86}Rb as a physiological substitute for ^{42}K. Especially in short-term experiments with relatively low concentrations of potassium in the absorption solutions, this tracer substitution is fully justified.

The reason why almost all uptake experiments were with excised roots instead of intact plants is to prevent the shoot from interfering with the uptake capacity of the root. However, the work with excised roots or decapitated root systems, bathing completely in the absorption medium as described by Epstein et al. (1963), has often been criticized, because:

1. a release or leakage of potassium by the cut xylem vessels of excised roots and epicotyls, which are in open connection with the absorption medium, might result in incorrect data (Pitman et al., 1971). This xylem or vascular flux has been investigated in particular by exudation experiments (Chapter 6);
2. after decapitation, the downward phloem stream of assimilates from shoot to root has been cut off; the latter can exhaust the excised root material (lack of carbohydrates) and subsequently salt absorption rate tapers off.

Results from the different experiments indicate that vascular efflux through excised roots will be negligible if the absorption experiment is done either with low-salt roots during an absorption period of up to 8 h, or with high-salt roots for up to 6 to 8 h with tracers.

These $CaSO_4$ roots do not seem to lack carbohydrates during short-term experiments. Long-term absorption experiments with roots having a higher salt content did not show a reduction in uptake rate until 20-24 h after decapitation (Fig. 46). These findings justify the use of excised roots in all short-term influx experiments with tracers. On the other hand, in simultaneous influx-efflux experiments the use of excised roots can be risky. With the technique employed, the efflux rate was measured after absorption for at least 10 h. So measured efflux could be composed of both a true (Jackson & Stief, 1965) and a vascular efflux component.

7.1.1 Phases in the time course of the uptake

After the root material is transferred from a diluted to a more concentrated solution, there is a period of rapid uptake of potassium, followed by a period of less rapid and constant uptake (Chapter 5). In literature, this rapid uptake during the first period is called initial uptake, whereas the constant uptake during the subsequent phase is indicated as steady-state or stationary uptake. Not only time courses of the influx show these two phases, but also efflux. Roots transferred from a labelled absorption solution to an equivalent unlabelled solution show a rapid loss of activity, followed by an almost constant release of label.

In potassium absorption experiments (Section 5.1) with continuous titration, an intermediate transition phase was detected. As reported by Lüttge & Pallaghy (1972) and Heller et al. (1973), a first short period of rapid uptake is followed by extremely slow potassium uptake during a second phase of about 15 min. During the subsequent third phase, uptake rate was constant with time. This second phase is not a real transitional phase between a process of initial uptake (filling of AFS) and a subsequent second process of

steady-state uptake (Fig. 7). The filling of the AFS seemed to be completed after only about 1 h, while real accumulation of potassium exists from the beginning of the absorption period. Also the time course of accumulation itself indicates the existence of this second phase, characterized by a low influx rate of potassium. This implies uptake kinetics more complicated than postulated by the three-compartmental model (Cram, 1968; Pitman, 1963). This model of free space - cytoplasm - vacuole in series does not apply under all circumstances and for all root material. More discussion about the kinetics of the three - phase course will be presented in the next section.

The significance of these three phases in the uptake - time curve lies mainly in deriving information about the system of ion absorption. Under normal steady-state conditions of plant growth, the stationary salt uptake will be by far the most important component. Only under non-equilibrium, e.g. just after supply of fertilizer or water, a fast accumulation or depletion of salt in the root tissue (AFS) will occur, comparable with the initial salt flux. The initial uptake component allows the following phases to proceed and regulates supply of salts to be absorbed during the subsequent phase(s) of ion absorption. In salt absorption experiments with low-salt roots, the initial phase must be seen more or less as an 'artificial' period (artefact) before real ion accumulation.

7.1.2 Uptake kinetics

As most attention in this study is concentrated on the stationary uptake and steady-state accumulation of salts within the root, the initial phase of salt absorption will be only briefly discussed. Only those features of the initial uptake will be presented which participate in or are associated with subsequent phases of salt absorption. Some speculations about the mechanism of the second phase will be presented together with discussions on the transport kinetics of salts during the third phase of steady-state.

7.1.2.1 Initial uptake and apparent free space

As reported by Briggs et al. (1961), Grobler (1959), Nobel (1970) and Vervelde (1952), initial uptake or the filling of AFS is a physico-chemical process. Solute and solvent move readily from the external solution into the water free space (WFS) of the root tissues, in contrast to transport to the part of the root tissue or root cells into which the solvent, but not the solute, readily penetrates. This space, the Donnan free space (DFS), in the cell wall and probably part of the cytoplasm, shows the features of a Donnan distribution.

Experiment 9 indicates that absorption of cations during initial uptake is highly positively correlated with the pH of the medium. This contrasts with the absorption of anions during this phase, which is low and nearly independent of pH. This may indicate, that compounds such as fixed carboxyl groups or carboxylates, amino acids or proteins are responsible for the fast absorption or adsorption of cations during the initial phase, because the degree of dissociation of these compounds and thus the number of negative charges is strongly dependent of pH. Theoretical calculations about Donnan distributions, made for different pH of the external medium and for different pK of the hypothetical

indiffusible or restrained acids gave results also in agreement with the experimental data.

In contrast with the results of Ighe & Pettersson (1974), data gathered in Experiments 27, 30 and 33 show that initial uptake of potassium is not significantly affected by the temperature of the absorption solution nor by treatment of the roots with glucose or inhibitors as CN and DNP. So initial uptake, contrary to steady-state, is a binding of ions in the AFS of the root, which is not directly linked to metabolism. However, Ighe & Pettersson (1974) and Pettersson (1977) postulated the existence of a close relationship between exchangeable labile bound sulphate and rubidium, and the rate of subsequent uptake of these ions.

A change in membrane permeability likewise did not affect initial uptake. Obviously, a changed permeability of cellular membranes for electrolytes, effected by a calcium deficiency of the plant cell or by a treatment of the excised maize roots with one of the surface active chemicals like glyceryl triacetate, did not change the adsorption capacity of the cells for potassium ions.

This means that permeability does not play a substantial role in the initial adsorption of potassium. The conclusion could be that the AFS does not extend to any appreciable depth and mainly occupies the cell wall and the outer part of the cytoplasm. Potassium then does not have to travel very far before being adsorbed and is little retarded by a diminished permeability. This idea runs somewhat parallel to the idea of Dainty & Hope (1959) that the AFS occupies the cell wall only.

As expected, the amount of potassium adsorbed during the initial phase will be negatively correlated to the potassium status of the root tissue.

7.1.2.2 Steady-state of ion uptake

Potassium accumulated during the steady-state of ion uptake, in contrast to the potassium in the AFS, is not directly nor completely exchangeable against a 10 mmol l^{-1} KCl solution (Figs. 21 and 45). Macklon & Higinbotham (1970) found, for ^{42}K accumulated in the free space, cytoplasm and vacuole of excised pea epicotyls, half time exchange values ($T_{\frac{1}{2}}$) of 2.53, 69 min and 737 h, respectively. This means that, on a short-term, the passage across the plasmalemma by solutes is almost irreversible. After passing this barrier, ions can move to 3 different destinations by

1. accumulation in the cytoplasm;
2. passing the tonoplast and accumulation in the vacuole;
3. symplasmic transcellular radial transport, and long-distance longitudinal transport through xylem.

In short-term uptake (Chapter 5), the net solute transport during steady-state will be composed of the fluxes ϕ_{oc} , ϕ_{co} , ϕ_{cv} , ϕ_{vc} , while the long-distance xylem transport can be neglected there.

Potassium uptake isotherms show clearly a dual character (Fig. 15). In general, the lower isotherm, hereafter referred to as System 1 is operational at concentrations below 1 mmol l^{-1}, whereas the upper isotherm (System 2) is operative at above 1 mmol l^{-1}. Both systems are characterized by different K_M and V_{max} , but are also differently dependent on the anions present (Lüttge, 1973). The classical interpretations of the dual isotherm

have, until recently, rested on the concept of the cell as three compartments, cell wall, cytoplasm and vacuole, between which the transport of ions must necessarily be sequential (Torii & Laties, 1966a). According to this concept, at lower concentrations of the absorption solution, the rate of ion uptake is regulated at the outer membrane, the plasmalemma, while at high concentrations, as a result of a high rate of passive diffusion of ions through the plasmalemma, the real barrier for ion uptake is formed by the tonoplast (System 2).

Criticism both about the existence of the dual mechanism and the correctness of the static three-compartment model of the plant cell has recently changed the view of many plant physiologists about the uptake mechanism and the site of uptake isotherms. Cram & Laties (1971), Leigh et al. (1973) and MacRobbie (1970) justify the assumption of a direct pathway, possibly by way of membrane-bound vesicles, from the external solution to the vacuole. In this view, the cytoplasm does not behave as a single homogeneous compartment, but as a biphasic one, composed of a ground cytoplasm and a number of micro-vesicles or minivacuoles. A simple picture presented by Cram (1973a) is that of minivacuoles accumulating Cl from the external solution or cytoplasm, restricting exchange of this Cl with the cytoplasmic Cl while crossing the cytoplasm, and finally discharging the Cl into the main vacuole(s).

The existence of such vesicles or minivacuoles, proved by freeze-etch micrographs by Leigh et al. (1973) can possibly explain the dip or second phase in the influx-time curves. Despite a slight accumulation of potassium during the initial phase, it probably takes some time before the main cytoplasmic salt transport by vesicles starts, as described by Cram (1973a). Consequently, after the very fast filling of the AFS, salt accumulation will be diminished. This will be expressed by a dip in the uptake - time curve.

There is room for a better explanation of the differences in mechanism and location of the two systems, leading to the dual isotherms. Although no final proof is available from the present study, an attempt will be made to formulate such an explanation.

The probability that at high salt concentrations System 1 and System 2 act simultaneously and that the total salt absorption is the resultant of both systems, does not favour the idea that System 2 is a modification of System 1, which modification then would come about when the external potassium chloride concentration exceeds 1 mmol l^{-1}. Suggestions depicting such a modification as a qualitative change in cell structure or cell contents cannot be supported therefore.

Part of the explanation for the dual character of the isotherm, if it is not an artifact of low-salt roots, is that the two systems have different locations in the root. The idea that the two Systems 1 and 2 act at the two membranes of the cell, being the plasmalemma and the tonoplast, respectively, in parallel or in series, postulated by Torii & Laties (1966a), Osmond & George (1969), in fact is such an explanation on the basis of spatial differentiation. One additional part of the explanation that is suggested here, is based on the variation between root tissues. In longitudinal as well as in radial direction, cells are different in structure, size and function. Differences which might easily contribute to the dual character of uptake isotherms, are existent between the young non-vacuolated cells of root tips (only System 1) and mature vacuolated parenchymatous cells (both systems), as has been proved before by Torii & Laties (1966a). The ideas

of a differentiation between cell compartments and a differentiation between tissues have in common the difference in symplasmatic compartment and the enclosed sap compartment.

The next part of the explanation suggested here is, that System 1 mainly consists of exchange of potassium from the surrounding DFS with symplasmatic cations (mainly H^+), whereas System 2 is the separation of vascular or vacuolar sap from the surrounding symplasm. The separation just mentioned may proceed in the normal gradual way or, if the external conditions have been changed drastically as in some experiments, in a more turbulent way. But in both cases the separation means a withdrawal of equivalent amounts of mineral cations and anions from the symplasmatic compartment which, due to the principles of the Donnan distribution, contains fewer anions than cations, the more so as the external salt concentration is less. The availability of anions thus limits sap separation. At low external concentrations sap separation may almost cease in spite of a high difference in osmotic pressure between existing sap and the external solution. At low external concentration System 2 works slowly. In fact, System 2 may have a rate which is more or less proportional to the concentration of symplasmatic anions as determined by the external salt concentration. This also explains why potassium uptake rates of System 2 depends on the kind and the concentrations of anions in the external solution (Hiatt, 1968; Exp. 34). Thus it is not just the tonoplast that governs the rate of System 2, but it is the conductance of the adjacent cytoplasmatic compartment for salts. As the concentration of the external solution increases, the passage of anions through the symplasm will be less discriminated, at the same time modifying the rate and nature of on-going vacuolation.

The dynamics of K influx across the plasmalemma (ϕ_{oc}) in relation to internal concentration $[K_i]$ of the excised roots was examined in Experiment 14. As Glass (1975) found for barley roots, Figure 23 shows that the plasmalemma influx ϕ_{oc} is inversely correlated with $[K_i]$. The marked decrease in tracer influx as the internal ion concentration increases suggests negative feedback by the cytoplasmic or vacuolar ion concentrations. A vascular efflux from the roots with high internal potassium concentration possibly added to the measured influx inhibition.

Some factors, probably involved in cell membrane behaviour affect influx and efflux of potassium quite differently. A reduction in cation influx ϕ_{oc} with decreasing external pH was observed. However, efflux under the same conditions did not show any pH effect. So during these short-term influx - efflux experiments, low pH effects are not caused by injury and consequent leakage of the root tissue. Types of injury suggested by Rains et al. (1964) (for instance denaturation of proteins, nucleic acids, phospholipids and other polymers in membranes), will possibly be involved only in the long term. Obviously, pH affects the potassium absorption by H^+ - K^+ competition, at least during these short-term experiments. A second but less probable explanation for the reduced K uptake is reduction of initial uptake or adsorption.

The idea that during short-term absorption experiments Ca affects the uptake of monovalent ions mainly through its effect upon permeability of the plasmalemma (Waisel, 1962), is not very attractive in the light of efflux experiments. The hypothesis of Kahn & Hanson (1957) that Ca present in the absorption solution increases the affinity between K and a postulated carrier seems to be more probable in short-term experiments (Viets effect).

Roots of plants, grown for longer periods before the experiment on a medium without calcium, show a decrease in Ca content and a strongly reduced influx during subsequent absorption of K. If an efflux or exchange period of 1 h directly follows the 4 h influx, a tendency for slow and gradual K - Rb exchange in the Ca-rich roots can be observed, whereas the Ca-starved maize roots have a fast 'explosive' release of Rb. This may indicate that Ca deficiency in the root results in an enhanced permeability of the outer cell membrane, the plasmalemma.

Changes in membrane permeability have been achieved artificially by the surface-active compound glyceryl triacetate. Kuiper (1967) showed release of solutes by bean roots to be promoted by solutions of acetylated glycerol compounds. I studied the effect of this surface-active chemical on membrane permeability indirectly by influx and efflux measurements of solutes by the root. Results (Fig. 33) illustrate that roots, bathing in an absorption solution containing 10^{-2} mol l^{-1} triacetin, show a significant increase in influx and a strong reduction in efflux, both resulting in a significant increase in net uptake of K. The obligatory presence of the surfactant in the absorption solution makes it reasonable to assume that the triacetin is directly coupled to either the solute or to the membrane (compounds). The formation of a surfactant - solute complex and a subsequent accelerated influx of this complex through the apolar interior of the lipid bilayer of the cell membrane, as described for the ionophore valinomycin - potassium complex by Kinsky (1970), is not very plausible for this glyceryl triacetate, because the structure of this chemical is totally different from an annular or ringlike compound like valinomycin. An alternative and more probable mode of action of triacetin is incorporation of several of these molecules into the membrane to form a physical channel in the membrane, a transit pore which admits cations and small uncharged molecules. A mechanism for the activity of acetylated compounds, as suggested by Kuiper (1972), in which surface active chemicals interfere with the hydrophobic regions of the membrane, resulting in a more hydrophyllic character of the membrane and an increased permeability to water and salt is another possibility. The negative influence of treatment of the roots on subsequent uptake from an absorption solution without surfactant can be the result of a 'wash-out' of dissolved membrane compounds. This could result in a loss of structure or in a structural collapse of the membrane and a subsequent increased efflux of the root cells. Since the effect of triacetin on influx and efflux is quite different for different ions means that also the selectivity of the biological membranes has been changed by this surfactant.

The effect of metabolic inhibition on influx and efflux of potassium was studied with CN and DNP, which inhibit respiratory electron-flow and uncouple oxidative phosphorylation, respectively, and by using chilled absorption solutions. Together with the results of Lüttge & Laties (1967), Drew & Biddulph (1971) and Lüttge (1975),the data confirm the inhibition of solute influx. Special attention was paid to the effect of these agents on efflux. As opposed to CN treatment, treatment of the roots of intact plants with increasing concentrations of DNP significantly increased the fraction of K (^{86}Rb) released immediately during a 'rinse-exchange' period of the roots after the uptake experiment. So high concentrations of DNP interfere by uncoupling energy transfer, and also have an indirect effect on root tissue, probably by a changed membrane structure. Treatment of roots with DNP

changes the normal shape of the uptake isotherm (Fig. 36). The metabolically driven part of the ion uptake is switched off and the remaining part of the K uptake consists of electrochemical transport.

The temperature of the absorption solution strongly affects the rate of net K uptake. Temperature mainly modified influx and not efflux.

A stimulatory effect on potassium influx was achieved by incubation of roots in a 1% (*W/V*) glucose solution. These low-salt roots with their high carbohydrate content show a positive effect of glucose. With light and dark treatment of intact plants, the glucose effect is not significant after a continuous lighting of the plant, but after a long dark-period glucose significantly increases the rate of K uptake by excised roots. These dark and light effects, just like the day and night sequence in K uptake indicate that glucose is an energy source for salt transport. Especially the high content of carbohydrate in the $CaSO_4$ roots in relation to roots of normally grown plants (Breteler, 1975) makes the behaviour of the gypsum roots quite different from high-salt roots. For example, a carboxylate effect on potassium uptake, as found by Breteler (1975), did not show up with this low salt material.

7.2 TRANSPORT MECHANISM OF SALT IN ROOTS

Besides the uptake of salts in the root, the transport of ions from the root (source) to the upper part of the plant (sink) is an essential process, because the bulk of inorganic salts can reach the growing shoot only by a longitudinal transport through xylem. The fact that both uptake and transport of salts are coupled, while the transport of salts is also coupled to the uptake and transport of water, makes it impossible to confine this transport model only to longitudinal transport of salt.

Most of the transport experiments were with complete root systems with cut stump derived from plants 5 weeks old, grown on complete nutrient solution (Table 1), until one week before the beginning of the experiment. With these older complete root systems, large volumes of exudate could be collected easily and accurately. In a few experiments, salt transport was studied with excised, low-salt roots, identical to the root material used in the uptake experiments, to get an impression about the rate of vascular salt efflux during uptake experiments. In study of simultaneous transport of water and solutes with decapitated root systems a considerable part of the water transport, related to transpiration, is eliminated. Consequently, the remaining xylem transport in exudation experiments is induced by osmosis.

While in uptake experiments, steady-state was reached within 1 h (Fig. 7), for salt transport it took 10 h or more. Exudation experiments must continue for at least 20-24 h. Because all transport experiments were with solutions of one salt and plants were grown for the previous week on a low-salt medium, $[K_i]$ and $[K_o]$ were indicators for π_i and π_o, respectively (Munting, 1977).

7.3 MODEL OF SALT AND WATER TRANSPORT

The common view how a root transports water from the external solution to the xylem stream has been described by Anderson (1975a), House & Findlay (1966) and Slatyer (1967). Water flow J_v across the root to the xylem can be described for intact plants and decapitated root systems by Equation 6 and 7 of Chapter 6.

$$J_v = L_p \ (\Delta P - \sigma n R T \Delta c_s) + \Phi_o \qquad (6)$$

$$J_v = L_p \ (-\sigma n R T \Delta c_s) + \Phi_o \qquad (7)$$

Excised roots behave like an osmometer. Salts are accumulated in the xylem sap so that the osmotic equivalent of the xylem sap (Π_i) exceeds the osmotic equivalent of the external medium (Π_o). As water moves by osmosis into the xylem, the xylem sap exudes out of the stump. Consequently, the substance flux of ions J_s is equal to the product of exudation rate J_v and the substance concentration of ions in the exudate c_i, or

$$J_s = J_v \cdot c_i \qquad (8)$$

Equation 7 indicates that the volume flux of water into the xylem depends on the osmotic equivalent difference $\Delta\Pi$ and on permeability of the root to water.

Experiment 34 confirmed the validity of Equations 7 and 8. Immediately after the start of uptake, a fast initial ion uptake together with a negligible salt transport in xylem result into a fast accumulation of salts and the build-up of an osmotic gradient. However, this process of salt accumulation will enhance both Π_i and J_s. The latter will then reduce the rate of salt accumulation. After a certain time Δt, net accumulation stops, because of the steadily rising transport in xylem J_s (Fig. 47). A steady-state is thus reached, characterized by:

- equal rates of uptake and transport;
- a steady flow of water and of exudation J_v;
- no net accumulation of salt within the root;
- a constant concentration of K in xylem $[K_i]$.

Obviously, the plant achieves steady-state by the substance concentration gradient of salts, as reflected in the osmotic equivalent difference between xylem sap and root medium. Under these conditions, the flux of salt in xylem is dependent only on the rate of salt absorption by the root, because amounts of salt equal to the freshly absorbed salt have to be transported upward to keep a constant Δc_K. Another characteristic of transport is that in steady-state Δc_K is almost equal for all external concentrations of salt within the range 0.1-10 mmol $K^+ \ l^{-1}$. So root cells have a mechanism that regulates uptake, accumulation and transport of salts, almost independently of the external concentration. Despite this constant Δc_K the volume flux of water J_v is not really constant for all external concentrations in the range 0.1-10 mmol l^{-1}. Looking back to Equation 7, with a constant $\Delta\Pi$ and the assumption of a small and constant Φ_o, a variation in J_v can only be the result of a variation in L_p, σ or both. Thus, as suggested by Klepper (1967), L_p

seems not to be constant for different concentrations of salt in the root medium.

The role of the anion on the transport pattern of both solute and solvent is significant (Fig. 56). In steady-state, Δc_K is constant for different external concentrations of some kinds of salt such as KCl or KNO_3, but totally different for other salts like K_2SO_4. Plants supplied with K_2SO_4 reach equilibrium at much lower $\Delta\Pi$ than plants supplied with KCl or KNO_3. As in equations 7 and 8, the low $\Delta\Pi$ with K_2SO_4 reduced J_v and even more J_s.

The difference between plants with KCl and K_2SO_4 could be caused by the differences in anion uptake. With KCl, uptake and transport of cations are almost equal to those of anions. With K_2SO_4, the absorption of the associated anion is far less and organic acids are synthesized in quantities equivalent to the cation excess (Hiatt, 1968; Lüttge, 1973). The concentration between K and SO_4 in xylem sap of K_2SO_4 plants was indeed different. A considerable part of the salts in the xylem sap is present in organic form, mainly as malates (Table 11). These carboxylates may either be directly involved in the accumulation and transport of water and solutes or indirectly by a change in L_p or σ. Another anion effect is the rate of translocation of freshly absorbed potassium from the root to the upper part of the plant. As in tobacco roots (Wallace, 1967), potassium with nitrate as counterion was transported much faster in maize roots than potassium accompanied by Cl or SO_4 (Table 10). This indicates that $K_{nitrate}$, in contrast to $K_{chloride}$ and $K_{sulphate}$, is transported almost directly from the outer solution through apoplasm and symplasm into the xylem stream, without first being mixed with or exchanged against a pool of potassium already present in the vacuoles of cortical cells. Potassium from a KCl salt was mixed with potassium from the pool before being transferred to the exudate.

The existence of this exchange supports to the views expressed in Section 7.1, indicating that errors in absorption data, caused by vascular efflux, are negligible in absorption experiments with tracers. A vascular efflux of potassium was absent during the first 8 h of an exudation experiment with excised low-salt gypsum roots (Fig. 49). On the other hand, maize roots loaded with potassium have a considerable vascular efflux of potassium within 1 to 2 h. However, since only a small fraction of the xylem K is labelled after 6 h, most of the potassium present in the exudate is derived from a potassium pool already present in the root tissue, while freshly absorbed, labelled potassium is accumulated temporarily in the root before being transferred to the xylem stream. In this way, all absorption data for up to 6 h and obtained by tracer techniques are free from errors that might arise from the considerable vascular efflux of K at the cut end. As found by Meiri (1973) and Hodges & Vaadia (1964), loading of roots with a potassium salt reduces the time to reach equilibrium in transport of K. Uptake and transport isotherms then show the same pattern (Fig. 51).

The isotherms presented in Figure 51 lack a dual character, supporting the view that duality could be an artefact with excised low-salt roots, or with short-term absorption experiments, because System 1 then has an abnormally predominant position.

Xylem sap was similar in composition to root-cell sap. Concentration of K in the cell sap of root systems, after 24 h of absorption of K from different solutions (Experiment 40), was 17.3-33.3 mmol l^{-1} and of xylem sap (Fig. 55) it was 18-34 mmol l^{-1}.

Despite high variance, time courses of absorption, accumulation and xylem transport

of K for decapitated root systems (Fig. 46, 47, 48), and for excised low-salt roots (Fig. 49) show clear similarity of interrelationships. So maize roots obey certain general rules as described before, irrespective of salt status, age, completeness or excision, branching or not.

Absorption rates of potassium by excised low-salt maize roots (Chapter 5) were about 10 mmol kg^{-1} DM h^{-1} but for decapitated roots were 15-65 mmol kg^{-1} DM h^{-1}, usually 25-30 mmol kg^{-1} DM h^{-1}. Perhaps attached and detached roots may differ slightly in ion absorption and ion transport rates, at least if expressed on basis of dry mass of roots. Substance flux of K and volume flux of water were both inhibited immediately after the addition of CN and DNP to the absorption solution (Exp. 41). After addition of glucose, a new steady-state was reached, because of the increased internal osmotic equivalent Π_i. In this new situation, both influx ϕ_{oc} and xylem flux J_s were higher than before, because absorption was stimulated by glucose.

Extrapolation of salt and water transport in excised roots and root systems with cut ends to transport in intact plants is difficult. Transpiration in intact plant would enhance the longitudinal upward water stream and dilute the internal concentration of salt (Munting, 1977). In intact plants, Equation 6 will apply, but the hydraulic component will, dependent on the rate of transpiration of the shoot, account for the majority of the volume flux of water J_v. With this passive upward flow of water, the majority of the absorbed salt is also transported upwards. As a consequence, the internal concentration in entire transpiring plants will be low and the role of osmosis in water and salt transport will be smaller in intact plants than in excised roots. Even so, it would be nice to know more about the contribution of the osmosis to salt and water transport.

Summary

Uptake of nutrients in roots and subsequent transport of these substances from root to aerial parts of plants form the foundations of mineral nutrition of plants. Especially the first step, absorption of ions into roots is a process governed by a complex of internal and external factors. The second phase, transport of ions towards xylem vessels (radial transport) and upward transport within xylem vessels (longitudinal transport), is closely linked to ion absorption. After passing the plasmalemma of root cells salts are, either unchanged or after transformation, translocated to aerial parts of the plant.

In the literature, uptake and transport of minerals in plants have been described in detail. However, little effort has been paid to simultaneous study of absorption and transport of ions.

This report deals with effects of various internal and external factors on absorption of potassium in maize roots (Chapter 5). Absorption - exudation experiments are described and simultaneous absorption, accumulation and xylem transport of potassium in maize roots are discussed (Chapter 6). Most exudation experiments were with complete root systems cut from maize plants 5 weeks old.

Absorption of potassium in excised roots of maize plants (about 10 days old) was studied after altering the permeability, internal salt concentration and energy status of root cells. Permeability of cell membranes (plasmalemma, tonoplast) was altered by incubating maize roots in solutions different in pH and temperature, by withholding calcium and by treating with the inhibitors cyanide (CN) and dinitrophenol (DNP) and the surface-active chemical triacetin. Energy status of the roots was altered by treatment of the roots in solutions of DNP, CN and glucose. Cellular salt concentration in general, or concentrations of potassium, organic nitrogen compounds and inorganic and organic anions particular were altered by different treatments of the roots.

Effects on fluxes of potassium in excised maize roots low in salt (Chapter 5) seemed to be mostly restricted to influx in the short-term (4-10 h). Addition of the surface-active chemical triacetin to the absorption solution simultaneously increased influx, decreased efflux and thus stimulated net absorption of potassium in the maize root. Low pH of absorption solution and low temperature as well as calcium starvation of roots significantly inhibited influx of potassium. On the other hand, increased endogenous concentrations of glucose and amino acids increased influx of potassium. Besides the role of DNP as metabolic inhibitor, this chemical seemed at high concentrations to alter membrane permeability.

Relative to uptake kinetics, titration - uptake experiments (Section 5.1) demonstrated a three-phase absorption - time curve rather than the traditional two-phase one (initial phase and a steady-state). One of the features of the high-salt absorption isotherm (System 2), e.g. anion-dependent cation uptake, was confirmed in short-term absorption

experiments, whereas in longer absorption-transport experiments, no dual isotherms could be demonstrated.

Absorption - exudation experiments with complete root systems of maize plants 5 weeks old (Chapter 6) indicated the following.

- In potassium-starved roots, absorbed potassium all accumulated initially. Consequently, cellular concentration of salt and osmotic equivalent increased with time. So exudation rate and upward transport of potassium increased steadily. In steady-state, upward transport of potassium in xylem equalled absorption of potassium in the root, while net accumulation of potassium stopped and concentrations of potassium in xylem sap stayed constant.
- Roots rich in potassium demonstrated a considerable upward transport of potassium in xylem directly after starting the experiment, because of the high internal concentration of salt. However, experiments with labelled potassium (^{86}Rb) indicated that only a fraction of the freshly absorbed labelled potassium was transported straight to xylem and subsequently upwards in the xylem vessels. Part of the freshly absorbed potassium exchanged with potassium already present in the root cells. So part of the freshly absorbed potassium was stored temporarily in a pool, probably in vacuoles of cortex cells, and net upward transport of potassium kept constant.
- This exchange of freshly absorbed potassium with potassium already present in root cells, depended on the nature of the anion. So potassium with nitrate as counterion exchanged more slowly with the pool than potassium with sulphate.
- Different external concentrations of salt resulted in equal osmotic equivalent differences between outer solution and xylem sap. However, the osmotic equivalent in steady-state was different for KNO_3 and KCl solutions from that for K_2SO_4 solutions. The difference was expressed in rate of exudation and of transport of potassium upward in xylem.

Although transport of salts in excised root systems differs from transport in intact transpiring plants in some respects, the data have contributed to a better understanding of salt transport in maize plants. This salt transport from the roots resembles the part of the salt uptake known as System 2.

Samenvatting

De opname van voedingsstoffen door de wortel en het transport van deze stoffen vanuit de wortel naar de bovengrondse delen zijn de belangrijkste processen in de mineralenvoorziening van een plant. Vooral de eerste stap, de opname van ionen door de plantewortel, is een proces dat sterk afhankelijk is van tal van interne en externe factoren. De tweede fase, transport van ionen naar de houtvaten (radiaal transport) en opwaarts transport in de houtvaten (longitudinaal transport), sluit aan op de opname. Na het passeren van de plasmalemma van de wortelcellen worden zouten, al dan niet na omzetting, afgevoerd naar bovengrondse delen van de plant.

De opname en het transport van mineralen en water in de plant worden in de literatuur uitvoerig beschreven. Onderzoekingen naar de directe samenhang tussen opname en transport van zouten in de plant zijn echter schaars.

Dit verslag behandelt enerzijds de invloed van een aantal inwendige en uitwendige factoren op de opname van kalium in de maiswortel (hoofdstuk 5), anderzijds worden een aantal opname - exudatieproeven beschreven waarin de gelijktijdige opname, accumulatie en transport (afvoer) van kalium in de maiswortel gemeten zijn (hoofdstuk 6). Dit laatste gebeurde veelal met behulp van volledige, afgeknipte wortelstelsels van vijf weken oude maisplanten.

De opname van kalium in afgeknipte wortels van maisplanten (ongeveer 10 dagen oud) werd bestudeerd nadat al dan niet ingegrepen was in de membraanpermeabiliteit, het interne zoutgehalte en de energievoorziening van de wortelcellen. Wijzigingen in de membraanpermeabiliteit (plasmalemma, tonoplast) werden bewerkstelligd door dompeling van de maiswortels in oplossingen (verder buitenoplossingen genoemd) van verschillende zuurgraad en temperatuur, door onthouding van calcium en door een behandeling met de remstoffen CN en DNP en de oppervlakte-actieve stof triacetin. In de energievoorziening werd ingegrepen door toevoeging van glucose, DNP en CN aan de buitenoplossing. Het interne, cellulaire zoutgehalte in het algemeen, of het gehalte aan kalium, organische stikstofcomponenten en anorganische en organische anionen in het bijzonder, werd gevarieerd door middel van diverse voorbehandelingen van het wortelmateriaal.

Influx- en effluxmetingen van kalium wezen uit dat gedurende de kortdurende experimenten (4-10 h) effecten veelal beperkt bleven tot de influx. Een duidelijke wijziging in zowel influx als efflux van kalium werd verkregen door toevoeging van triacetin tijdens de opname. Lage pH en lage temperatuur van de opname-oplossing, evenals calciumgebrek van de wortel remden de influx van kalium duidelijk af. Daarnaast had een verhoging van het inwendige suiker- en aminozuurgehalte der cellen een positieve invloed op de kaliuminflux. Duidelijk werd dat DNP niet alleen invloed had op de energievoorziening van de wortel, maar eveneens op de membraanpermeabiliteit (hoofdstuk 5).

Verder bleek de opname van kalium bij hoge concentraties in de buitenoplossing afhankelijk te zijn van de opnamesnelheid van het begeleidend anion (systeem 2). In tegenstelling tot het traditionele 2-fasen-beeld van de opname - tijd-relatie, namelijk een initiële en een steady-state-fase, werd in dit onderzoek een 3-fasen-verloop aangetoond (hoofdstuk 5).

Opname - exudatieproeven met volledige wortelstelsels van vijf weken oude maisplanten (hoofdstuk 6) wezen uit dat:
- bij kalium-arme wortels de opgenomen kalium aanvankelijk geheel accumuleerde in de wortel. Als gevolg hiervan nam het interne zoutgehalte in de wortel en dus de osmotische spanning toe. Dit resulteerde in een toename van de exudatiesnelheid en het opwaarts transport van kalium. Een dynamisch evenwicht werd bereikt, waarbij opwaartse afvoer van kalium gelijk was aan de kaliumopname door de wortel. Vanaf dat ogenblik vond er geen netto accumulatie van kalium in de wortel meer plaats. Bovendien werd deze steady-state-fase gekenmerkt door een constante kaliumconcentratie in het xyleemsap.
- kalium-rijke wortels als gevolg van een reeds hoog intern cellulair zoutgehalte vanaf de aanvang van de proef een aanzienlijk opwaarts transport van kalium vertoonden. Proeven met gelabeld kalium (^{86}Rb) wezen echter uit dat niet alle vers opgenomen kalium rechtstreeks via het xyleem opwaarts afgevoerd werd, maar voor een gedeelte omwisselde met kalium die reeds in de wortelcel (vacuole) aanwezig was. Op deze wijze werd een gedeelte van de vers opgenomen kalium tijdelijk in de wortelcellen opgeslagen, terwijl de netto afvoer van kalium via het xyleem constant bleef.
- deze omwisseling van vers opgenomen kalium met reeds in de wortelcel aanwezige kalium afhankelijk was van de aard van het begeleidend anion. Zo bleek kalium met het begeleidende anion nitraat deze omwisseling veel minder intensief te vertonen dan bij de aanwezigheid van sulfaat.
- verschillen in externe concentraties van één zout leidden tot eenzelfde osmotisch drukverschil tussen buitenoplossing en xyleemsap. Dit osmotisch drukverschil nam echter een andere waarde aan bij de aanwezigheid van een ander zout. Dit leidde onder steady-state-condities tot grote verschillen in opwaarts transport van kalium en water voor deze beide zouten.

Alhoewel het transport van zouten in afgeknipte wortelstelsels in sommige opzichten afwijkt van dat in normale, intakte transpirerende planten, dragen de op deze wijze verkregen resultaten bij tot een verruiming van het inzicht in het zouttransport in de maisplant. Dit zouttransport vanuit de wortels gelijkt het meest op het als systeem 2 aangeduide deel van de zoutopname.

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