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A new concept of root exudation[1]

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ABSTRACT

After discussing numerous models for exudation from the xylem of roots, we present a new biphasic exudation model based on osmoregulation of the root symplast by stretch-activated ion channels (SA channels). We tested some features of the model in maize roots. (1) Using a microdrop recorder we showed that bathing the roots in 50 mmol m^{-3} gadolinium ions, known to inhibit some SA channels, inhibited xylem exudation by over 80% after 24h application. (2) Measuring xylem exudation from single roots into an attached micropipette revealed the capacity of the roots to perform strong autonomous exudation pulses. (3) In partially encased roots, the rhizodermis exuded water concurrently to xylem exudation. These results were regarded as supporting our model. An interesting observation with the microdrop recorder, which does not address the theory, is that addition of a variety of inorganic ions to distilled water as the roots' bathing medium instantaneously and reversibly increases xylem exudation, evidently nonosmotically.

Key-words: root exudation; stretch activation; ion channels; gadolinium; exudation pulses; rhizodermis exudation; *Zea mays*.

INTRODUCTION

The secretion of water from the vessels of excised roots, root xylem exudation, is a ubiquituous phenomenon in cormophytes (Wieler 1893). For the past few decades, the osmometer model of root xylem exudation (see below) has become the leading paradigm, but its insufficiency is obvious. Searching for a more satisfying explanation of root exudation we tried to test the possible explanations.

We postulate that the exudation involves at least a three-compartment system with: C_1 = outside medium; C_2 = symplast of the root parenchyma cells and living vessel elements; and C_3 = exudation fluid. The compartments are aligned in series, separated by two membranes (M_{12}, M_{23}). M_{12} is represented by the rhizodermis plasma membranes at the root surface, M_{23} consists of the plasma membranes inside the root that secrete the exudation fluid. According to this three-compartment exudation system, possible models can be classified as mono- or biphasic. Monophasic models propose a unidirectional water flow; biphasic models anticipate that the water uptake of the symplast alternates with its water release. So far, only monophasic models have received appreciable attention; however, the results of Mozhaeva & Pil'shchikova (1972) and Lazareva, Borisova & Zholkevich (1986), who report auto-oscillations of xylem exudation, and a phase shifting of water uptake and exudation, in sunflower and maize roots respectively, justified consideration of biphasic models as well. Their results can be much more easily explained by a biphasic model.

Mono- and biphasic models may be more closely specified by the mechanism of water flow across the two membranes, and by the kind and location of the driving force of water flow.

In principle, water flow may be convective or diffusive. Convective flow is controlled by the influence of an external force; for example, a pressure or temperature gradient. The movement of the single molecules gains a preferential direction by impulse transfer from other molecules. Diffusive flow across a membrane is a purely statistical process based on the kinetic movement of the molecules. It is usually called osmotic if the membrane exhibits a higher permeability for the solvent of an adjacent solution than for its solutes. Osmotic flow follows a gradient in the water potential $\psi = P - \sigma \cdot \pi$, where P is the hydrostatic pressure, σ the reflection coefficient of the membrane and π the osmotic pressure. The reflection coefficient σ is the ratio of the osmotic pressure of the solutes retained by the membrane to the osmotic pressure of all solutes that hit the membrane. σ is lower than 1 if not all solutes are reflected by the membrane. The so-called active transport may be classified as convective water transport, for the water molecules are not moving along their own diffusive gradient, but are transported by impulse transfer from other molecules. Active water transport is almost exclusively carried out by ions, and therefore, it is called 'electro-osmosis'.

Monophasic xylem exudation models

The osmometer model

The most simple monophasic model is the already mentioned osmometer model, which supposes the driving force of xylem exudation to consist in the difference

[1] Essential parts of this paper are based on the dissertation of Schwenke (1990).

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in the osmotic potential between the exudation fluid (π_3) and the outside medium (π_1). Exudation only can occur if $\pi_3 > \pi_1$. Pfeffer (1877) formulated the basic principle of the osmometer model, but he (Pfeffer 1897) and also Wieler (1893) regarded it as unsuitable to explain xylem exudation, because they found π_3 to be very low in some vigorously exuding species, especially in vine.

Later on, the model was elaborated and disseminated by Atkins (1916), Priestley (1922) and Sabinin (1925; see Lyalin 1989). It has become a kind of a doctrine in plant physiology since the 1950s. However, in its basic version the osmometer model was already refuted by Van Overbeek (1942), who observed xylem exudation against a concentration gradient between outside medium and exudation fluid, which we call 'uphill exudation' in the following text (see also Broyer 1951; Mozhaeva & Pil'shchikova 1972, 1976, 1979; Sinitsyna, Peisakzhon & Zholkevich 1977; Zholkevich *et al.* 1979). In order to explain uphill exudation by a monophasic model, active transport by ions (electro-osmosis), or standing concentration gradients seem to be the only realistic possibilities.

Electro-osmosis

Active water transport by ion movement from the outside medium to the exudation fluid via the cell wall, as suggested by Tyree (1973), is restricted to xylem exudation near the root tip, where the Casparian strip has not yet formed.

An exudation model based on electro-osmosis via channels in M_{23} seems to suffer from a much too low coupling ratio (i.e. number of water molecules transported per ion) (see Dainty 1963). The problem of low coupling ratio could be circumvented by 'ion recycling' through backtransport of ions without adhering water molecules. Küppers, Plagemann & Thurm (1986) suggested such a mechanism for the water uptake of Lepismatidae. One main disadvantage of this concept in respect to root exudation lies in its expenditure of energy. Therefore, it should be considered only if a more economical model is not available.

Standing gradient concepts

It is not the average solute concentration of a compartment, but only the solute concentration at the membrane which is relevant to osmosis. So-called standing gradient concepts of water transport operate with a higher or lower osmotic pressure at the membrane surface in comparison to the average osmotic pressure of the compartment. First, we will review models that propose a standing gradient within C_3. Pfeffer (1877, 1897) and Wieler (1893) have already considered concentration gradients in C_3, but thought that they could be only transient.

Anderson, Aikman & Meiri (1970) proposed a concentration gradient within the vessel lumens due to solute transport from the symplast, by analogy with Diamond & Bossert (1967). This assumption was made improbable by the results of Sinitsyna *et al.* (1977) and Zholkevich *et al.* (1979), who cut roots at different distances from the root tip, collected the exudation fluid, and failed to detect the predicted rise in exudate concentration towards the root tip. Furthermore, there may be doubt whether any appreciable vessel lumen exists in young exuding roots, for the large late metaxylem vessel elements in roots of maize and soybean have been found to persist as living cells quite far from the root tip and therefore belong to the symplast (St Aubin, Canny & McCully 1986; Kevekordes, McCully & Canny 1988).

The reverse-osmosis model of Lyalin (1989) likewise explains uphill exudation by a concentration gradient in the vessel lumens. Passive solute efflux to the vessels and the outside medium is an essential feature of this concept. The vessel lumen is treated as limited and unmixable, the outside medium as practically infinite. As a consequence, solute efflux leads to a concentration gradient in the vessel lumen but not in the outside medium. In the light of the previously mentioned results of Sinitsyna *et al.* (1977) and Zholkevich *et al.* (1979) such a gradient does not occur in the vessel lumen, but it could be localized in boundary layers of M_{23}. However, membrane boundary layers might also exist at M_{12}. In addition, the model faces difficulties if most of the vessel space belongs to living vessels.

The model of Katou, Taura & Furumoto (1987) represents a further development of the concept of Anderson *et al.* (1970) mentioned above. It is based on a concentration gradient within the cell wall inside the Casparian strip of the endodermis, which is caused by solute transport from the symplast. The main failure of this model may be that it cannot apply to exudation from other parts of the plant where there is no Casparian strip. As described by Wieler (1893) and Pfeffer (1897), exudation occurs in almost all plant organs (e.g. in twig segments) of which one end has been sealed. It seems very likely that the mechanism of exudation is the same, everywhere in the plant. In addition, one has to conclude from the work of Perumalla & Peterson (1986) that root xylem exudation can already be found when the Casparian strips have not yet, or not completely developed.

The test tube model (Hylmö 1953) that has been revived by McCully & Canny (1988) assumes xylem exudation to be a consequence of vessel maturation. Hylmö takes the driving force of exudation to consist in the expansion of the intact tonoplast after cell wall rupture. To our mind, this is not an optimal version of the model. As far as we can see, the postulated mechanism would mainly lead to an increase of the volume of the maturing vessel at the expense of the water column within C_3. If tonoplast or plasmalemma remain intact during maturation most of the water will be withdrawn from the apoplast, especially from the

lumens of mature vessels, and not from the symplast, for the water potential may usually be much lower in the symplast (down to −0·5 MPa, see e.g. Ursprung & Blum 1925) than in the xylem sap (frequently some −0·1 MPa). The model might be more powerful if it were designed as a standing gradient concept: From the rupture of the plasmalemma and tonoplast of maturing vessels arise short-time concentration gradients in C_3, for the concentration of the immature, living vessels is much higher than of the exudation fluid. If vessel maturation takes place continuously, generation of new gradients at new sites will occur, resulting in a macroscopically uniform exudation flow. However, Hylmö's model is not suitable to explain exudation from plant organs lacking maturing vessels.

Based on the work of Hofmeister (1862) and Sachs (1865), Pfeffer (1890, 1892, 1897) designed an exudation model based on a concentration gradient within C_2. The osmotic pressure of C_2 is assumed to be lower at M_{23} than at M_{12}. If the high hydrostatic pressure generated at M_{12} is transmitted throughout C_2 to M_{23}, then a secretion of water across M_{23} into C_3 can occur. Uphill water transport is possible. This 'pressure secretion' model prevailed at the end of the last century and was also favoured later on by Ursprung & Blum (1925). To our mind, it offers the neatest explanation of uphill exudation, but there are no data available that prove the existence of osmotic and hydrostatic gradients within the symplast able to account for pressure secretion.

Reflection coefficient models that are designed to cope with uphill exudation may also be classified as standing gradient models. One can distinguish two types of reflection coefficient exudation models.

The first such model operates with a M_{23} containing pores that allow for substantial convection. Hence their reflection coefficient must be very close to zero. (See the calculations of diffusive and convective flows as a function of pore radius by Atlan & Thoma 1985). The hydrostatic pressure in the symplast causes a convective flow of water and ions into the vessels. Similar to the 'pressure secretion' model, the concentration gradient which enables uphill exudation is located within C_2 at M_{12}. The prototype of this concept is Münch's pressure flow hypothesis of phloem transport (Münch 1926). Better known in general physiology is the similar model of Curran & MacIntosh (1962). Ginsburg (1971) modified their model and transferred it to root exudation. He supposed open plasmodesmata ending in the vessels to be the membrane pores allowing the convection. However, it is very unlikely that plasmodesmata will remain open after vessel maturation.

The second reflection coefficient model assumes that only osmotic forces govern exudation. To our knowledge, this concept has not been discussed explicitly in the literature. Its basic feature consists in a greater leakage of solutes into C_3 than into C_1, due to a lower reflection coefficient of M_{23}. To account for uphill exudation it has to postulate a concentration gradient in C_3 at M_{23} established by solute leakage from C_2, similarly to Lyalin's model.

Biphasic exudation models

From the preceding discussion, we may conclude that only osmotic flow, but not active transport or hydrostatic convection, should be considered as responsible for exudation. Consequently, biphasic models should only be based on water potential oscillations. If the water potential is $\psi = P - \sigma \cdot \pi$, oscillations in water potential could result from changes of concentration, turgor, or reflection coefficient.

Concentration oscillations

Godlewski's model (Godlewski 1884), and similarly, later on, Lundegårdh's concept of 'extra water' exudation (Lundegårdh 1950), suggest concentration alternations in the symplast, caused by de- and repolymerization of symplast molecules. First, there is no hint of such a polymerization oscillation in root cells, and second, the model does not explain how the water flow can take a preferential direction (see Wieler 1893). This could be realized only by a mechanism simultaneously changing the hydraulic conductance of the membranes and the concentration oscillations.

Turgor oscillations

Bose's pulsation model (Bose 1923), also considered later by Mozhaeva & Pil'shchikova (1972) and other Soviet investigators, assumes turgor alternations by muscle-like contractions of the root cells. In principle, such contracts could be performed by the cytoskeleton, but they appear to be impossible in plant cells with rigid cell walls. Therefore, the results of Lazareva *et al.* (1986) and Zholkevich (1991) who found cytoskeletal agents to extinguish the auto-oscillations of xylem exudation have to receive another explanation. The problem of a preferential direction of water flow remains unsolved, as in Godlewski's model.

The new model: oscillations of the reflection coefficient

A third kind of biphasic exudation model could be based on oscillations in the reflection coefficient of M_{12} and M_{23}. In biological membranes the reflection coefficient mainly depends on opening and closing of ion channels. In a new exudation model, we regard exudation as a consequence of symplast osmoregulation by stretch-activated channels [SA channels] (for a review of mechanosensory ion channels see Morris 1990). Hence, we postulate opening and closing of the membrane channels to be triggered by symplast turgor. If a certain threshold turgor in C_2 is reached, ion channels sensitive

to membrane-stretch open, and water and ions are expelled from the symplast into C_1 and C_3. In a more complicated variant, the channels that carry out water and ion outflow, might be activated by a SA channel-triggered signal transduction chain (Christensen 1987). After turgor reduction, the channels close and volume flow through the lipid bilayer into C_2 starts again. The new approach does not depend on any root-specific features like Casparian strips or maturing vessels to explain exudation, and therefore, may be applied to exudation from all plant organs. The model may provide an explanation for the influence of cytoskeletal agents on exudation mentioned above, for there is some evidence that SA channel function depends on the cytoskeleton. Since volume decrease by SA channels can be regarded as an archaic strategy of cells (Schultz 1989), our model would make it easy to explain the widespread occurrence of exudation. A biophysical account of the model is presented in the appendix including a possible explanation of uphill exudation.

Here we report on the first experiments carried out to test some features of the model in maize roots.

MATERIALS AND METHODS

Roots

We used the primary roots of 4-d-old maize seedlings (*Zea mays* L., cv. Brio, Asgrow Co., Buxtehude, Germany), which were germinated and grown on vermiculite with distilled water as described in Kutschera & Schopfer (1985).

Chemicals

In the experiments with the microdrop recorder (see below), the following substances were used: barium chloride (Sigma, Deisenhofen, Germany), caesium chloride (Roth, Karlsruhe, Germany), gadolinium chloride hexahydrate (Aldrich, Steinheim, Germany), lanthanum chloride (Sigma), sodium chloride (Roth), potassium chloride (Merck, Darmstadt, Germany), quinine hydrochloride (Sigma), and tetra-ethylammonium chloride [TEA] (Sigma).

Cumulative xylem exudation rate of several roots: the microdrop recorder method

The collective xylem exudation rate of 24 or 30 roots was measured simultaneously at a constant temperature of 23 °C with a microdrop recorder developed according to Zhu, Wang & Lou (1986). The measuring principle is the following: A tiny drop emerges from a capillary tip, touches a piece of cardboard and is immediately sucked into it. This short-time bridge between the capillary tip and the wet cardboard closes a circuit. The current is used as the signal for one drop. Drop size is well defined by the distance between capillary orifice and cardboard. The arrangement of the apparatus is shown in Fig. 1. Experimental control and data processing were done by a specifically developed PC-program, which allowed selection of measuring cycles in steps of one second. Calibration of drop volume can be done by injection of a small known volume into the system. The drop volumes usually ranged between 10 and 30×10^{-12} m^3, but values below 1×10^{-12} m^3 may be obtained. In our experiments, the optimum diameter of the capillary orifice lay between 70 and 100 μm.

The experiments with the microdrop recorder were carried out in the following manner: The seedling boxes were flooded with tap water, the vermiculite was loosened by hand, the seedlings were removed from the vermiculite, and their main roots were cut with surgical scissors and briefly immersed in aerated distilled water in a black plastic pot until use. Using a syringe, the collecting spindle was filled with distilled water and hung over a pot filled with distilled water. The roots were attached to the collecting spindle by means of rubber tube segments. The collecting spindle was then connected with the drop recorder, whose lumen had previously been filled with a 20 mol m^{-3} KCl-solution. An air bubble was enclosed at the connection site to separate the distilled water from the KCl solution. The roots were placed in the aerated control medium in a black pot. After optimal positioning of the capillary tip, found out by artificial drop creation with a syringe, the measurement was started. In general, exudation started after some hours and increased gradually to reach a relatively stable plateau after 6–12 h. During this preliminary run, corrections in the positioning of the capillary tip were sometimes necessary to obtain a satisfactory drop formation. When exudation had reached a rather constant level, the test substances were added to the root medium or to another pot which was then exchanged with the first pot.

Xylem exudation rate of single roots: the micropipette method

The xylem exudation rate of a single maize root was too low to be measured by the microdrop recorder. Therefore, we used a micropipette method that required a lower exudation rate and permitted a higher resolution, but was much more demanding to work with.

Fourteen roots were immersed in aerated distilled water in a black pot at a constant temperature of 23 °C, and were attached to one-way-micropipettes with an inner diameter of 0·12 mm (Hilgenberg, Malsfeld, Germany) by means of silicon rubber tube segments and flexible polyethylene tubing. Tubes and pipettes were filled with distilled water. A small drop of methyl acetate stained with Sudan black (1 kg m^{-3}) was enclosed as a marker in the pipettes. An air-filled, sealed glass tube connected to a micropipette, which was placed in the same pot as the roots, served as a control. The 15 pipettes were mounted side by side on a support and all

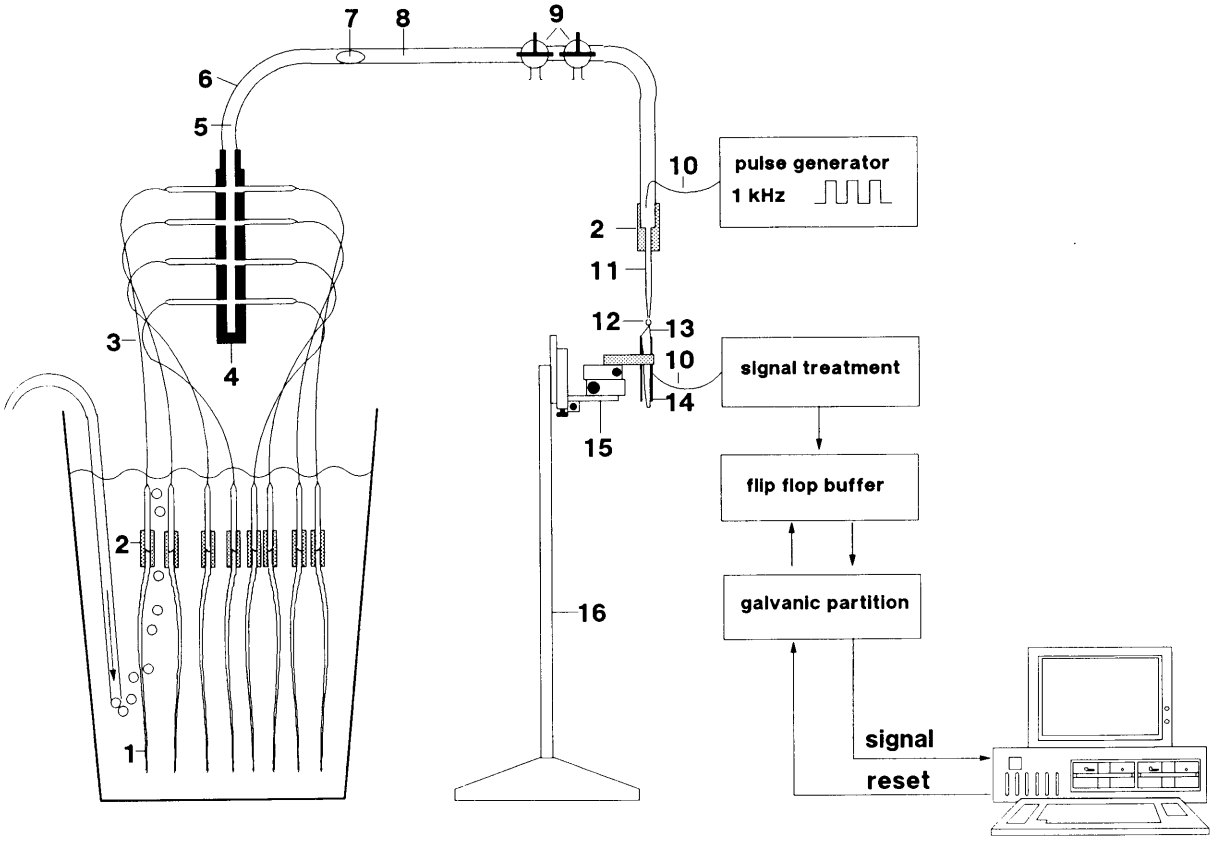

Figure 1. Flow diagram of the microdrop recorder and the maize root incubation system: (1) maize root; (2) rubber joint; (3) polyethylene tubing; (4) collecting spindle; (5) exudation fluid; (6) glass tubing; (7) air bubble separating exudation fluid from KCl-solution; (8) 20 mol m^{-3} KCl- solution; (9) three-way-cocks; (1) platinum electrode; (11) capillary; (12) drop; (13) cardboard; (14) metal sleeve; (15) micromanipulator; and (16) support.

together photographed at intervals of 30s with a clock included in the field. The camera used was a Contax 139 Quarz with a Yashica ML 1,4/50mm objective and the microfilm Agfa Copex Pan A.H.U. TRI 13. The positions of the stained droplets were determined by means of an image analysing system combining a 'Göttinger Meßtisch' (Gebr. Merzhäuser Wetzlar GmbH, Wetzlar, Germany; see Pretzsch 1990), a wild Makroskop M 420, and a Panasonic WY-CCD-50 video camera. The standard error of these measurements was estimated with the concomitant measurement of a scale on the film.

We also recorded single micropipettes attached to roots with a video camera (Grundig-FA82-CCD) at 20× magnification and studied single root exudation with a microscope using a measuring ocular and different pipette types with an inner diameter of up to 0·58mm.

Rhizodermis exudation

The experimental setup is shown in Fig. 2a. The upper end of the root was connected via a rubber tube segment to a 100mm^3 pipette. The upper half of the root was enclosed in a glass tube sealed to the root above and below using a paste (Silopren Paste VP I 4036, Bayer, Leverkusen, Germany). The lower part of the root was immersed in aerated distilled water at constant temperature of 23°C. The experiments lasted 2·5d and were carried out with 25 roots.

RESULTS AND DISCUSSION

Blocker effects

According to our model, exudation is the immediate consequence of symplast volume decrease. It should be inhibited by blocking of the SA channels that are supposed to carry out or trigger this volume decrease. After 24h, 50 mmol m^{-3} gadolinium chloride was able to reduce xylem exudation below 20% of a control set (Fig. 3). However, the speed of inhibition could not be accelerated markedly by millimolar concentrations of Gd^{3+}. Quinine and Ba^{2+} yielded a comparable exudation inhibition only at a concentration near 1 mol m^{-3} in the bathing medium, though especially quinine acted much faster than Gd^{3+}. For La^{3+} and TEA, we observed

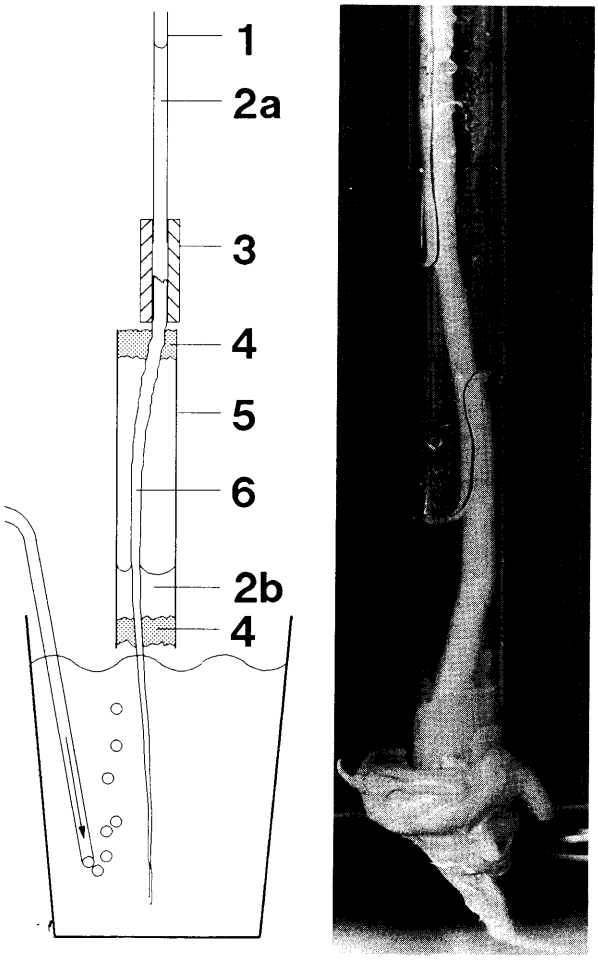

Figure 2. (a) Experimental setup for demonstrating rhizodermis exudation: (1) capillary; (2a) xylem exudation; (2b) rhizodermis exudation; (3) rubber joint; (4) silicon rubber cement; (5) glass cylinder; and (6) maize root.
(b) Photograph of rhizodermis exudation of an excised maize root after 2·5 d in distilled water at 23 °C.

no substantial inhibition effect on xylem exudation at millimolar concentrations.

Gd^{3+} is a well known blocker for SA channels (Gustin *et al.* 1988; Yang & Sachs 1989). The work of Moran, Fox & Satter (1990) proved that Gd^{3+} can strongly block K^+ currents that might be responsible for cell volume decrease in *Samanea saman* leaflets. From their work, it remains unclear whether Gd^{3+} interacts directly or through calcium-activation with a voltage-dependent plasmalemma K^+ channel capable of carrying the large K^+ currents that flow from shrinking cells during leaflets movements. If Gd^{3+} actually inhibits exudation by channel blocking at the exudating plasma membranes, it has either to pass the apoplast or to enter the symplast of maize roots. The former would require the absence of Casparian strips in the experimental maize roots. The capacity of Gd^{3+} to enter the symplast of maize roots in at least small amounts has been proven by Quiquampoix *et al.* (1990). The low membrane permeability they found may account for the long lag time required of the Gd^{3+}-induced inhibition of xylem exudation to appear. Since Gd^{3+} acts at quite low concentrations in the bathing medium, its effect seems to be specific. However, the long lag time even at millimolar concentrations necessitates further investigation. The antagonism of initial stimulation (see below) and later inhibition of exudation by Gd^{3+} may be significant in resolving this problem.

The xylem exudation inhibition by quinine and Ba^{2+} may be a consequence of ion channel blocking, but it must be suspected that these substances exhibit strong toxic effects on the root cells at millimolar concentrations in the bath, so that we do not want to present and discuss these results in detail. The almost negligible inhibition effects of TEA and La^{3+} might be due to a low specificity for the putative SA channels or a weak penetration into the roots.

Instantaneous xylem exudation increase after salt application

Except for quinine hydrochloride, all salts tested ($BaCl_2$, CsCl, $GdCl_3$, KCl, $LaCl_3$, NaCl, TEA), caused a marked instantaneous increase in xylem exudation after addition to distilled water as the bathing medium. The long-term inhibitory effects of Ba^{2+} and Gd^{3+} only set in later on. The instantaneous exudation increase already appeared at concentrations below 0·1 mol m^{-3}. It could be weakened, fail to appear or even become negative when another salt was already present in the incubation medium. In the case of CsCl (Fig. 4), $LaCl_3$, KCl and NaCl, we alternately applied the salt solution and the control medium and found a reversibility of the stimulatory effect.

These strong salt effects may be the result of changes in the electrostatic properties of the outside plasma membranes leading to activation of ion pumps or channels. An osmotic action of the salts can be ruled out, because some ions tested only slowly penetrate the plasma membranes (e.g. Cs), and the effect already occurred at submillimolar concentrations. There has been a considerable discussion in the context of the osmometer model whether potassium is an ion necessary for exudation because of its contribution to the osmotic pressure of the exudation sap (see van Steveninck, van Steveninck & Läuchli 1988, and the literature cited there). Our results show that potassium and other ions can strongly stimulate exudation probably by acting at the root surface and evidently not by increasing the osmotic pressure of the exudation sap.

Xylem exudation pulses of single roots

The simultaneous xylem exudation measurements of single roots with the micropipette method revealed the existence of extremely strong autonomous exudation pulses (Fig. 5), which are nonetheless not a necessary

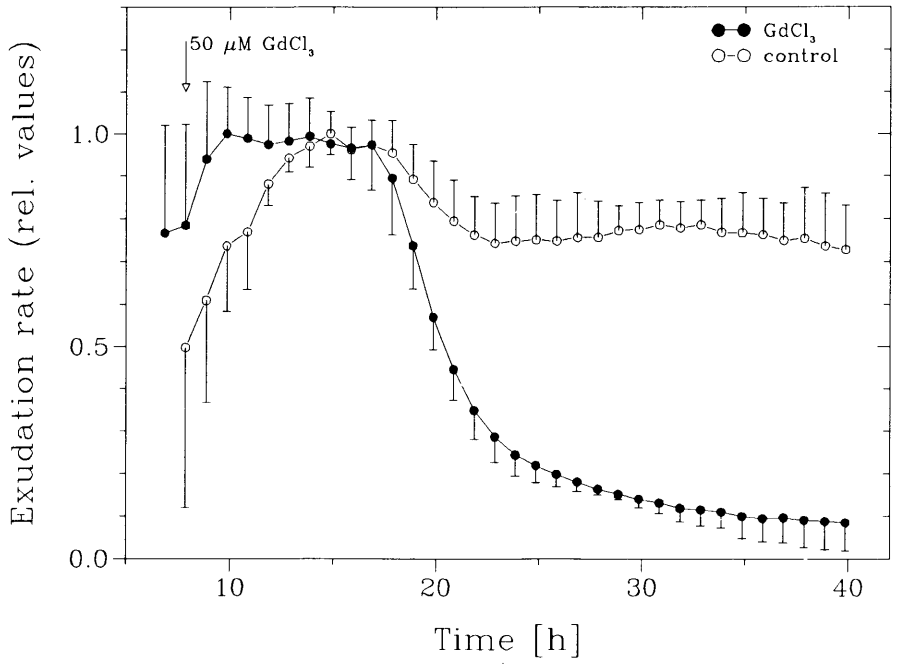

Figure 3. Microdrop recording of maize root xylem exudation at constant temperature of 23°C with and without 50 mmol m^{-3} $GdCl_3$ in distilled water. The curves represent the mean of three independent experiments with 24 roots each. Plot points are hourly cumulative values of one minute interval recordings. $GdCl_3$ was dissolved in 20 cm^3 of distilled water and added to the bathing medium (see arrow). Since the curves are standardized to the highest y-value and not to the exudation level immediately before addition of $GdCl_3$, the initial exudation increase after addition (second point of the curve) is not significant according to the standard deviation bars. In fact, the first derivative of the gadolinium curves increases for the average of 0–30 min after addition in relation to the respective value for the preceding 30 min by +0·49±0·21.

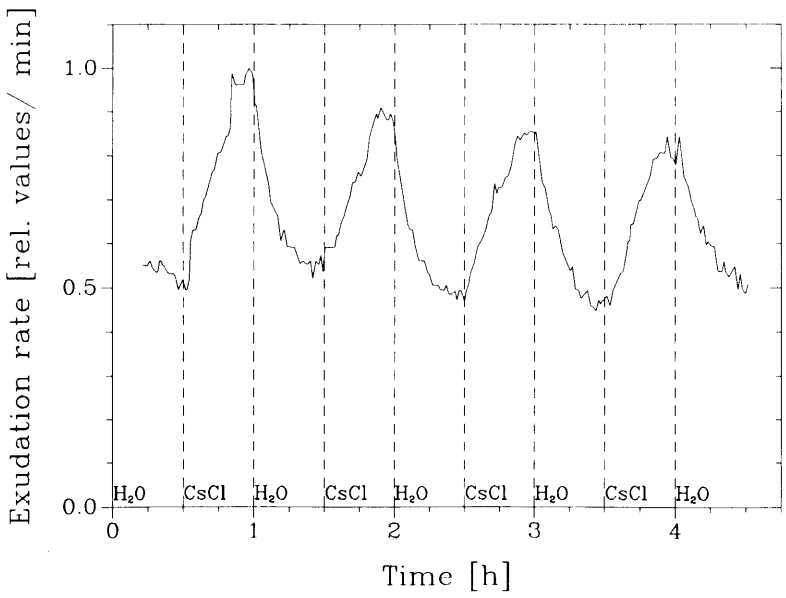

Figure 4. Microdrop recording of the change in xylem exudation of 30 maize roots at constant temperature of 23°C after application of distilled water (H_2O) alternating with 1 mol m^{-3} CsCl as incubation medium. The alternation of the incubation medium was carried out by pot change.

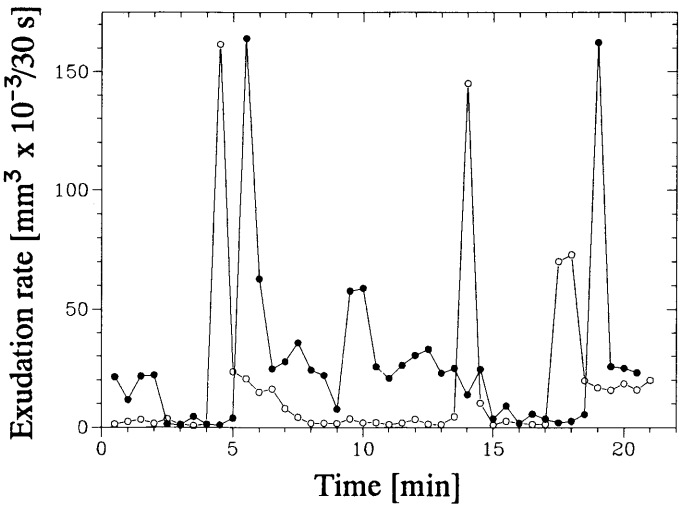

Figure 5. Rate of xylem exudation of two single maize roots in distilled water at constant temperature of 23°C measured simultaneously by the micropipette method; standard error is $2{\cdot}5 \times 10^{-3}$ mm³.

condition of exudation. Five of the seven roots that performed considerable exudation during the measuring time of 21 min showed 2, 4 pulses on average; one root exhibited only a kind of negative peak and another root very even exudation without any pulse. In between pulses, exudation was sometimes negligible, especially immediately before a pulse. It is unlikely that the observed exudation pulses are due to periodic flow resistances in the thin capillaries, because we could observe similar pulses by the microscope with single pipettes of 0·58mm inner diameter. The microscopic observations gave the impression of a split-second movement of the root which was corroborated by some video recordings. By video recordings, it could also be documented that the stained drops in the micropipettes mostly were in a slow, but continuous motion before a pulse, so that we do not believe blockage to be the cause of the pulses.

Concerning our model we want to stress that in rapid nastic movements the underlying omsotic changes seem to be triggered by SA channels (Edwards & Pickard 1987; Stoeckel & Takeda 1989). Since xylem exudation of the experimental roots appeared to function also without exudation pulses, we regard the pulses as a consequence of synchronization of the whole root exudation activity. It is not a necessary condition of a biphasic model that the whole root performs a biphasic mode of water pumping. Because the symplast of roots is far from being an ideal hydrostatic and diffusive continuum, a rather independent osmoregulative behaviour of cells or cell groups may occur. Consequently, without synchronization the overall exudation rate will not reflect the cellular exudation oscillations. The cytoskeleton might be a factor in synchronization of single cell activity, for Lazareva *et al.* (1986) and Zholkevich (1991) report cytochalasin B and colchicine to extinguish auto-oscillations of xylem exudation.

Another explanation of the observed exudation pulses would be provided by Hylmö's test tube model, but the pulses seem to be too large to be caused by the rupture of the one vessel element.

Concerning the cause of the xylem exudation pulses, we may add that in recent experiments using the microdrop recorder we could show that a very strong xylem exudation pulse of a collection of 24 maize roots could reliably be triggered if the roots were transferred from warm (22°C) to cold (4°C) water (D. Blasel, unpublished results). The pulse starts at the very moment of immersing.

Rhizodermis exudation

The most striking observation of our investigation was the discovery of rhizodermis exudation. We carried out a screening experiment to reproduce the results of Lazareva *et al.* (1986) concerning phase shifting of water uptake and exudation. Micropipettes were attached to the root's tip and cut end, while the middle part of the root was encased in a water-free, air-filled jacket, in which it could expand and contract without influencing the fluid volume in the micropipettes. Surprisingly, after several hours, we detected water in the air-filled compartment. The simpler arrangement shown in Fig. 2a was set up to confirm this observation.

Rhizodermis exudation occurred in all 25 maize roots tested. It may be ruled out that rhizodermis exudation is an artefact caused by condensation, for the inner surface of the glass tubes encasing the roots showed only drops at areas touched by the root and touching was not a necessary condition for rhizodermis exudation. In

recent experiments (D. Blasel, unpublished results), we found no rhizodermis exudation from boiled maize roots. We also observed rhizodermis exudation in several woody species.

We believe the simultaneous occurrence of rhizodermis and xylem exudation can hardly be explained by a monophasic exudation model, and strongly favours a biphasic concept. Rhizodermis exudation may be the cause of the formation of wet, coherent soil sheaths around grass roots, as described by McCully & Canny (1988). The coherence of these sheaths seems mainly to be due to their high water content. M.J. Canny (personal communication) suggested that our exudation concept could explain not only formation of the soil sheaths (wetting of the soil by rhizodermis exudation), but also their location. Soil sheaths occur in root zones where the late metaxylem is closed, and therefore, the resistance to upward movement of xylem sap is very high. It can be derived from our model that rhizodermis exudation increases with growing resistance to xylem exudation. This would explain why the soil sheaths form in regions of closed metaxylem. Rhizodermis exudation may also apply to hydraulic life, where, so far, the root has been considered to be only a passive water bridge (Richards & Caldwell 1987; Caldwell & Richards 1989).

CONCLUSION

The results gave some good evidence supporting our new exudation model. First, a biphasic exudation mode appears most probable following the discovery of rhizodermis exudation concomitant to xylem exudation. Second, the assumption that SA channels are involved in exudation was backed by the experiments with the SA channel blocker gadolinium. Third, the observed split-second xylem exudation pulses can be explained well by a biphasic exudation model, and recalled rapid nastic movements which are thought to be triggered by SA channels. After these results, we regard the new exudation model as much more promising than any other exudation model considered before.

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APPENDIX

We consider the root as a three-compartment-system with the compartments C_i (outside medium = C_1, symplast = C_2, exudation fluid = C_3) and the membranes M_{ij} (outer membrane = M_{12}, inner membrane = M_{32}). The membranes can hold two different states s: open channels (s = o) and closed channels (s = c).

At the membrane M_{ij}, the compartment C_i exhibits a water potential:

$$\psi^s_{i,M_{ij}} = P_i - \sigma^s_{M_{ij}} \cdot \pi_i \qquad (1)$$

Our model postulates a biphasic water flow from C_1 to C_3, which is driven by alternations of ψ_2. In the first phase, C_2 takes up a certain amount of water, which is released in the second phase. The basic condition of a water flow from C_1 or C_3 to C_2 is:

$$(\psi_i - \psi_2)^s_{M_{i2}} > 0 \qquad i = 1, 3 \qquad (2)$$

of a flow from C_2 to C_i correspondingly:

$$(\psi_2 - \psi_i)^s_{M_{i2}} > 0 \qquad i = 1, 3 \qquad (3)$$

As a mean feature of our model, the sign change in the difference of water potential:

$$(\psi_2 - \psi_i)^s_{M_{i2}} = (P_2 - P_i) - \sigma^s_{M_{i2}} \cdot (\pi_2 - \pi_i) \qquad (4)$$

is caused by a change of σ. It is suitable to obtain a flow from C_i to C_2 that the channels are closed, i.e. σ holds the higher value, for P_2 normally is larger than P_i. If $\pi_2 > \pi_i$, σ^c has to fulfil the following unequation (see Eqn 1):

$$\sigma^c > \frac{P_2 - P_i}{\pi_2 - \pi_i} \qquad i = 1, 3 \qquad (5)$$

By analogy, to achieve an outflow from C_2 by channel opening, necessarily:

$$\sigma^o < \frac{P_2 - P_i}{\pi_2 - \pi_i} \qquad i = 1, 3 \qquad (6)$$

A flow $J_{i\to j}$ from one compartment C_i into another compartment C_j is given by:

$$J_{i\to j} = (\psi_i - \psi_j)^s_{M_{ij}} \cdot L^s_{M_{ij}} \cdot A_{M_{ij}} \qquad (7)$$

where $L^s_{M_{ij}}$ indicates the hydraulic conductance of membrane M_{ij} in the state of opened and closed channels respectively, and A the membrane area. The inflow into C_2 is composed of the volumes $V_{1\to 2} = J^c_{1\to 2} \cdot \Delta t^c$ and

$V_{3\to2} = J^{c}_{3\to2} \cdot \Delta t^{c}$, the outflow consists of $V_{2\to1} = J^{o}_{2\to1} \cdot \Delta t^{o}$ and $V_{2\to3} = J^{o}_{2\to3} \cdot \Delta t^{o}$.

A sufficient condition of a netflow from C_1 through C_2 to C_3 can be given under the assumption that there is a definite relation between P_2 and the volume V_2 which is stored in C_2, and the additional assumption, that the environmental conditions (P_1, π_1, P_3, π_3) and π_2 can be taken as constant for the time of one pumping cycle. Then the volume $V_{1\to2} + V_{3\to2}$, taken up by pressure increase from P_2 to $P_2+\Delta P$, matches the released volume $V + V_{2\to3}$ after pressure decrease from $P_2+\Delta P$ to P_2:

$$V_{1\to2} + V_{3\to2} = V_{2\to1} + V_{2\to3} \tag{8}$$

A netflow from C_1 to C_3 will occur, when $V_{2\to3} > V_{3\to2}$. With Eqn 8 this can be written as:

$$\frac{V_{1\to2}}{V_{2\to1}} > \frac{V_{3\to2}}{V_{2\to3}} \tag{9}$$

Together with (Eqn 7), it follows that:

$$\frac{[(\psi_1 - \psi_2)^{c} L^{c} A]_{M_{12}}}{[(\psi_3 - \psi_2)^{c} L^{c} A]_{M_{23}}} > \frac{[(\psi_2 - \psi_1)^{o} L^{o} A]_{M_{12}}}{[(\psi_2 - \psi_3)^{o} L^{o} A]_{M_{23}}} \tag{10}$$

The hydraulic conductance L^{c} for closed channels is assumed to be equal for both membranes. Besides the water potentials of the compartments at the respective membranes, the hydraulic conductance of the membranes with open channels (L^{o}) remains the only determining factor of a netflow from C_1 to C_3 during a pumping cycle.

Given equal reflection coefficients at both membranes, uphill transport from C_1 to C_3 can nevertheless take place, if $L^{o}_{M_{23}}$ is larger than $L^{o}_{M_{12}}$, to an extent given by:

$$L^{o}_{M_{23}} > L^{o}_{M_{12}} \cdot \frac{(\psi_3 - \psi_2)^{c}_{M_{23}}}{(\psi_1 - \psi_2)^{c}_{M_{12}}} \cdot \frac{(\psi_2 - \psi_1)^{o}_{M_{12}}}{(\psi_2 - \psi_3)^{o}_{M_{23}}} \tag{11}$$

A difference in L^{o} could be realized by different densities of open channels of M_{23} and M_{12}.