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RESEARCH PAPER

Ion-mediated flow changes suppressed by minimal calcium presence in xylem sap in *Chrysanthemum* and *Prunus laurocerasus*

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Abstract

After the discovery of ion-mediated changes in xylem hydraulic resistance a few years ago, a number of research papers were published that related ion-mediated flow changes in the xylem to various aspects of whole plant functioning and evolutionary diversification of vascular cells. Ion-mediated changes in xylem hydraulic resistance are commonly quantified as the percentile change in hydraulic resistance, relative to the hydraulic resistance measured using a reference fluid, usually (ultra) pure deionized water. In this research the impact was investigated of the complete absence of all ions in deionized water compared with reference fluids containing a minimal amount of free calcium on the quantification of ion-mediated flow changes in stem segments of Chrysanthemum (*Dendranthema*× *grandiflorum* Tzvelev) and Prunus L. (*Prunus laurocerasus* L.). The addition of 10 mM KCl to deionized water significantly increased flow rate in Chrysanthemum (17–24%) and Prunus L. (16%). The addition of 1 mM $CaCl_2$ to the reference fluid reduced this KCl-mediated increase in flow rate to 1–2% in both species. 1 mM Ca^{2+} is within the lower range of Ca^{2+}-concentrations normally measured in xylem sap of many plant species, and three times lower than the original Ca^{2+}-concentration measured in the xylem sap of Chrysanthemum plants used for the present measurements. The present results indicate that the complete removal of cations from the xylem fluid with deionized water causes the major part of the ion-mediated flow change previously reported in the xylem of plants. It is concluded that the use of deionized water as a reference fluid should be avoided. Earlier proposed relationships between ion-mediated changes and water flow in xylem of plants should be re-evaluated if they were based on deionized water as the reference fluid.

Key words: Calcium ions, Chrysanthemum, deionized water, hydraulic resistance, ion-mediated changes, Prunus, xylem.

Introduction

For decades, hydraulic resistance of xylem in plants has been considered to be mainly dependent on the anatomical characteristics of the xylem vessels and on the viscosity of the fluid (Pickard, 1981; Zimmermann and Milburn, 1982). Almost 40 years ago, during experiments to determine the hydraulic resistance of stem segments (R_x), Zimmermann (1978) observed that the use of deionized water significantly increased R_x. He suggested that this phenomenon might be based on swelling and shrinking of the vessel-to-vessel pit membranes, but did not discuss the problem further. A few years ago, Van Ieperen *et al.* (2000) showed that the observed differences in R_x were not caused by changes in the osmotic potential of the fluid interacting with the pit membranes, but by the presence of cations in the fluid decreased R compared with pure deionized water. Zwieniecki *et al.* (2001) confirmed this finding in a broad range of species and also showed the occurrence of ion-mediated flow changes in tobacco stems that were still attached to plants. These authors also provided an elegant theoretical framework to explain this phenomenon: R_x changed due to swelling and shrinking of the pectin gel in the bordered pit membranes, in response to the presence of cations in the xylem fluid. They suggested that the observed phenomenon could substantially contribute to the

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regulation of water flow through plants (Zwieniecki *et al.*, 2001). Based on these observations and the theoretical framework provided, the role of ion concentration on xylem hydraulic resistance became a topic of interest: Zwieniecki *et al.* (2003) reported that the lateral exchange of water between vascular bundles was influenced by the ion concentration in tomato (*Lycopersicon esculentum* Mill.) and suggested a role in nutrient distribution among sectors of a plant. Based on experiments with branches of *Acer rubrum* L. and *Acer saccharum* Marsh. Zwieniecki *et al.* (2004) proposed a functional link between the hydraulic resistance of the xylem and neighbouring phloem, mediated by the active exchange of ions from phloem to xylem. Boyce *et al.* (2004) correlated the extent of ion-mediated changes in R_x with variation in the distribution of lignins and pectins in cell walls between taxa, and suggested a relationship with the evolutionary diversification of vascular cells between Gymnosperms, Angiosperms, and Pteridophytes. In all the above-mentioned publications, quantification of the ion-mediated changes in R_x was done using solutions with an ionic strength comparable with that in xylem sap in transpiring plants, while deionized water was used as the reference fluid: the magnitude of the ion-mediated response (usually induced with 10–25 mM KCl) was expressed as the percentage change of R_x compared with deionized water. The choice of deionized water seems logical from a reference point of view, but can also be questioned: even xylem sap of low ionic strength (caused by high transpiration rates) usually contains small amounts of different ions such as calcium and magnesium (Herdel *et al.*, 2001; Schurr and Schultze, 1995), which, in principle, could interact with the observed effect. The separate effects of several mono- and divalent cations on R_x have briefly been investigated. Although the magnitude of the decrease in R_x was usually comparable between equimolar solutions containing ions (K^+, Na^+, Mg^{2+}, Ca^{2+}), divalent cations caused changes in R_x that required more time to reverse after changing back to deionized water (Van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001). As far as is known, interactions between different ions with respect to changes in R_x have not been reported yet. In particular, calcium might be of interest, because it is always present in the xylem sap and calcium is involved in the pectin gel in mainly primary cell walls and as a structure-providing component (Grignon and Sentenac, 1991; Willats *et al.*, 2001). Ca^{2+}-ions cross-link galacturonic acid groups of antiparallel chains of poly-galacturonic acid (PGA) together and provide the structural framework for the pectin gel. Remaining negatively charged groups within the chains attract water and repulse each other, which contribute to a volume increase of the gel. Electro-shielding of these negatively charged groups by positive counter ions can decrease the volume significantly. Calcium is very effective in suppressing the swelling of artificial pectin films at low concentrations (Ryden *et al.*, 2000), which are comparable to those measured in xylem sap (Atkinson *et al.*, 1992; Ehret and Ho, 1986; Pomper and Grusak, 2004). The aim of this research was to investigate the impact of using deionized water as the reference fluid on the quantification of ion-mediated changes in R_x, instead of a reference fluid that mimics minimal calcium concentration in the xylem sap. Specifically, the hypothesis was tested that a low concentration of Ca^{2+}-ions in the reference xylem fluid does not significantly influence the percentile change in xylem hydraulic resistance (R_x) induced by physiologically relevant changes in K^+-concentration in the xylem fluid, during measurements in stem segments of two model species: Chrysanthemum (*Dendranthema*×*grandiflorum* Tzvelev) and Prunus L. (*Prunus laurocerasus* L.).

Materials and methods

Plant material and sample preparation

Single stem Chrysanthemum (*Dendranthema*×*grandiflorum* Tzvelev cv. Cassa) plants were grown in pots on commercial potting soil in a greenhouse at Wageningen University in the autumn and winter of 2005 (Van Meeteren *et al.*, 2000). Additionally, *c.* 50–70 cm long branches of Prunus L. (*Prunus laurocerasus* L.), were harvested early in the morning in October 2005 from plants in the garden of the Horticultural Production Chains group at Wageningen University. Before further use, these branches were stored in cold tap water in the dark at high humidity to minimize the presence of air embolisms in the xylem vessels.

Stem segments, 12–13 cm (Chrysanthemum) or 7–9 cm long (Prunus L.), were cut under tap water from fully turgid plants and branches to minimize the presence of air embolisms in the samples. The length of the samples ensured that most of the xylem vessels were much shorter than the sample length. The Chrysanthemum samples consisted of 3–4 internodes, and were cut from positions starting between the 4th and 6th leaf, counted from the soil surface, while Prunus L. samples (1 internode) were cut from positions between 6–10 leaves, counted from the top of the branches. After cutting, a short (3 cm) piece of silicone tubing was tightly wrapped over the upper cut end of the stem segment and connected to the apparatus to measure xylem hydraulic resistance (Fig. 1). All cuts were made under water with new razors.

Measurement of xylem hydraulic resistance (R_x)

Different artificial xylem fluids were consecutively pulled through a stem segment by a constant suction pressure difference over the segment (ΔP) of approximately 20 kPa, while flow rate (F) was recorded (Fig. 1). The fluid was pulled through the stem segments to mimic the situation in transpiring plants as closely as possible and to prevent flooding of the apoplast outside the xylem vessels as far as possible. During the flow measurements, pressure varied less than 50 Pa. Flow rate was measured by the change of weight of the container with artificial xylem fluid (Balance Sartorius LC3200D, Göttingen, Germany) over time intervals of 15 s. Flow rate was adjusted for the evaporative loss of water from the vessels on the balances, which was measured before and after a measurement cycle on a stem segment. Measurements were done in a climate-controlled room at constant temperature and relative humidity to avoid changes in evaporative loss during measurements. Evaporative loss was constant and very low (<1% of flow rates measured through the stem segments). Xylem hydraulic resistance (R_x) was calculated as $R_x=\Delta P/F$. To change the fluid composition during the experiments the container with fluid on

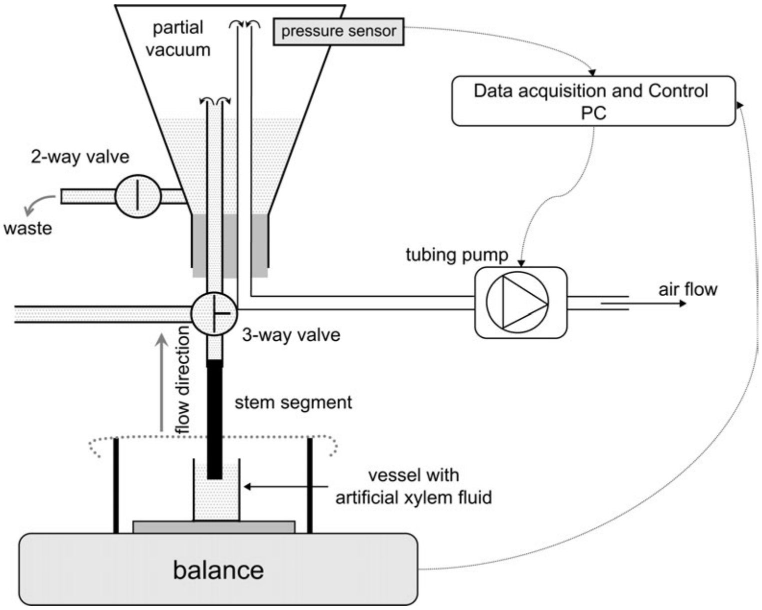

Fig. 1. Apparatus to measure hydraulic resistance in stem segments at constant below-atmospheric pressure. Xylem fluid is pulled through the stem segments at below atmospheric pressure to mimic the situation in xylem in *planta*.

the balance was replaced by another one, after complete depressurizing of the stem segment to avoid the entrance of air in the xylem vessels at the lower cut end of the stem segment during the change. The effect of changes in xylem fluid composition on R_x was expressed as the percentile change of R_x, relative to the xylem hydraulic resistance measured with a reference fluid ($R_{x\ \mathrm{reff}}$): $100\times(R_x - R_{x\ \mathrm{reff}})/R_{x\ \mathrm{reff}}$. This equals $100\times([F_{\mathrm{reff}}/F]-1)$, in which F_{reff} is the flow rate measured with the reference fluid, while F is the flow rate measured with the fluid of interest at exactly the same pressure difference over the same stem segment.

Reference fluids were: ultra-pure deionized water (MilliQ ultrapure water system, Millipore, France), ultra-pure deionized water with a 0.1 mM $CaCl_2$ or ultra-pure deionized water with 1 mM $CaCl_2$ as a background concentration. Solutions to induce the ion-mediated flow changes were based upon one of these three reference solutions, enriched with 10 or 100 mM KCl. The pH of all solutions was between pH 6 and 7.

Calcium concentration of the xylem sap

Ca^{2+}-concentration in the xylem sap of Chrysanthemum plants, similarly grown and treated as the ones used for flow experiments, was measured as verification for the physiological relevance of the calcium background concentrations used in the reference fluids. 20 cm-long stem segments were cut in air, washed with deionized water, and carefully dried with tissue paper. The stems, including 6–8 leaves, were tightly fixed in a pressure bomb with the distal cut end outside. The bomb was pressurized at a rate of 0.2 MPa min^{-1} to a final pressure of 0.6 MPa. The first drop appearing at the cut end of the segment was neglected, and the second and third drops were collected separately in preweighed 2 ml cuvettes. The volume of the collected drops were determined by accurate weighing. Ca^{2+} concentration was determined using a colorimetric assay: 2 ml of a 100 mM imidazol buffer solution (pH 6.5) containing 0.12 mM Arsenazo III was added to the cuvette with one drop of xylem sap and the absorbance change was measured at 652 nm (Cary 4000, Varian inc, USA). The Ca^{2+} concentration was calculated using a predetermined calibration curve for Ca^{2+} concentrations and corrections for the volume of the drop of xylem sap (Malone *et al.*, 2002).

Experiments with different reference fluids

Ion-mediated changes in R_x due to 10 mM KCl were investigated in stem segments of Chrysanthemum and Prunus L. using deionized water, or 1 mM $CaCl_2$ as the reference fluid in October 2005. After harvest and preparation as described above, stem segments were flushed with deionized water or 1 mM $CaCl_2$ to determine the reference flow rates. After the initial flow rates were stabilized (usually between 5 min and 15 min), artificial xylem fluids were exchanged for new fluids, consisting of the previously used reference fluid enriched with 10 mM KCl. Changes in the flow rate were measured. Percentile changes in R_x with reference to the appropriate reference fluid, were calculated and analysed (Student *t* test, unpaired; P=0.05).

In addition to these experiments a more detailed experiment was carried out in January 2006, using Chrysanthemum stem segments and three reference fluids: ultra-pure deionized water, 0.1 mM $CaCl_2$, and 1 mM $CaCl_2$. Ion-mediated changes in R_x were determined by adding 10 or 100 mM KCl to the different reference fluids. All measurements with reference fluids containing $CaCl_2$ were preceded by a measurement step with deionized water, in order to be able to calculate the part of the ion-mediated change in R_x that could be attributed to $CaCl_2$ separately from KCl. Percentile changes in R_x (with reference to deionized water and to the reference fluids containing $CaCl_2$) were calculated and means compared (Student *t* test, paired and unpaired; P=0.05).

Concentration responses to KCl and $CaCl_2$

The responses of R_x in Chrysanthemum stem segments to KCl-concentration (0–100 mM) and $CaCl_2$ concentration (0–10 mM) with deionized water as the reference fluid, were investigated in December 2005. Stem segments were harvested and treated as described above. Flow measurements started with deionized water until stability was reached (10–30 min). The KCl concentration in the xylem fluid was subsequently increased in steps (0.1, 0.5, 1, 5, 10, 50, and 100 mM KCl) and the flow rate at each step was measured after stability was reached (usually within 10 min). A negative exponential function was fitted to the results and the plateau (maximal change in R_x) and half-concentration (K^+-concentration at which half of the change in R_x was reached) were determined. The response to

Ca^{2+}-concentration (0.1, 0.5, 1, 5, and 10 mM $CaCl_2$) was similarly determined and analysed.

Statistical analysis

Curve fitting and statistical analyses were performed using Prism software (GraphPad Prism version 4.00; GraphPad Software, San Diego, CA).

Results

Calcium concentration measured in the xylem sap of Chrysanthemum plants, grown and similarly treated as plants used for measurements to quantify ion-mediated flow changes, was approximately 3 mM (2.9±0.3 mM; *n*=5). Examples of the time-courses of flow rate through Chrysanthemum stem segments, measured before and after the addition of 10 mM KCl to the xylem fluid, are presented in Fig. 2. After starting to flush stem segments with deionized water, flow rates decayed to a lower constant level. This initial decay was observed in the majority of stem segments that were flushed with deionized water, but was usually absent in stem segments that were flushed with 1 mM $CaCl_2$ after harvest. The addition of 10 mM KCl to deionized water significantly increased the flow rate through the stem segments within minutes. However, this increase was largely reduced when 10 mM KCl was added to xylem fluid containing 1 mM $CaCl_2$, instead of deionized water (Fig. 2). Similar results were obtained in stem segments of Prunus L.: the average reduction in ion-mediated decrease in R_x due to the use of a reference fluid containing 1 mM $CaCl_2$ instead of pure deionized water was approximately 87% in Chrysanthemum and 95% in Prunus L. (Fig. 3).

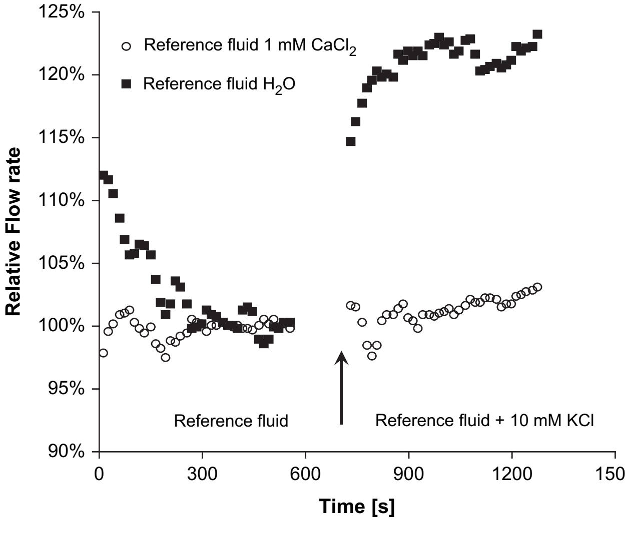

Fig. 2. Time-course of changes in relative flow rate in Chrysanthemum stem segments before, and after the addition of 10 mM K^+ in the xylem fluid. The reference flow rate was measured (average over the last 10 individual measurements before a change in fluid) just before the change in solution composition (indicated by the arrow) for both samples. All flow rates were measured and plotted over 15 s intervals.

A more detailed experiment was carried out in January 2006 with Chrysanthemum. The response of R_x was measured after 10 and 100 mM KCl was added to the reference fluids containing (i) pure deionized water, (ii) deionized water with 0.1 mM $CaCl_2$, or (iii) deionized water with 1 mM $CaCl_2$. With all three reference fluids no statistically significant differences in percentile changes in R_x were observed between the addition of 10 and 100 mM KCl (Student *t* test; unpaired; *P*=0.05). This indicated that the response of R_x was saturated at 10 mM KCl in the xylem fluid. A statistically significant decrease in R_x was found after the addition KCl to each of the three reference fluids (Student *t* test, paired; *P*=0.05). The percentile decrease in R_x was large with deionized water, intermediate at 0.1 mM $CaCl_2$, and small when KCl was added to a reference fluid containing 1 mM $CaCl_2$ (Fig. 4). Because in all stem segments the flow rate with deionized water was also measured prior to measurements with the reference fluids

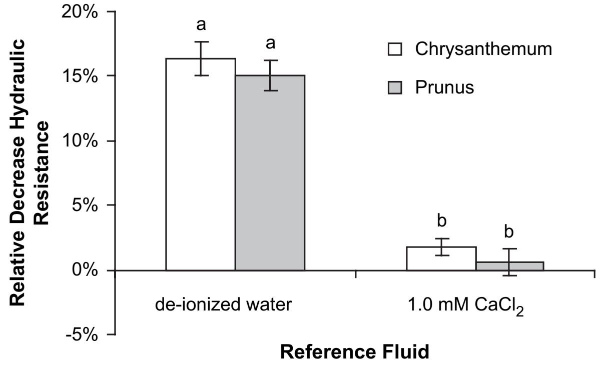

Fig. 3. Percentile decrease in xylem hydraulic resistance (R_x) in Chrysanthemum and Prunus L. stems due to the addition of 10 mM K^+ to different reference xylem fluids. Means ±SEM; *n*=6; different letters indicate significant differences between treatments and species; Student *t* test, *P*=0.05.

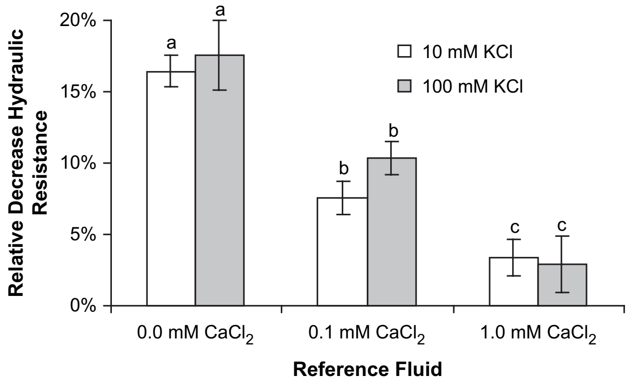

Fig. 4. Percentile decrease in xylem hydraulic resistance (R_x) in Chrysanthemum stem segments due to the addition of 10 and 100 mM K^+ to reference fluids containing background concentrations of 0.0, 0.1, and 1.0 mM Ca^{2+}. Means ±SEM; *n*=6; different letters indicate significant differences between treatments; Student *t* test, *P*=0.05.

containing $CaCl_2$, all changes in R_x could be expressed relative to R_x with deionized water: the part of the total decrease in R_x (reference fluid+KCl) that could be attributed to calcium was large (87.1±6.1%; *n*=12), when 1 mM $CaCl_2$ was present in the reference fluid, and substantial (60.7±4.1%; *n*=12) when only 0.1 mM $CaCl_2$ was present in the xylem fluid. These results also show that very low concentrations of Ca^{2+} ions in the xylem fluid may significantly decrease variations in xylem hydraulic resistance due to variations in the K^+-concentration in the xylem fluid. In a separate experiment with Chrysanthemum stem segments, the relative decrease in R_x clearly depended on the KCl-concentration (Fig. 5A). However, most pronounced effect was observed at KCl-concentrations below 1 mM, while the maximal decrease in R_x was measured at the highest KCl concentration applied (approximately 24% at 100 mM KCl; Fig. 5A). A one-phase negative exponential function described the relationship between changes in R_x and KCl concentration well, particularly below 1 mM KCl (R^2 >0.90; shared for all stem segments). The half-concentration (the KCl concentration at which half of the decline in R_x was accomplished) was 0.40±0.09 mM KCl (mean ±SEM; *n*=6). The log plot (Fig. 5B), on the other hand, indicates a two-phase response, which may point to an additional effect of KCl-concentration on R_x at higher KCl concentrations. However, the most pronounced effect occurred at low concentrations (1 mM KCl). An alternative fit using a two-phase negative exponential function resulted in a curve that also matched the measured data well (Fig. 5, dashed curve; R^2 >0.96; shared for all stem segments). The half-concentration of the first phase was approximately similar to the half-concentration of the one-phase negative exponential fit, while the half-concentration of the second phase (136 mM) was estimated to be outside the range of KCl concentrations which was measured and may therefore be unreliable. A similar experiment to explore the relationship between $CaCl_2$ concentration and its associated decline in R_x resulted in a more or less similar plateau (approximately 20%) and a half-concentration of 0.45±0.09 mM $CaCl_2$ (mean ±SEM; *n*=7) (Fig. 6). In this experiment $CaCl_2$ concentration was not raised above 10 mM. Therefore, no additional two-phase exponential fit was done.

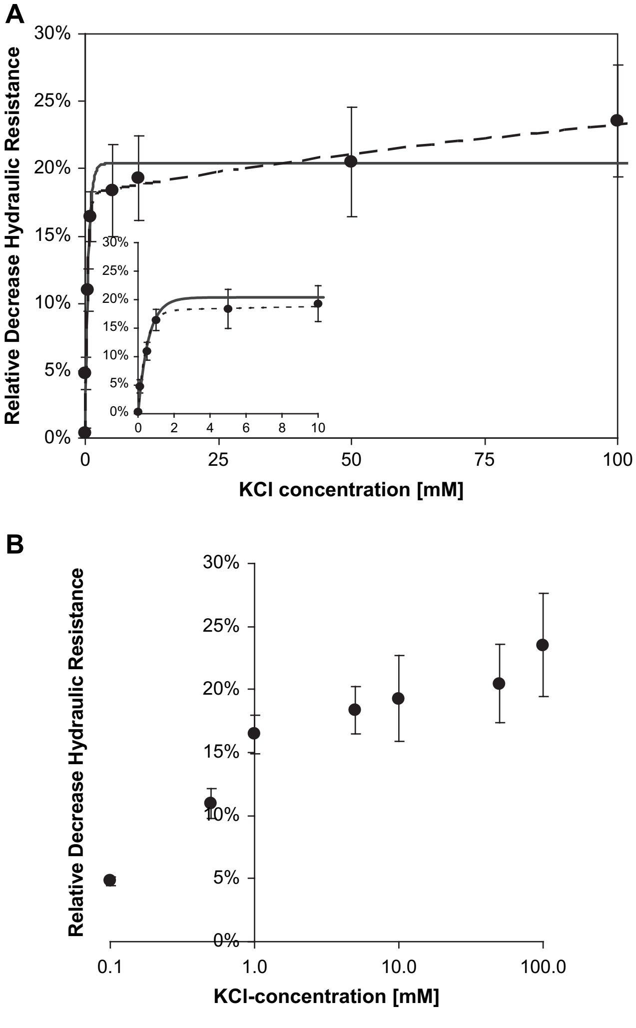

Fig. 5. (A) Relationship between KCl-concentration in the xylem fluid and the percentile decrease in xylem hydraulic resistance (R_x) in Chrysanthemum stem segments (means ±SEM; *n*=7). The insert shows the relationship at KCl-concentration lower than 10 mM. The solid curve is one-phase negative exponential fit (shared on all stem segments), the dashed curve represents a two phase exponential fit (shared on all stem segments). (B) The log curve of the relationship in (A).

Discussion

The 3 mM Ca^{2+}-concentration, which was measured in the xylem sap of Chrysanthemum stem segments prior to use, was within the range usually measured in the xylem sap of plants. The lowest free Ca^{2+}-concentrations measured in the xylem sap are, to our best knowledge, in the order of 0.1–0.2 mM (Siebrecht *et al.*, 2003). More commonly measured are concentrations between 0.5 and 15 mM (Atkinson *et al.*, 1992; Ehret and Ho, 1986; Pomper and Grusak, 2004). The Ca^{2+}-concentration in xylem sap varies between species and diurnally. Diurnal variation is not necessarily negatively correlated with transpiration rate (Köstner *et al.*, 1998) as usually observed for total ion concentration, but others report differently (Herdel *et al.*, 2001; Schurr and Schultze, 1995; Siebrecht *et al.*, 2002). Ca^{2+}-concentration is generally much lower than the K^+-concentration (Herdel *et al.*, 2001).

The Ca^{2+}-concentration used as background in the reference fluid in this paper was relatively low (1 mM) or very low (0.1 mM). It provided a level of free Ca^{2+} ions in the xylem fluid that could be expected to be present in the xylem sap of plants, even at high transpiration rates. The concentrations applied were certainly much lower than the 3 mM Ca^{2+} that was present in the xylem sap of Chrysanthemum plants used for the measurements.

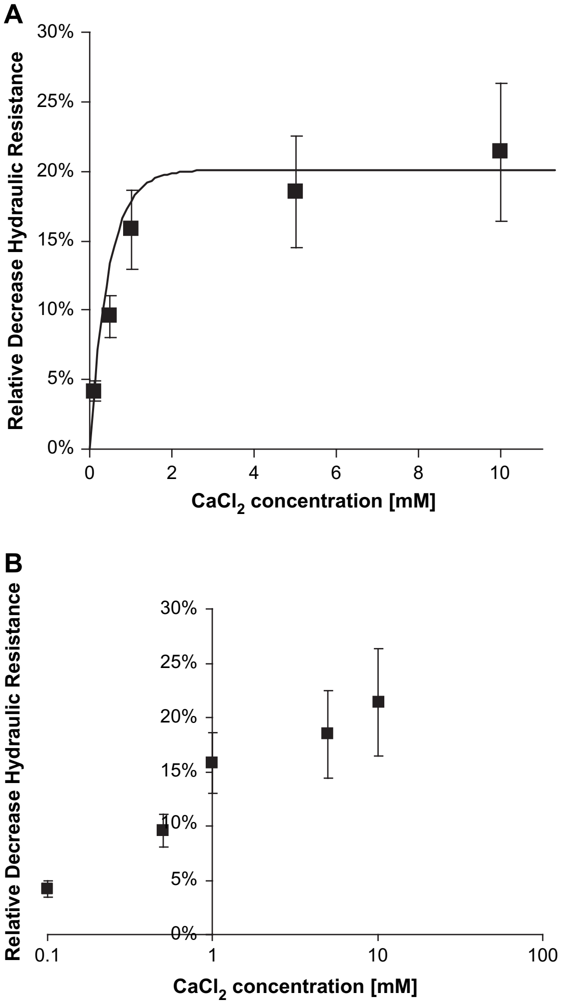

Fig. 6. (A) Relationship between Ca^{2+}-concentration in the xylem fluid and the percentile decrease in xylem hydraulic resistance (R_x) in Chrysanthemum stem segments (means ±SEM; n=7). The curve is one-phase negative exponential fit (shared on all stem segments).

Possible reductions in ion-mediated flow changes after harvest caused by increased rigidity of pectin gel in the pit membranes by additional cross-linking of pectin chains (Ryden *et al.*, 2000) due to 0.1 or 1.0 mM Ca^{2+} in deionized water compared with the situation before harvest (3 mM Ca^{2+}), is therefore very unlikely. The calcium concentration in xylem sap of Prunus L. was not measured. There is, however, no reason to expect that the calcium concentration in xylem sap of Prunus L. significantly differed from the broad range measured in many other plants.

The strong reduction in the ion-mediated flow response, caused by minimal calcium presence in the xylem fluid, shows that subnormal levels of free Ca^{2+} ions in the xylem fluid are sufficient to largely decrease the significant ion-mediated fluctuations in xylem hydraulic resistance that were measured when deionized water was used as the reference fluid in Chrysanthemum and Prunus L. The present results clearly show that the use of deionized water as the reference fluid, which has been common practice until now (Boyce *et al.*, 2004; Van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001, 2003, 2004) would have caused very serious overestimations of the ion-mediated flow changes occurring *in planta*. It is doubtful whether ion-mediated flow changes, due to variations in cation concentration upon a normal cation background presence in xylem sap, would have appeared at all in Chrysanthemum plants.

The commonly used procedure to quantify ion-mediated changes in R_x (based on deionized water) shows potential ion-mediated changes in R_x rather than ion-mediated changes in R_x flow that could occur in the presence of xylem sap. The potential fluctuations in R_x clearly seem in accordance with the swelling and shrinking behaviour of the pectin gel in bordered pit membranes, in response to a cation presence in the xylem fluid (Zwieniecki *et al.*, 2001). The present results suggest that the small amount of cations, usually available in xylem sap, already maximizes the electrostatic screening of the repulsing negative de-esterified groups in the pectin matrix (which cause the swelling), and therefore minimizes pectin gel volume and, consequently, R_x. In theory, Ca^{2+}-ions may act differently from monovalent cations: besides the electrostatic screening effect, Ca^{2+}-ions may alternatively cause additional cross-links between neighbouring pectin chains, which will increase the rigidity of the pectin gel. This would also have caused a reduction of the swelling potential of the pectin gel (Ryden *et al.*, 2000). Inducing xylem flow with a fluid not containing any Ca^{2+} ions, on the other hand, may also have caused a concomitant loss of Ca^{2+} from the pectin matrix (Tagawa and Bonner, 1957; Probine and Preston, 1962). In some test measurements with Prunus L. and Zinnia (*Zinnia elegans* L.), a considerable efflux of Ca^{2+} ions from stem segments was indeed measured after changing the xylem fluid from deionized water to 10 mM KCl (W van Ieperen, unpublished results). K^+ is known to loosen the cell wall matrix by reducing its Ca^{2+} concentration (Tagawa and Bonner, 1957; Probine and Preston, 1962; Ryan *et al.*, 1992). Loss of Ca^{2+} may initially cause a loss of the degree of cross-linking of the pectin chains, which decreases the rigidity of the pectin gel resulting in more swelling with deionized water and, therefore, a larger ion-mediated response in R_x. This could explain the earlier observation in Chrysanthemum (Fig. 4 in Van Ieperen *et al.*, 2000) that K^+-induced changes in flow rate finally resulted in a lower flow rate with deionized water than the original flow rate, which was measured with deionized water immediately after harvest. This extra reduction in flow rate with deionized water was not observed when Ca^{2+} was used to induce the changes in flow rate. Loss of Ca^{2+}, induced by high KCl-concentration may finally cause leakage of pectin polymers from the cell wall (Gillet *et al.*, 1992) and may therefore cause a permanent reduction in R_x. Another possibility is that high KCl-concentrations induced osmotic-driven shrinking of the pectin gels (Ryden *et al.*, 2000).

Variations in high salt concentrations were observed in the xylem sap of two mangrove tree species (López-Portillo *et al.*, 2005) and was related to changes in R_x. Their results indicated osmotic-induced changes in flow rate, which are consistent with the swelling and shrinking behaviour of synthetic pectin gels (English *et al.*, 1996; Ryden *et al.*, 2000). However, the low osmotic potentials of xylem sap in mangrove tree species are exceptional compared with other species, and osmotic driven changes in R_x are not likely to appear at the normal ionic strength of xylem sap in other land plants (Van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001).

The response of R_x to increasing concentrations of Ca^{2+} and K^+ shows saturation of the most pronounced ion-mediated changes in R_x at concentrations, which are below the concentrations usually observed in the xylem. The combination of electrostatic screening at low KCl-concentration combined with osmotic-driven volume changes of the pectin gel and/or solubilization of pectins at high KCl-concentration could, in principle, explain the two-phase response of changes in R_x with increasing KCl-concentration, as observed in the present experiments and previously in *Laurus nobilis* (Fig. 1B, C in Zwieniecki *et al.*, 2001). However, additional research over a wider range of KCl concentrations is needed to confirm the present results and their interpretation.

Conclusions

Investigating the possible contribution of ion-mediated changes in R_x to the regulation of water flow *in planta* requires the use of appropriate reference fluids to be able to apply the results obtained from hydraulic resistance measurements to the physiological processes occurring *in planta*. These reference fluids should at least have the minimal basic ionic strength observed in the xylem sap of the plants under investigation, and mimic a natural mixture of mono- and divalent cations to avoid any swelling behaviour of the pectin gels that would not occur *in planta*.

These results in Chrysanthemum and Prunus L. suggests that ion-mediated changes in R_x might be rare *in planta*, despite the strong ion-mediated changes in R_x measured with deionized water as the reference solution. Earlier proposed relationships between the role of ion-mediated changes in flow in plants and various other aspects of whole plant functioning in the literature should be re-evaluated insofar as they were based on measurements with deionized water as reference fluid.

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