Embolism repair in cut flower stems: a physical approach

W. van Ieperen, U. van Meeteren *, J. Nijsse

Horticultural Production Chains Group, Department of Plant Sciences, Wageningen University, Marijkeweg 22,
6709 PG Wageningen, The Netherlands

Abstract

The role of xylem anatomical properties on air embolism removal and xylem hydraulic conductance recovery from cut flower stems during the first hours of vase life was studied from a physical point of view. A model based on physical processes was developed and tested using chrysanthemum (*Dendranthema × grandiflorum* Tzvelev) cut flowers. The model predicts that the repair process takes place in two major phases. During the first few seconds after replacing in water, initially air-filled vessels at the cut surface partly refill with water. Consequently, reconnections are established between the vase water and the non-cut water-filled xylem vessels just above the cut surface and hydraulic conductance is partly recovered. During the following hours, air partly or completely dissolves into the surrounding water in the stem and hydraulic conductance recovery gradually takes place. The results of the model agreed well with dynamic measurements of hydraulic conductance recovery on chrysanthemum stem segments after aspiration of air. Visual detection of air emboli by cryo-scanning electron microscopy showed that after 1.5 h of repair, air was only present in large-diameter vessels at a position relatively distant from the cut surface of the stem. According to the model, hydraulic conductance repair occurs more readily in stems with smaller diameter vessels. Model calculations and experiments showed that the height of water in the vase influences recovery of water uptake more in stems with large-diameter vessels than in stems with small-diameter vessels. It is concluded that the anatomical structure of the xylem plays an important role in the rehydration capability of cut flowers. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Dendranthema × grandiflorum; Air embolism; Embolism repair; Cryo-scanning electron microscopy; Cut flowers; Hydraulic conductance; Model; Postharvest; Rehydration; Water relations

1. Introduction

Insufficient water uptake due to xylem occlusion is one of the main reasons for inferior cut flower performance during vase life. Causes of xylem occlusion are various: microbial growth, deposition of materials such as gums and mucilage in the lumen of xylem vessels, formation of tyloses and the presence of air emboli in the vascular system (Van Doorn, 1997). Air emboli are present in vessels that are cut open at the base of flower stems but their presence is (partly) reversible. Sufficient removal of air emboli has been

* Corresponding author. Tel.: +31-317-482403; fax: +31-317-484709.

E-mail address: uulke.vanmeeteren@hpc.dpw.wag-ur.nl (U. van Meeteren).

proposed as a prerequisite for restoration of water uptake and a positive water balance during vase life (Durkin, 1979, 1980; Van Doorn, 1990; Van Meeteren, 1992; Van Meeteren and Van Gelder, 1999). The presence of air emboli might also be a prerequisite for the formation of other, permanent types of xylem occlusion (Van Doorn and Cruz, 2000). Variations in rate and level of embolism removal are thought to contribute to differences in water uptake between various cultivars of cut roses (Evans et al., 1996). Previous calculations with a physical model (Yang and Tyree, 1992) showed that the process of air removal from embolised xylem vessels in trees depends on a combination of physical and plant anatomical factors. In cut flowers, similar factors in both plant material and vase water may play a role. However, after harvest all emboli are situated near the cut surface. Therefore, after replacement in vase water, xylem pressures around emboli are relatively high (presumably even positive instead of negative) instead of the negative xylem pressures that are generally expected around emboli in trees.

In moderately desiccated chrysanthemum cut flowers (Nijsse et al., 2001a) and in detached stem segments (Van Ieperen et al., 2001) we showed that almost all vessels that are opened by cutting fill with air. These cut vessels completely embolised, while no air passed into non-cut xylem vessels as might be expected, because air–water interfaces could not pass vessel-to-vessel connections at low pressure differences (Zimmermann, 1983).

Thus, at the start of vase life, air cannot simply be pressed out of the cut open vessels. Instead, it will be trapped between the entering water column and the pit membranes of the vessel-to-vessel connections. Dissolution of air into surrounding water is necessary for removal of air from these cut, open vessels. In chrysanthemum, a positive correlation between emboli presence and a subsequent negative water balance during vase life was shown (Nijsse et al., 2000).

The water balance and water uptake after entrance of air in chrysanthemum cut flowers is positively influenced by cutting height (Marousky, 1973; Van Meeteren and Van Gelder, 1999). This might be related to the gradual basipetal increase in xylem diameter in chrysanthemum cut flower stems (Nijsse et al., 2001b).

The aim of the present paper is to describe and explain the processes involved in air embolism removal in cut flowers at the start of vase life from a physical point of view. A model is used to simulate the complex interactions between air removal and physical and anatomical factors of the xylem and vase water properties. Simulation results are tested by measuring the dynamics of hydraulic conductance of chrysanthemum stem segments. Visualisation of air emboli presence in a recovering chrysanthemum stem segment using cryo-scanning electron microscopy (cryo-SEM), and results of a vase life experiment, in which the water height in the vase was varied, nicely agreed with model predictions.

2. The model

2.1. Model description and simulation results

The process of hydraulic conductance recovery in the basal cut-end of a flower stem at the start of vase life can be separated into two sub-processes:

1. reconnection of the vase water with the water in intact xylem vessels above the cut surface through redistribution of air and water in (the network of) cut and air-filled xylem vessels ends, and
2. dissolution of trapped air from (partly) embolised xylem vessels into their surroundings and subsequent further refilling with water.

The processes under step 2 are derived from a previously described model for embolism recovery in intact trees after cavitation (Yang and Tyree, 1992). The processes under step 1 are specific for cut flowers at the start of vase life. Immediately upon reapplying water at the start of their vase life, water tends to rise in the cut xylem vessels of flower stems. This tendency is caused by capillary forces in the cut vessels and the positive pressure due to the height of the vase water above the cut surface of the stem. The rise of water in the cut open vessels compresses the air and generates an

above atmospheric air pressure, which is the driving force for the later dissolution of air. Because the cut xylem vessels act in a network in which they are interconnected to non-cut vessels, both sub-processes influence the hydraulic conductance of the xylem in the flower stem. The model below describes these processes, and enables some quantification of air removal as dependent on xylem anatomical properties. To improve understanding of factors and processes involved, a simplified system description concerning one individual vessel is presented first and later this model is extended to a multi-vessel system.

2.1.1. Simplified system description: initial rise in an individual air-filled cut open vessel

At the start of vase life of a flower stem, an individual air-filled cut open vessel can be regarded as a closed chamber for air (Fig. 1). Upon reapplying water to the cut-end of such a vessel, air becomes trapped because air–water interfaces cannot pass the bordered pits at vessel-to-vessel connections. However, partial refilling of such a cut open vessel still occurs due to a combination of forces, which force the air–water meniscus to rise in the vessel. These forces include the capil-
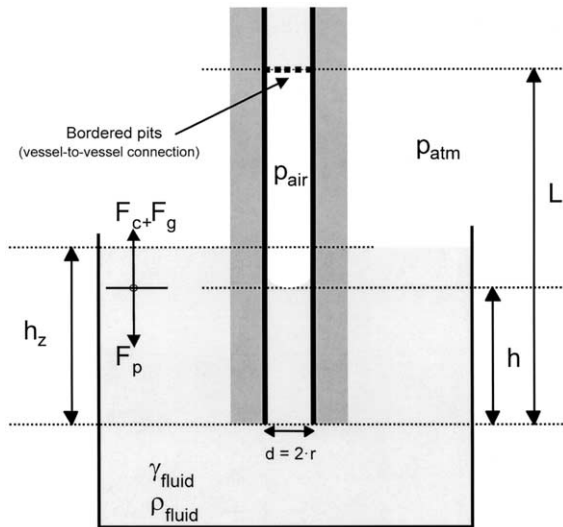

Fig. 1. Schematic representation of physical forces and parameters involved in the rise of water (h) in an individual air-filled cut open vessel (for parameters, see text and Table 1).

Table 1
Description of input variables and parameters including standard values for the parameters used in model calculations

γ	Surface tension of the solution in the vessels (water at 20 °C)	0.0728 N m^{-1}
η	Viscosity of the solution in the vessels	0.001 Pa s
ρ	Density of the solution in the vessels	998.2 kg m^{-3}
Θ	Contact angle between meniscus and xylem cell wall	50°
g	Gravity constant	9.8 m s^{-2}
P_{atm}	Atmospheric air pressure	101 325 Pa
h_z	Height of the water level in the vase above the cut-end of the flower stem	0.1 m
R	Radius of the vessel	20 μm
L	Length of the vessel	0.03 m
k_a	Solubility of air in water	7.75×10^{-6} mol m^{-3} Pa^{-1}
D_a	Diffusion constant for air in water	1.95×10^{-9} m^2 s^{-1}

Standard values are used in the model calculations unless explicitly mentioned else.

lary force (F_c (N) Eq. (1)), promoting partial refilling, and the gravitational force (F_g (N); Eq. (2)), defilling or promoting partial refilling depending on the height of the water level in the vase (h_z (m)). Refilling also causes compression of air inside the captured air-bubble, which results in an additional force that counteracts the rise (F_p (N); Eq. (3)).

$$F_c = 2\pi r \gamma \cos \theta \qquad (1)$$

$$F_g = \pi r^2 (h - h_z) \rho g \qquad (2)$$

$$F_p = \left(\frac{L}{L-h} - 1 \right) p_{atm} \pi r^2 \qquad (3)$$

A description of the variables and parameters including their standard values used in model calculations, is presented in Table 1. The contact angle of the water-wall interface, (Θ (degree)) has often been assumed to be 0°. However, recently, it was shown in various plant species that Θ might be much higher (42°–55°; Zwieniecki and Holbrook, 2000). In this model Θ is assumed to be 50° as has previously been estimated for xylem vessels of chrysanthemum cut flowers (Van Ieperen et al., 2001). Vessels are assumed circular in cross-section (with radius r (m)).

Providing no air disappears from the trapped air volume, a static equilibrium is reached when the net force on the meniscus is 0 ($F_c + F_g + F_p = 0$). This situation is accomplished when the meniscus has reached height h (m) in a cut open vessel of length L (m) (Eq. (4)).

$$h = \frac{-Y + \sqrt{Y^2 - Z}}{-2g\rho r}$$

$$Y = p_{atm}r + (L + h_z)gr + 2\cos$$

$$Z = 4(gr)(L2\cos + h_z L\, gr) \qquad (4)$$

Calculations with Eq. (4) show that the rise h increases with vessel length L and decreases with vessel radius r (Fig. 2(A)). However, air pressure at static equilibrium mainly depends on vessel radius (Fig. 2(B)). The influence of the contact angle on rise is substantial, especially in small-diameter vessels (Fig. 2(C)). The influence of the water level in the vase (h_z) on rise (h) is small in small-diameter vessels (results not shown). However, increasing h_z from 1 to 10 cm increases the air pressure inside the air-bubble more than 30% in large-diameter vessels ($r > 30$ μm). This substantially increases the driving force for air solution from trapped air in large-diameter vessels into surrounding water.
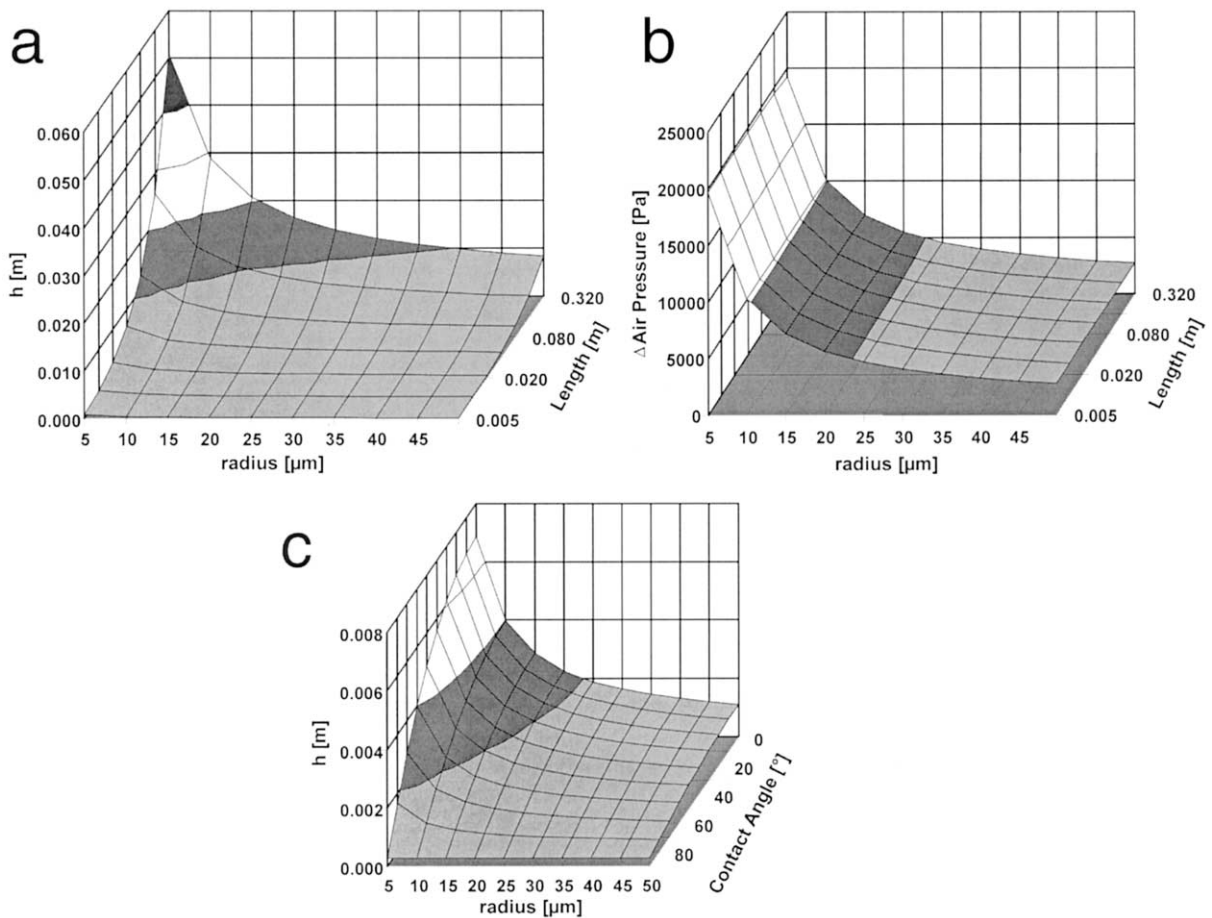

Fig. 2. Simulated effects of length and radius of individual embolised cut open xylem vessels on the rise of water (h) in the vessel (A) and on the above-atmospheric air pressure developed inside the trapped air-bubble (B) at static equilibrium after applying water at the cut surface. Effect of the contact angle on the relationship between radius and rise h is given in (C).

Besides the forces F_c, F_g and F_p, an other important force influences the dynamics of rise: the friction force (F_w (N); Eq. (5)). F_w describes the friction that a flowing water column in a circular tube experiences.

$$F_w = \frac{8\pi d\rho h v^2}{Re} \tag{5}$$

$$Re = \frac{d\rho v}{\eta} \tag{6}$$

Re is the Reynolds number (Eq. (6)), a dimensionless number that describes flow characteristics, e.g. it indicates whether flow will be laminar or turbulent. The Reynolds number depends on the diameter of the vessel ($d = 2r$ (m)), the velocity of the moving water column (v (m s^{-1})), and the density ρ and viscosity (η (Pa s)) of the fluid (vase water). The position of the meniscus in the vessel determines the length h of the water column, which in turn influences F_w. The model calculates the acceleration (a (m s^{-2})) of the rising water column based on the sum of all forces (F_{net}) and the mass of the moving water column (Eq. (7)).

$$F_{net} = \frac{a}{h\pi r^2} \tag{7}$$

The acceleration a is used to calculate the velocity v of the meniscus, which in turn is used to calculate the rise of water and the position of the meniscus in the vessel.

Computer simulations with a model based on Eqs. (1)–(3), (5)–(7) show that initial rise is completed within a period of usually a few seconds (maximally a few minutes). The period is mainly influenced by the length of the air-filled vessel (Fig. 3).

2.1.2. Extended system description: initial rise in a set of adjacent air-filled cut open vessels with different diameter and length

Fast partial repair of hydraulic conductance may take place when a rising water column in a single air-filled vessel connects via bordered pits with a neighbouring non-cut and thus, water-filled xylem vessel. The probability and impact of this type of reconnection on hydraulic conductance repair depends on the network anatomy and the
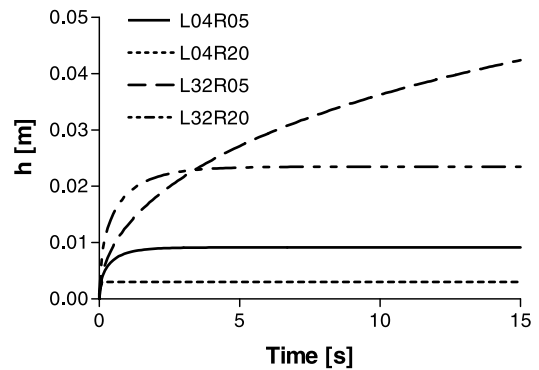

Fig. 3. Simulated time course of rise of water (h) in individual, initially air-filled cut open xylem vessels of different anatomical properties ($L32R20$ = length 32 cm, R radius 20 μm and so on; for other parameters, see Table 1).

magnitude of the rise in individual vessels and thus, on vessel anatomy as described above.

The rise of water in an individual air-filled vessel might be enhanced if the air in this vessel could be pushed into a neighbouring air-filled vessel. This may only occur when bordered pits are at least partly empty. It can be calculated that relatively small negative pressures (-40 to -400 kPa) already empty the cavities on both sides of the pit membrane (Van Ieperen et al., 2001). The only obstacle left for air transport is the presence of water in the small pores of the cellulose mesh in the pit membrane. Walls of xylem vessels in chrysanthemum are almost completely covered by bordered pits (Nijsse, 2001) and the total area of pit membranes between two adjacent air-filled vessels covers a large percentage of the vessel area. The intake of dry air after cutting followed by a desiccated period as well as the very small amount of water in the pit membrane pores might result in small holes in the water film in the cellulose mesh. Very few of these holes are sufficient for air transport among adjacent vessels. Once air transport takes place, it might enhance the formation of other holes. If air exchange via bordered pits occurs, then the total volume that is compressed in the two vessels might be considered as one. In that case, the air pressure counteracting the rise of the menisci in the two vessels is similar in both vessels (Fig. 4). Hence, refilling patterns and capillary rise in adjacent communicating ves-

sels may differ from non-communicating vessels of similar dimensions. To test this theoretically, a model was developed to simulate the refilling process due to capillary rise in clustered vessels. Compared to the single vessel version of the model the calculation of F_p changes. As long as the rise in one vessel does not exceed the top of the other vessel, F_p is determined by the increase in air pressure of the total volume in both vessels. Several diameter and length combinations of two adjacent air-filled vessels were simulated to investigate the time course and final height of the water columns (Table 2). These simulations show that if a small-diameter vessel is shorter than the adjacent larger diameter vessel, the small-diameter vessel always completely refills by expelling its air into the adjacent vessel. If the smaller diameter vessel is longer than the adjacent vessel, it stops refilling shortly after the meniscus passes the top of the adjacent vessel. This may result in enhanced side-paths for water transport from vase water to water-filled vessels, which start close to the basal cut surface of the flower stem (Fig. 5). It

Table 2
Simulated water height (h), percentile refilling (R_{fp}) and above atmospheric air pressure (ΔP) in various combinations of two adjacent, initially air-filled cut open vessels after a 60 s simulation

Radius (μm)	Length (cm)	h (cm)	R_{fp} (%)	ΔP (Pa)
Effect of vessel radius in three combinations of a short and long vessel				
5	4	4.0	100	–
30	20	0.7	3.3	4035
5	4	4.0	100	–
20	20	0.8	4.1	5581
5	4	4.0	100	–
10	20	0.9	4.7	10 251
Effect of vessel length in two combinations of a narrow and wide vessel				
5	4	4.0	100	–
30	4	0.1	1.3	4095
5	20	5.8	28.9	17 166
30	4	0.1	1.8	4093

is possible that the larger diameter vessel completely empties again due to the entrance of air originating from an adjacent refilling smaller diameter vessel. The model does not account for the subsequent release of air into the vase solution.
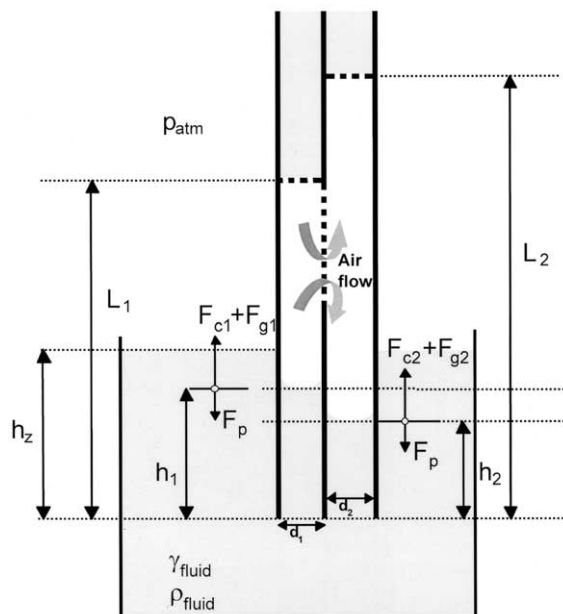

Fig. 4. Schematic representation of rise of water in a system of two adjacent communicating cut open xylem vessels assuming free air exchange via bordered pits (parameters see text and Table 1).
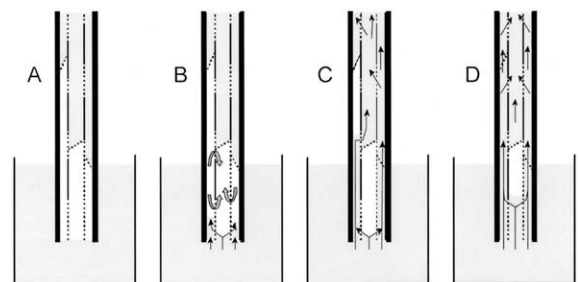

Fig. 5. Schematic representation of different subsequent phases of hydraulic conductance recovery after reapplying water to the basal cut surface of a system of three communicating cut open xylem vessels of different diameter. (A), (B) and (C) occur during the first few seconds to minutes after reapplication of water and show the processes of redistribution of air (grey arrows in (B)) and water in the communicating cut open vessels, which results in partial restoration of the water connection between vase water and xylem water column above the air emboli (visualised by the arrows in (C)). The time between (C) and (D) may be several hours. (D) shows the (partial) dissolution of trapped air.

Air pressure inside the remaining air-bubbles in cut open vessels depends mainly on the diameter of that vessel. The period to reach maximal water height in a vessel varies between seconds to a minute and mainly depends on the length and diameter combinations of the vessels involved.

2.1.3. The last step: air solution and diffusion from trapped air-bubbles in cut open xylem vessels

The mechanism of air removal from trapped air inside an embolised xylem vessel involves two steps. (1) The dissolving of gas into water at the surface of the air-bubble, followed by (2) the diffusion of dissolved gas to the surrounding environment. Due to air removal, the water column in the vessel rises.

According to Henry's law, the equilibrium solubility of gas at a gas–water interface is proportional to the gas pressure. The equilibrium air concentration (C_a (mol m^{-3})) at the air–water interface depends on the air pressure (p_{air}) and the solubility of air in water (k_a (mol m^{-3} Pa^{-1})) as shown in Eqs. (8) and (9).

$$C_a = k_a p_{air} \tag{8}$$

$$k_a = 0.8 k_{N_2} + 0.2 k_{O_2} \tag{9}$$

The solubility of air in water (k_a) is defined as the average solubility of the two major gasses in air (N_2 and O_2). The time to reach equilibrium between the gas phase and the liquid at the air–water interface is assumed negligible. The atmospheric pressure (p_{air}) in the air-bubble generates a concentration gradient between the water interfaces at the air-bubble and the surrounding tissue, which is the driving force for dissolved air movement by diffusion. The rate of diffusion ($\Delta N_a/\Delta t$ (mol s^{-1})) is determined by the concentration gradient ($\Delta C/\Delta x$ (mol m^{-4})), the diffusion area (A (m^2)) and diffusion constant (D_a (m^2 s^{-1})) for dissolved air in surrounding tissues, according to Fick's Law (Eq. (10)).

$$\frac{\Delta N_a}{\Delta t} = D_a A \frac{\Delta C}{\Delta x} \tag{10}$$

The diffusion area (A) is an uncertain variable since the transport path between air-filled vessels and surrounding tissue involves transport through cell walls. These walls are highly lignified and have a low water content and therefore, a very low diffusion constant for air. It is likely that only direct water connections via the bordered pits and at the cross-sectional area of the rising water column are available for diffusion. Diffusion area (A) will also decrease in time, when the water column rises due to dissolution and diffusion of air from the air-bubble into the environment.

Partial repair of hydraulic conductance by the reconnection of partial refilled cut vessels with water-filled xylem vessels may also enhance the diffusion process: flow of water continually refreshes the solution close to the embolism and therefore, shortens the diffusion path.

2.2. Model summary and discussion

Repair of cut open xylem vessels in the basal part of a cut flower stem may be separated into two processes. Fast partial repair of mostly small-diameter vessels leads to reconnections between vase water and non-cut vessels (of all diameters) above the cut surface. Possible expulsion of air into adjacent air-filled vessels of larger diameter could further improve this process. This process takes place during the first seconds to minutes after reapplying water. It results in a xylem network in the basal part of the stem in which most small-diameter vessels conduct water over a large percentage of their length while larger diameter vessels remain, at least for a part of their length, air-filled (Fig. 5). The effect on hydraulic conductivity is complex. In xylem systems without air emboli most of the water is transported through only a few large-diameter vessels (Zimmermann, 1983; Gibson et al., 1984). Therefore, it could be expected that removal of air emboli from large-diameter vessels largely contribute to the recovery of water uptake. However, in the network of many parallel, interconnected xylem vessels of different lengths and diameters (Zimmermann, 1983), partial or complete refilling of small-diameter vessels or a small rise of water in some large-diameter vessels may also significantly contribute to hydraulic conductance recovery.

The process of hydraulic conductance recovery continues with the much slower processes of solution and diffusion of trapped air from the captured air-bubbles into their surroundings. It was calculated before that in trees the process of air removal from embolised vessels has a time constant of several hours (Yang and Tyree, 1992). Anatomical properties of xylem vessels clearly influence the long-term dissolution processes. There are several reasons why air disappears faster from small-diameter vessels. (1) They develop a higher p_{air} in the trapped air-bubble. (2) They have a longitudinal surface area–volume ratio that enhances the rate of air removal compared to large-diameter vessels. (3) They have a higher chance to reconnect with non-cut vessels because initial rise is higher, which enhances the dissolution process by shortening of the diffusion path. Vessel length is less important: increasing vessel length almost proportionally increases the diffusion area, and therefore, the rate of air removal. This compensates for the larger volume of air in longer air-filled vessels. The contact angle between water and wall material in embolised vessels also strongly influences p_{air} in the trapped air-bubble and thus, the processes of solution and diffusion. Chemical contents of the cell wall as well as chemo-physical properties of the entering fluid (vase water) might influence this contact angle, p_{air} and thus, the rate of air removal.

When postharvest treatments affect the physical processes involved in embolism repair (like vase water height, water temperature, surfactants) it may be expected that the outcome of these treatments will interact with the anatomical properties of the flower stems.

3. Materials and methods

3.1. Plant material

Chrysanthemum (*Dendranthema × grandiflorum* Tzvelev cvs. Cassa and Vyking) plants were grown in a greenhouse at Wageningen University in 14-cm diameter plastic pots containing a commercial potting soil. The average temperature was 18 °C and a 18 h photoperiod was maintained until the plants had formed 15–17 leaves longer than 0.5 cm (3–4 weeks). Thereafter, a 9 h photoperiod was maintained until harvest. When necessary, lengthening of the natural photoperiod was achieved by high-pressure sodium lamps (Philips SON/T). Conversely, shortening of the natural photoperiod was achieved using black screens.

3.2. Measurement techniques

3.2.1. Hydraulic conductance dynamics

Stem segments for hydraulic conductance measurements were collected from plant material ('Cassa'), which was grown as described above and prepared (under water) according to Van Ieperen et al. (2001) to prevent initial air embolism. The hydraulic conductance of 20 cm long stem segments (K_h), cut at 15 cm height above the roots, was measured by pulling water through a stem segment at a known pressure difference (40.0 ± 0.01 kPa) using the apparatus described by Van Ieperen et al. (2000). Initial K_h was measured over 15 min. Then air entrance at the basal part of the stem segment was allowed for 3 min by removing the water supply from the basal cut surface while maintaining the pressure. Afterwards water was reapplied to the stem segment and the restoration of K_h was measured over ≈ 1.5 h at 30 s time intervals according to Van Ieperen et al. (2000).

3.2.2. Visualisation of air emboli by cryo-scanning electron microscopy

After 1.5 h of measuring hydraulic conductance the negative pressure was released from the top of a stem segment that was later used for visual detection of air emboli by cryo-SEM. After the water uptake of the stem segment had completely ceased, water supply was carefully removed from the basal cut-end of the stem segment, while leaving a drip of water at the cut surface to prevent occasional air entrance. Then the stem segment was plunge-frozen in liquid nitrogen (-196 °C) to immobilise the water in the vessels. At different distances from the cut surface, stem transverse sections were prepared for investigation in a cryo-SEM as described by Nijsse and Van Aelst (1999). In short, stem pieces of about 1 cm

were cut under liquid nitrogen using a circular diamond saw. These stem pieces were cryo-planed at -90 °C in a cryo-ultramicrotome (Reichert-Jung Ultracut E/FC4D) with a diamond knife (8 mm wide; Histo no trough 45°, Drukker International, The Netherlands). The planed surfaces were freeze-dried for 3 min at -89 °C and 10^{-4} Pa and sputter-coated with platinum in an Oxford CT 1500 HF cryo transfer unit. The surfaces were photographed in a cryo-SEM (Jeol 6300F) at -190 °C using a digital imaging system.

3.2.3. Water balance during vase life

To investigate the effect of vase water height on restoration, chrysanthemum cut flowers ('Vyking') were treated as described before (Van Meeteren and Van Gelder, 1999). In short, flowering stems were harvested at commercial maturity by cutting the stalks at their root–shoot junction (soil level) and brought to the laboratory as soon as possible. Thereafter, lower leaves were removed, cut stem ends were trimmed by 1 cm in air, and the stalks were placed for 3 h in a bucket with a mixture of ice and water (3:1 by volume) in darkness at 4 °C to regain full turgidity. The lower 0 or 12 cm of the stems were cut off in air after the hydration treatment. Thereafter, the fresh weight of the flowers was determined as the initial weight, flowers were moderately desiccated by placing the individual stems horizontally for 1 h on two wires (weight loss was $\approx 5\%$). Afterwards 2 cm from the cut-ends was recut in air to obtain fresh cut surfaces. The low water status of the stems ensured immediate air uptake in the fresh cut vessels. The flowers were placed in vases (4 cm diameter glass tubes) containing a solution of $CaCl_2$, $NaHCO_3$ and $CuSO_4$ (Van Meeteren et al., 2000) at 20 ± 1 °C, $60 \pm 5\%$ RH and a light intensity of 14 µmol m^{-2} s^{-1} (Philips, TLD 50W/84HF) with a light period of 12 h. The height of the solution in the vases was set to 5 or 18 cm above the cut surface of the flowers. Whenever the water level had lowered ≈ 1 cm, solution was added to the tubes to reestablish the correct water height. Water uptake after 24 h was calculated from the volume of added solution per individual flower. The percentage of initial weight of the flowers was determined after 24 h. Initial

weights were corrected for the weight of the cut 2-cm stem pieces. The design was a randomised complete block with three replicates per treatment in each of four blocks. Analysis of variance was applied to the percentage of initial flower weight and water uptake per flower after 24 h of vase life.

4. Results

4.1. Dynamics of hydraulic conductance restoration curves of stem segments

A typical time curve of hydraulic conductance (K_h) of a 20-cm long stem segment during a rehydration experiment is shown in Fig. 6(A). The hydraulic conductance without air in the xylem vessels was measured at the start of the measurement and set to 100%. Thereafter, air was aspired at the basal cut-surface for 3 min followed by replacement in water. The hydraulic conductance showed an initial fast recovery after the replacement in water (Fig. 6(A)). Like a saturation process, after a few hundreds of seconds, the increase levelled off and the hydraulic conductance tended to plateau at lower than 100% of the initial conductance before air entrance. As can be seen from the residuals (Fig. 6(B)), the data could not be fitted with a simple rectangular hyperbola or binding isotherm ($K_h = (K_{h,\,max} \times$ time)$/(T_{half} +$ time); $K_{h,\,max} =$ plateau of hydraulic conductance, $T_{half} =$ time required to reach half of $K_{h,\,max}$). However, extending the curve with a second hyperbola resulted in a close fit ($K_h = (K_{h,\,max\,1} \times$ time)$/(T_{half\,1} +$ time)$ + (K_{h,\,max\,2} \times$ time)$/(T_{half\,2} +$ time); $R^2 = 0.9915$; Fig. 6(A)) and a Gaussian distribution of the residuals ($P > 0.10$; Fig. 6(C)). The 95% confidence intervals of the parameters (Fig. 6(A)) show a short half time of a few seconds for the first hyperbola and a long half time of ≈ 2600 s for the second hyperbola.

4.2. Effect of de-gassed water on hydraulic conductance restoration curves

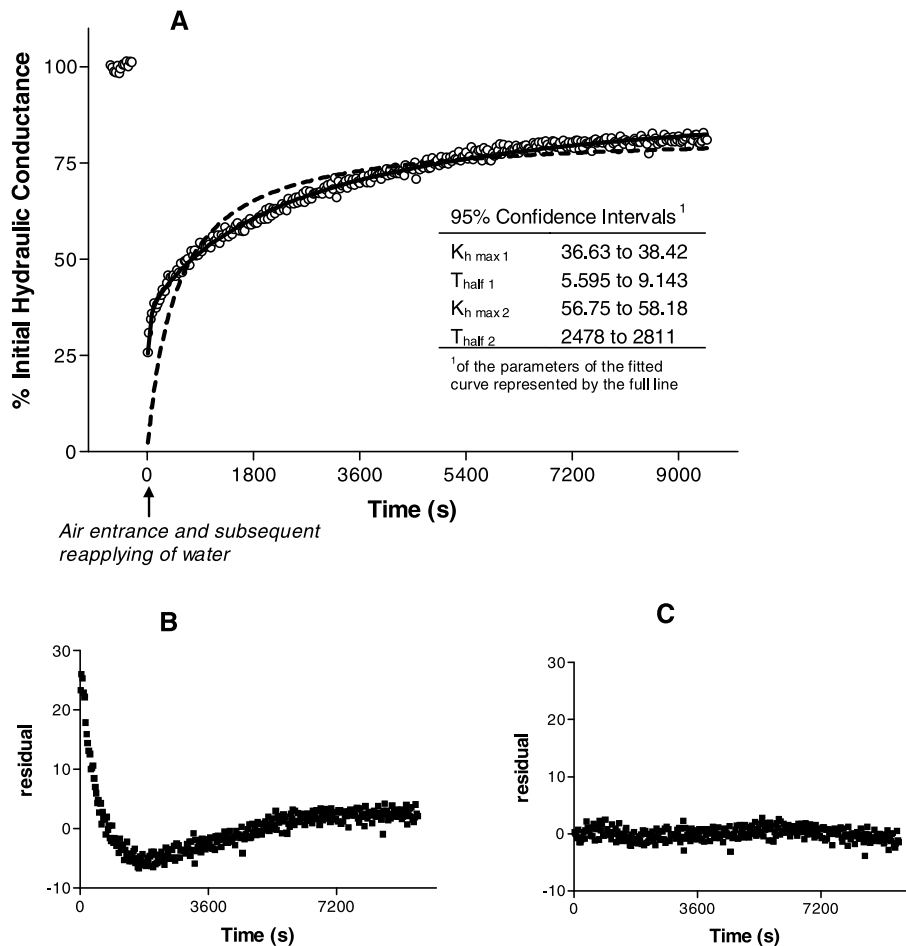If dissolving of air plays a role in the restoration of hydraulic conductance, changing the con-

Fig. 6. (A) Time curve of hydraulic conductance of a 20 cm chrysanthemum stem segment cut 15 cm above the roots. After measuring the initial hydraulic conductance without air in the xylem vessels, air was aspired at the basal cut-surface for 3 min. Thereafter water was reapplied. Time 0 is the time of reapplying water. ○ – measured data, dashed line – fitted rectangular hyperbola ($K_h = (82.83 \times \text{time})/(487.1 + \text{time})$), full line – fitted curve using a combination of two hyperbola ($K_h = (37.53 \times \text{time})/(7.37 + \text{time}) + (57.47 \times \text{time})/(2644 + \text{time})$). (B) Residuals of the fitting with one hyperbola. (C) Residuals of the fitting with two hyperbola.

centration gradient between trapped air and its surroundings will affect the dynamics of hydraulic conductance restoration. Therefore, an experiment was carried out in which the amount of air dissolved in the vase water was affected by de-gassing the water. Comparing the reapplication of de-gassed water after air aspiration with tap water (Fig. 7) demonstrated a large increase in the plateau of the restoration curve. When a double hyperbolic function is used and $T_{\text{half, 1}}$ is set to 7.4 s (because the first measured data after reapplying water were disturbed), the best fits of the curves for de-gassed and tap water are, respectively $K_h = (26.47 \times \text{time})/(7.4 + \text{time}) + (88.23 \times \text{time})/(221.5 + \text{time})$ and $K_h = (25.67 \times \text{time})/(7.4 + \text{time}) + (20.95 \times \text{time})/(684.7 + \text{time})$, both with a Gaussian distribution of the residuals. A fast increase in hydraulic conductance occurred when tap water was replaced by de-gassed water at 1930 s after reapplying water (Fig. 7). About half an hour after this change, it resulted in a conductance similar to that of the stem segment immediately

placed in de-gassed water after the air aspiration. The increase in hydraulic conductance halted instantaneously when de-gassed water was replaced by tap water (Fig. 7).

4.3. Presence of air at different heights above the cut surface

Transverse sections of a frozen stem segment were cut at 0.1, 1, 2 and 7 cm above the original base-cut-plane and studied by cryo-SEM. At 0.1 and 1 cm nearly all vessels were ice-filled. However, 2 and 7 cm above the cut-plane a number of xylem vessels were clearly without ice. Fig. 8 shows details at 1 cm (A) and 2 cm (B) above the cut-plane. In total, there were, respectively, 8, 29, 92 and 54 vessels without ice at 0.1, 1, 2 and 7 cm, respectively. They all belonged to the widest vessels of the particular stem segment. There was no air in small-diameter xylem vessels.

Fig. 8. Transverse sections of chrysanthemum stem segments examined by cryo-SEM. Segments were cryo-fixed by plunging into liquid nitrogen 1.5 h after placing the segment in water subsequent to air uptake. Frozen segments were planed at 1 cm (a) and 2 cm (b) above the original cut-plane for cryo-SEM study. Xylem fibres contain more solutes than vessels and show a dense crystallisation network. This network is absent in vessels. Vessels without ice can be recognised by a dark black colour in contrary to the smooth grey colour of the ice-filled vessels. Horizontal image widths are 500 μm.
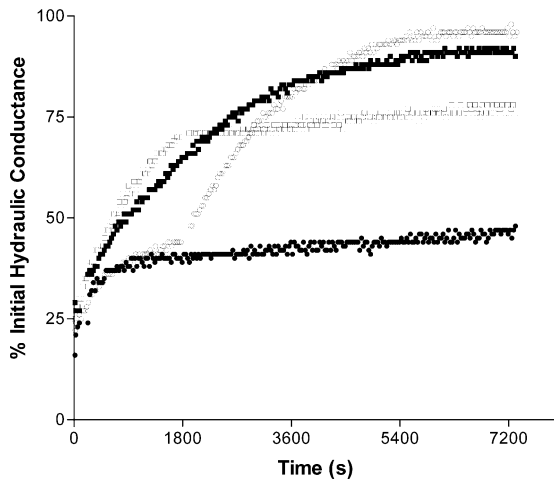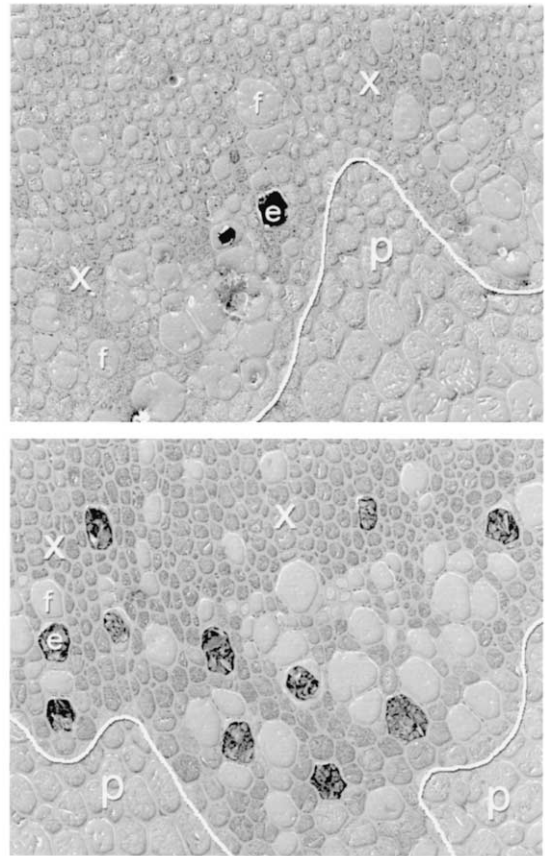

Fig. 7. Effect of the use of de-gassed water on time curves of hydraulic conductance recovery of a 20-cm chrysanthemum stem after air aspiration via the cut-surface. After measuring the initial hydraulic conductance without air in the xylem vessels, air was aspired at the basal cut-surface for 3 min. Thereafter water was reapplied. Time 0 is the time of reapplying water. The water used was either tap water ($\bullet$, $\circ$) or de-gassed water ($\blacksquare$, $\square$). In two treatments, 1930 s after reapplying water it was changed: tap water to de-gassed water ($\circ$) or de-gassed water to tap water ($\square$).

4.4. Water height

Water height (Fig. 1; h_z) in a vase will affect the air pressure inside xylem vessels, and therefore, it may be expected that water height would affect the dissolution of air. The simulation results showed that the effect would be small unless large-diameter vessels are present. Fig. 9 shows the effect of vase water height on fresh weight and water uptake during the first 24 h of rehydrating

cut chrysanthemum flowers that were kept dry for 1 h before rehydration. There was a positive effect of water height on fresh weight and water uptake, especially with flowers cut near their root–shoot junction. The interaction between vase water height and cutting height of the flowers at harvest was significant ($P < 0.001$).

5. Discussion

The physical description of the embolism repair processes in xylem vessels near the cut base of a cut flower predicted that, within a few hours after placement in water, only a part of the vessels would be completely refilled with water and other vessels incompletely. Consequently, hydraulic conductance will only be partly repaired. This agrees with the measurements of hydraulic conductance of stem segments (Fig. 6). The large improvement of conductance repair by the use of de-gassed water (Fig. 7) strongly indicates that the loss of conductance was indeed due to the presence of air in the vessels. The model assumed that refilling of xylem vessels will be due to two processes:

1. fast reconnection between vase water and non-cut xylem vessels above the cut surface by redistribution of air and water, followed by

2. relatively slow dissolution of trapped air and diffusion of dissolved air to the environment.

Time constants of both processes largely differ (seconds to minutes and several hours, respectively). This agrees well with the measured dynamics of hydraulic conductance of stem segments placed in water following air aspiration (Fig. 6). The results of cryo-fixation showed that near the stem base nearly all vessels were water-filled after 1.5 h on water, while some centimetres above the cut surface only large-diameter vessels were still air-filled (Fig. 8). The number of air-filled vessels was higher when comparing sections at 2 and 0.1 cm. At 7 cm above the cut surface the number was lower than at 2 cm. Taking into account the exponential decay in the number of air-filled vessels with height from the cut surface before reapplying water (Nijsse et al., 2001a,b), the amount of air-filled vessels at different heights support the model calculations: almost no air-filled vessels were observed close to the cut surface while higher in the stem a large percentage of the vessels that could contain air (since they were open from the cut surface) still contained air after 1.5 h of recovering. Only large-diameter vessels were filled with air after 1.5 h recovery. This shows that after 1.5 h, small-diameter vessels were completed recovered but does not necessarily
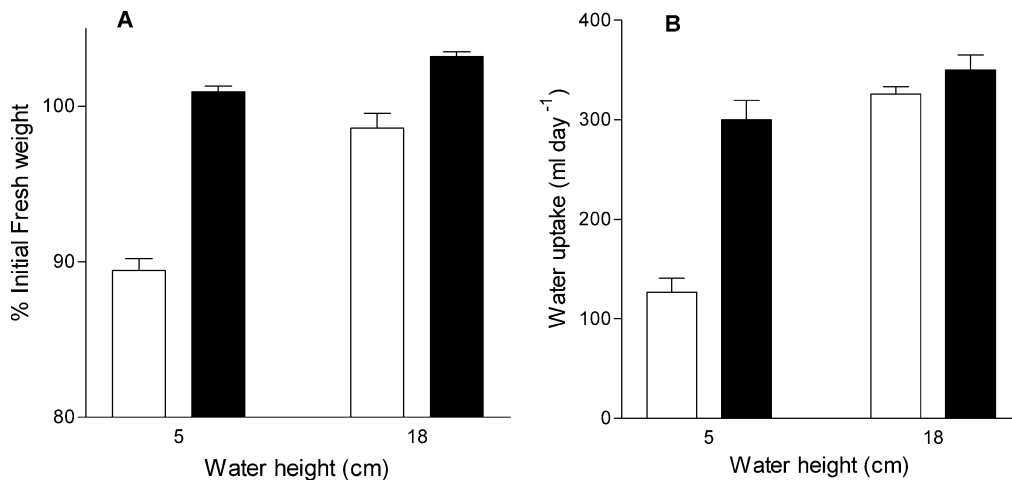

Fig. 9. Effect of height of vase water on fresh weight after 24 h, expressed as percentage of initial weight (A) and on water uptake (B) during the first 24 h after 1 h dry period of chrysanthemum flowers. Water height was 5 or 18 cm. Flowers were cut at 3 (open bars) or 15 cm (filled bars) above the root–shoot junction. Vertical bars represent standard error of the mean.

prove that the proposed exchange of air between adjacent air-filled vessels took place.

Vase water height had a positive effect on rehydration of flowers after a 1 h dry period (Fig. 9). Water height may have increased the air pressure inside xylem vessels and consequently, the dissolution of air. Simulation results showed that the effect would be small unless large-diameter vessels are present. In general, there is a gradual basipetal increase in xylem vessel diameter in plants (Aloni, 1987). This is also demonstrated in cut chrysanthemum flowers (Nijsse et al., 2001b). It explains the interaction between vase water height and cutting height (Fig. 9): higher cut stems have smaller diameter vessels that respond less to height differences in vase water because a lower percentage of the air pressure in the air-filled vessels results from vase water height. Another proposed reason for the water height effect on recovery could have been water uptake via the stem epidermis in contact with water. Van Doorn (1994) showed with roses, stems could take up water by exposure of more than about 60 mm^2 of xylem wall surface to water after removing the bark and closing the cut vessels at the cut surface with grease. However, it is doubtful that in our experiment with stems with open cut surfaces and intact bark, water import via the stem epidermis caused the different responses on water height after cutting at different heights above the soil.

The absence of air emboli is suggested to be a prerequisite for a positive water balance during the vase life of cut flowers (Durkin, 1979; Van Meeteren, 1992). Van Doorn (1990) concluded that in roses the presence of air in the lumen of the xylem elements in itself is not an obstacle to subsequent water uptake. In his experiment, however, the aspiration of air into the stem was measured and not the presence of air, which could be removed after replacing the flower stems in water.

We conclude that anatomical differences in xylem structure, especially vessel diameter distribution, play an important role in rehydration capability of cut flowers after air aspiration. During plant growth, new vascular tissues develop continuously in dynamic relationships with one another. This continuous development of new vascular tissues enables regeneration of the plant and its adaptation to interruptions and changes in the environment (Aloni, 1987). Therefore, it may be expected that differences in rehydration abilities will exist due to variations in growth conditions in the preharvest phase of cut flowers. Moreover, it can be expected that rehydration treatments will have different outcomes using different genotypes of flowers or even different lots of the same flower cultivar, as treatments will largely interact with xylem anatomical properties.

Acknowledgements

References

Aloni, R., 1987. Differentiation of vascular tissues. Ann. Rev. Plant Physiol. 38, 179–204.

Durkin, D.J., 1979. Effect of millipore filtration, citric acid, and sucrose on peduncle water potential of cut rose flower. J. Amer. Soc. Hort. Sci. 104, 860–863.

Durkin, D.J., 1980. Factors effecting hydration of cut flowers. Acta Hort. 113, 109–117.

Evans, R.Y., Zheng, J.-M., Reid, M.S., 1996. Structural and environmental factors affecting the postharvest life of cut roses. Acta Hort. 424, 169–173.

Gibson, A.C., Calkin, H.W., Nobel, P.S., 1984. Xylem anatomy, water flow, and hydraulic conductance in the fern *Cyrtomium falcatum* [Pteridophyta]. Amer. J. Bot. 84, 564–574.

Marousky, F.J., 1973. Recent advances in opening bud-cut chrysanthemum flowers. HortScience 8, 199–202.

Nijsse, J., 2001. Functional anatomy of the water transport system in cut chrysanthemum. Ph.D. Thesis, Wageningen University, The Netherlands.

Nijsse, J., Van Aelst, A.C., 1999. Cryo-planning for cryo-scanning electron microscopy. Scanning 21, 372–378.

Nijsse, J., Van Meeteren, U., Keijzer, C.J., 2000. Air in xylem vessels of cut flowers. Acta Hort. 517, 479–486.

Nijsse, J., Van Meeteren, U., Keijzer, C.J., 2001a. Quantification of emboli by visualization of air filled xylem vessels. Acta Hort. 543, 245–250.

Nijsse, J., Van der Heijden, G.W.A.M., Van Ieperen, W., Keijzer, C.J., Van Meeteren, U., 2001b. Xylem hydraulic conductivity related to conduit dimensions along chrysanthemum stems. J. Exp. Bot. 52, 319–327.

Van Doorn, W.G., 1990. Aspiration of air at the cut surface of

rose stems and its effect on the uptake of water. J. Plant Physiol. 137, 160–164.

Van Doorn, W.G., 1994. Vascular occlusion in cut flowering rose stems exposed to air: role of the xylem wall pathway for water. Physiol. Plant 90, 45–50.

Van Doorn, W.G., 1997. Water relations of cut flowers. Hort. Rev. 18, 1–85.

Van Doorn, W.G., Cruz, P., 2000. Evidence for a wounding-induced xylem occlusion in stems of cut chrysanthemum flowers. Postharvest Biol. Technol. 19, 73–83.

Van Ieperen, W., Van Meeteren, U., Van Gelder, H., 2000. Fluid ionic composition influences hydraulic conductance of xylem conduits. J. Exp. Bot. 345, 769–776.

Van Ieperen, W., Nijsse, J., Keijzer, C.J., Van Meeteren, U., 2001. Induction of air embolism in xylem conduits of pre-defined diameter. J. Exp. Bot. 358, 981–991.

Van Meeteren, U., 1992. Role of air embolism and low water temperature in water balance of cut chrysanthemum flow-ers. Scientia Hortic. 51, 275–284.

Van Meeteren, U., Van Gelder, H., 1999. Effect of time since harvest and handling conditions on rehydration ability of cut chrysanthemum flowers. Postharvest Biol. Technol. 16, 169–177.

Van Meeteren, U., Van Gelder, H., Van Ieperen, W., 2000. Reconsideration of the use of deionized water as vase water in postharvest experiments on cut flowers. Posthar-vest Biol. Technol. 18, 169–181.

Yang, S., Tyree, M.T., 1992. A theoretical-model of hydraulic conductivity recovery from embolism with comparison to experimental-data on acer-saccharum. Plant Cell Environ. 15, 633–643.

Zimmermann, M.H., 1983. Xylem structure and the ascent of sap. Springer, Berlin.

Zwieniecki, M.A., Holbrook, N.M., 2000. Bordered pit struc-ture and vessel wall surface properties. Implications for embolism repair. Plant Physiol. 123, 1015–1020.