PROCESSES AND XYLEM ANATOMICAL PROPERTIES INVOLVED IN REHYDRATION DYNAMICS OF CUT FLOWERS

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Abstract

In cut flowers, which are harvested in air and transported dry, all cut xylem vessels in the basal part of the stem contain air instead of water. These air-emboli initially block water transport at the start of vase life, but usually (partly) disappear during the first hours of vase life, resulting in rehydration of the flower. However, in some cases flowers are not able to sufficiently remove these air blockages, resulting in a poor water status expressed by wilting. Differences in rehydration ability are present between cultivars, but also between different lots of flowers within one cultivar as result of growing conditions.

Using chrysanthemum cut flowers, investigations are focussed on the dynamics of the flower water status during the first hours of vase life after air entrance in the xylem vessels via the cut surface. Role of xylem anatomy in the process related to the establishment of a good or bad rehydration are studied by means of cryo-SEM and other microscopic techniques, dynamic measurements of stem hydraulic resistance and ^{1}H NMR imaging. Modeling techniques are use to explore theoretical concepts and to integrate experimental results obtained by the different experimental techniques.

1. Introduction

One of the main factors which consumers clearly associate with cut flower quality is an adequate vase life. In many cut flowers the limiting factor of vase life is water stress, expressed as early wilting of leaves or flowers (van Doorn, 1997). Early wilting occurs as a result of premature loss of cell turgor and might appear when water uptake and transpiration are out of balance during a lasting period of time. The cause of this unbalance can be either high transpiration or limited water uptake. Limited water uptake is often due to a high hydraulic resistance (R_h) in the lower part of the cut flower stem. With a continuing unbalance between transpiration and water uptake water potentials continuously fall and cavitations might occur all along the water transport path. This reduces R_h further and finally leads the flower into an unrecoverable situation and the premature end of its vase life.

Water stress may show up after some days but also immediately at the start of the vase life. It is well known that in many species R_h gradually increases during vase life. This long term increase in R_h has been explained by the development of physical obstructions in the xylem, which might be due to physiological causes or due to blockage of the xylem vessels by bacteria, yeasts or fungi (Read *et al.,* 1996). In chrysanthemum this long term increase of R_h seem to be influenced by the composition of the vase solution (van Meeteren, 1999).

Besides this long-term increase in R_h, there seem to be also a short-term decrease in R_h, which occurs at the start of the vase life and seriously influence the water

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status of the cut flower. This decrease in R_h is caused by the removal of air blockages from the water transporting xylem vessels in the basal part of the cut flower stem. It is known for more then a century now that air embolism can cause removable blockage of water transport in the stems of cut flowers (De Vries, 1873). In practice this type of blockages arises at harvest or during dry storage when the stem is usually under negative tension and air is drawn into all cut xylem vessels-ends at the cut surface (Nijsse *et al.*, these proceedings). Therefore R_h of cut flower stems might be very high at the start of their vase life. This may cause serious water stress when air embolisms are not sufficiently removed during the first period of vase life. For some unknown reasons this unpredictably seem to occur in various modern chrysanthemum and rose cultivars. It was shown in chrysanthemum cut flowers that recutting of fresh, dry harvested shoots under water or vacuum dehydration or a 2 h pretreatment of cold water (0-5°C) could counterbalance the negative effect of air embolisms on the water balance (van Meeteren, 1992). High water temperature (40°C) or low pH could also obviate the effect of initial air embolisms (Reid *et al.*, 1996).

Anatomical properties of the xylem vessels largely determine the hydraulic resistance (R_h) of the water transport path in the cut flower stem. Poiseuilles law (Eq. 1) is often used to describe water flow through a xylem vessel

$$\Delta P = \frac{8\boldsymbol{h}Ql}{\boldsymbol{p}r^4} \tag{1}$$

where η is the viscosity of the flowing solution, ΔP the pressure difference between the ends of a circular tube with length l and radius r, and Q the volume flow. According to this often-used law, R_h largely depends on vessel diameter distribution, while it can be argued that far most of the transported water flows through a few large diameter vessels. It is known that large diameter vessels are more vulnerable for cavitations during periods of water stress (Zimmermann, 1983). Little is known about the role of xylem anatomical properties on the behavior of air embolisms in cut flower stems during the first hours of vase life. To gain understanding about processes and xylem anatomical properties involved in the recovering of chrysanthemum cut flowers during the first hours of vase life, we used a combination of computer modeling and advanced measurement techniques. In this paper general outline and preliminary results of this research are presented.

2.   General outline of the model & preliminary results

2.1. General model water balance of cut flower

The water status of a cut flower is the result of many interactions (Fig. 1). It is primary a function of transpiration and water uptake. Water uptake is mainly driven by transpiration and restricted by the hydraulic resistance (R_h) of the water transport path. Feedback mechanisms between the water status of the cut flower and stomatal opening usually prevent excessive water loss. A high R_h reduces water uptake and negatively influences the water status of the cut flower. If the water status becomes very low, R_h may progressively increase due to cavitations. The role of water status on an eventual decrease in R_h during the first hours of vase life is unclear.

At the start of its vase life the cut flower is positioned in an environment that enhances transpiration, while it is assumed that R_h is high due to air embolisms in the basal part of the stem. In this situation removal of air embolisms seems necessary to escape from a negative spiral of decreasing water status due to water uptake being lower than transpiration. Therefore the model focuses on the dynamic behavior of air embolisms in the basal part of the stem.

2.2. Initial condition: presence and size of air embolisms at the start of the vase life

The well-known equation for capillary rise can be modified to calculate the vulnerability of xylem vessels for air-entrance when they are cut at harvest (Eq. 2).

$$P_x = \frac{4\gamma}{d}\cos\Theta \qquad\qquad (2)$$

where P_x is the xylem tension at harvest, d is vessel diameter, γ surface tension (0.072 N.m^{-1}), and Θ is the contact angle between water and xylem wall (0°). Calculations with this equation showed that the tension, necessary to pull air into a xylem vessel, largely depends on vessel diameter. However, even with a moderate negative xylem tension (-30 kPa) all cut vessels larger than 10 μm diameter become air filled. It is thought that air-water interfaces cannot pass vessel-to-vessel connections because the force necessary to pull an air-water interface through the narrow pores of pit membranes in vessel-to-vessel connections (<< 1 μm) largely exceeds the pulling pressure in the xylem of a cut flower stem at harvest. The actual amount of air that enters a vessel depends on its length and diameter, which may largely vary. Research with the Cryo-SEM technique to check these assumptions and calculations clearly showed that indeed all cut xylem vessels at the basal cut surface are completely filled with air after harvest (Nijsse *et al.,* these proceedings). It may be clear that the actual R_h of the stem at the start of the vase life is a complex function of the length- and diameter distribution of air-filled vessel-ends in the basal part of the stem.

2.3. Model for the recovering from air embolisms during the first period of the vase life

The model for recovering from air embolisms describes the process of air removal as a result of capillary rise, followed by dissolution of air from the embolised vessels. After reapplying water to the cut flower stem, water tends to enter air-filled xylem vessel ends, mainly due to capillary forces and on the condition that the walls of the xylem vessels are wettable. This tendency is larger in small diameter vessels than in large diameter vessels. As a result hydraulic conductivity of the water transport path partly restore because the rising water column in embolised vessels may contact water in adjacent xylem vessels, which were not cut and embolised during harvest and storage. Model calculations showed that the proportion of the air-filled vessel that initially refills with water largely depends on vessel diameter. Small diameter vessels refill to a larger percentage of their length than large diameter vessels. Dynamic simulations showed that capillary rise is a relative fast process. The water column usually reaches its maximal height within several seconds. The time constant of the process mainly depends on vessel length and little on diameter. The second process involves air removal by dissolution. This process is much slower and may take hours to complete. Preliminary calculations showed that dissolution is much faster in small diameter vessels than in large diameter vessels. Calculations also showed that sometimes not all air embolisms completely disappear from large diameter vessels.

2.4. Measurements of the recovering from air embolisms during the first period of the vase life

To test the results of model calculations measurements of R_h were done on excised stem segments of chrysanthemum cut flowers (*Dendranthema x grandiflorum* Tzevelev cv. Cassa), which were cut at different heights from the roots. It is known that cut flower shoots, hich are harvested close to the roots, show more water stress problems during vase life than shoots, cut higher from the plant (Marousky, 1973), especially after air entrance (van Meeteren, 1989; van Meeteren and van Gelder, 1999).

The use of stem segments instead of whole cut flowers allows the use of a constant pressure gradient to induce water uptake instead of an unknown fluctuating driving force for water flow induced by transpiration. R_h was determined by measuring water flow through the stem segment while applying a constant pressure gradient of approximately 200 $kPa.m^{-1}$ (Fig. 2). The actual applied pressure and resulting flow rate were accurately measured at a frequency of 1 s^{-1} and averaged over 30 s. Flow rate was corrected for direct evaporation from the container with water on the balance and pressure was corrected for the actual air pressure in the measurement environment. Measurements were done at room temperature ($\pm$ 21°C). The solution was according to the specifications of 'standard vase water' (van Meeteren *et al.,* 1999).

Stem segments of approximately 20-cm length were harvested under water to prevent air entrance in the cut xylem vessels. The segments were connected to the system and an initial measurement of hydraulic resistance was done. Afterwards, the stem segments were lifted above the solution while remaining them under tension to allow air entrance for approximately three minutes. After that, flow measurements were resumed by reapplying water to the basal cut surface the stem segments and the recovering pattern of R_h was measured during approximately 1.5 hours.

Figure 3 shows the representative water flow patterns measured on a low and high cut stem segment. The presented results are normalized for the initial flow rate, which was higher in the low cut stem segment than in the high cut stem segment. This difference in initial flow rate (without air embolisms) demonstrates a lower R_h in low than in high cut stem segments. After air entrance, water uptake recovered to 20-30% of its initial value within seconds. After this quick initial rise, water uptake showed a slowly recovering pattern to a stable level, which varied between approximately 50-80% of its initial rate without air embolisms. The time span of the slow recovering process may exceed 2 hours. Stem segments of cut flowers that show water stress problems during vase-life (cut at 10-cm height) recover less than stem segments of cut flowers, which show no water stress problems during vase life (cut at 25 cm height).

Cryo-SEM images of chrysanthemum stem segments, which were allowed to recover from air embolisms for several minutes, show almost no air embolisms close to the basal cut surface. However, after recutting at 2 cm above the basal cut surface (Nijsse and van Aelst, 2000), several large diameter vessels were observed that were embolised (Fig. 4). Images of the rates of water flow (spatial resolved) through a cross section of a chrysanthemum stem segment, obtained by ^{1}H-NMR-imaging technique (Scheenen *et al.,* 1999), show that the rates of water flow in regions with many xylem vessels decreases after air entrance at the basal cut surface (Fig. 5).

3.   Discussion and conclusions

More than a century of research on water transport in cut flowers has indicated that in practice air embolisms largely influence the hydraulic conductance in the xylem, especially at the start of their vase-life. Because cut flowers are harvested in air, while the xylem system usually is under negative tension, air enters the cut xylem vessel-ends. The resulting increase in hydraulic resistance (R_h) largely obstructs water flow at the start of the vase life. However, this can be reversed by removal of the air embolisms from the xylem vessels during vase life. It is well known that not all species are sufficiently able to remove these blockages (Van Doorn, 1997). Consequently, some species are able to recover and restore a normal water status, and others not. They remain a high R_h instead and do not restore their water status but continuously lose water resulting in premature wilting. Differences are not only observed between species and cultivars but also between individual stems of the same cultivar. Therefore, Reid and coworkers (1996) concluded that it seems critically important to understand the structural, chemical, or physiological basis of these differences in restoration behavior, because such information may be important for breeders of cut flowers. Understanding of processes and plant properties that are important for early air removal from the xylem system could be important

to avoid conditions during the production and handling after harvest that increase problems with the removal of air.

To gain understanding, physical processes related to the removal of air embolisms during the first hours of vase life were modeled. Simulation results of this model were compared with measurements of hydraulic conductance recovering obtained from excised chrysanthemum stem segments. Dynamic calculations with the model showed that the process of hydraulic conductance recovery consists of an initial fast component followed by a slower second component, which may last hours. These fast and slow components were also observed in the measured hydraulic conductance recovery patterns of stem segments (Fig. 3). Cryo-SEM images of air embolisms in xylem vessels seem to confirm the prediction of the model that air gathers in the large diameter vessels during the first phase of the hydraulic conductance recovery process.

Model calculations also predicted that the success of air removal largely depends on xylem anatomical properties. Measurements on excised stem segments of one chrysanthemum cultivar, similarly grown, but harvested at different cutting heights clearly showed different restoration patterns. The lower hydraulic conductance recovery level of low harvested stem segments correlates with the frequent occurrence of early leaf wilting in low harvested chrysanthemum cut flowers (van Meeteren, 1989). It was found that a lower initial hydraulic resistance of a stem segment before air entrance usually correlates with a poorer recovery of hydraulic conductance after reapplying water. This properly agrees with predictions of the model.

Acknowledgment.

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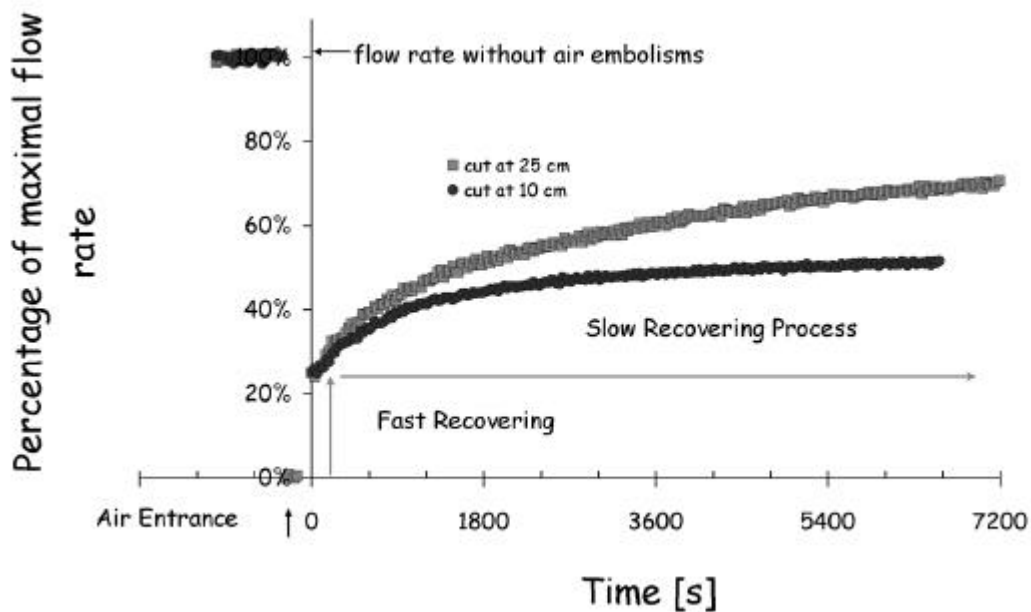Zimmermann, M.H., 1983. Xylem structure and the ascent of sap. Springer, Berlin.

Figures
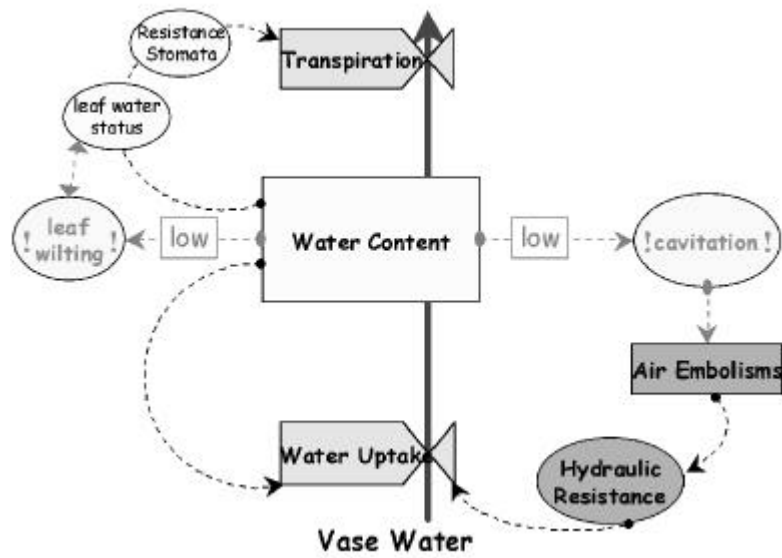
1. Schematical representation of the water relations of a cut flower.

$$K_h = \frac{J_v}{[(p_{atm} - P_{vacuum})/Length]}$$
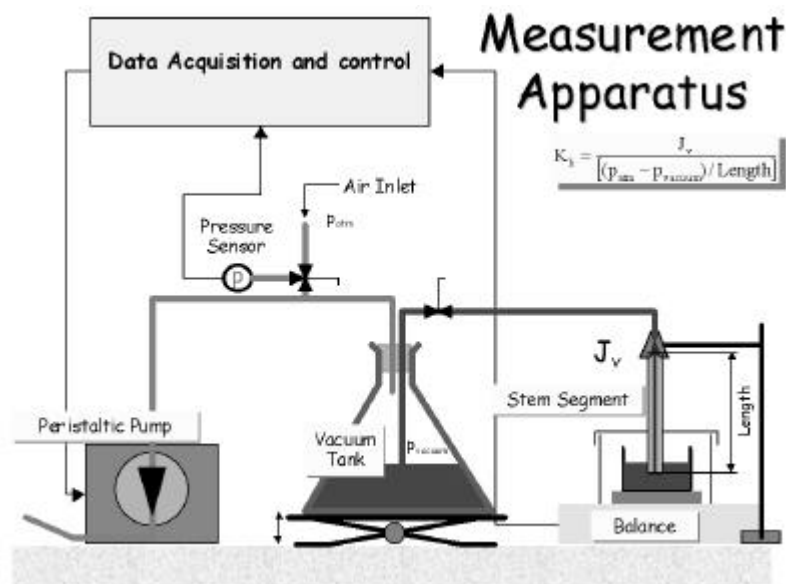
2. System to accurately measure changes in hydraulic resistance (R_h) of stem segments at a high sample frequency.

3. Examples of water flow rate patterns, measured in stem segments of chrysanthemum cut flower during recovering from air embolisms. Stem segments were cut at 10 and 25 cm height from the roots. In contrary to flowers harvested at 25 cm height, flowers harvested at 10 cm height often showed early leaf wilting during vase life experiments. Initial hydraulic resistances of stem segments harvested at 10 and 25 cm height are 1.45 and 2.15 $\mu mol.s^{-1}kPa^{-1}$ respectively.
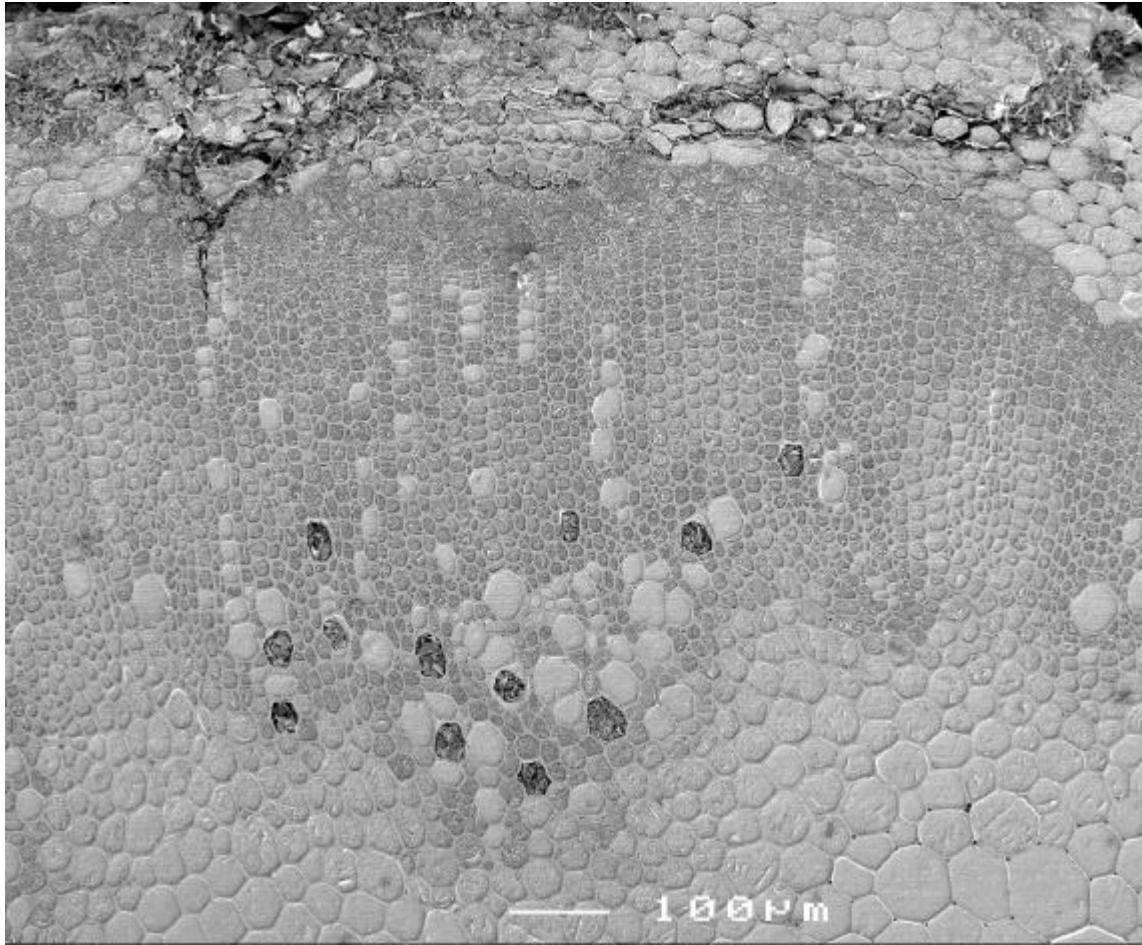
4. Cryo-scanning-electron microscopy (Cryo-SEM) image from a transverse section of a chrysanthemum stem segment located at 2 cm height above the basal cut surface. The sample is frozen several minutes after reapplying water (the start of the recovering process from air embolisms). Only large diameter vessels are air filled.
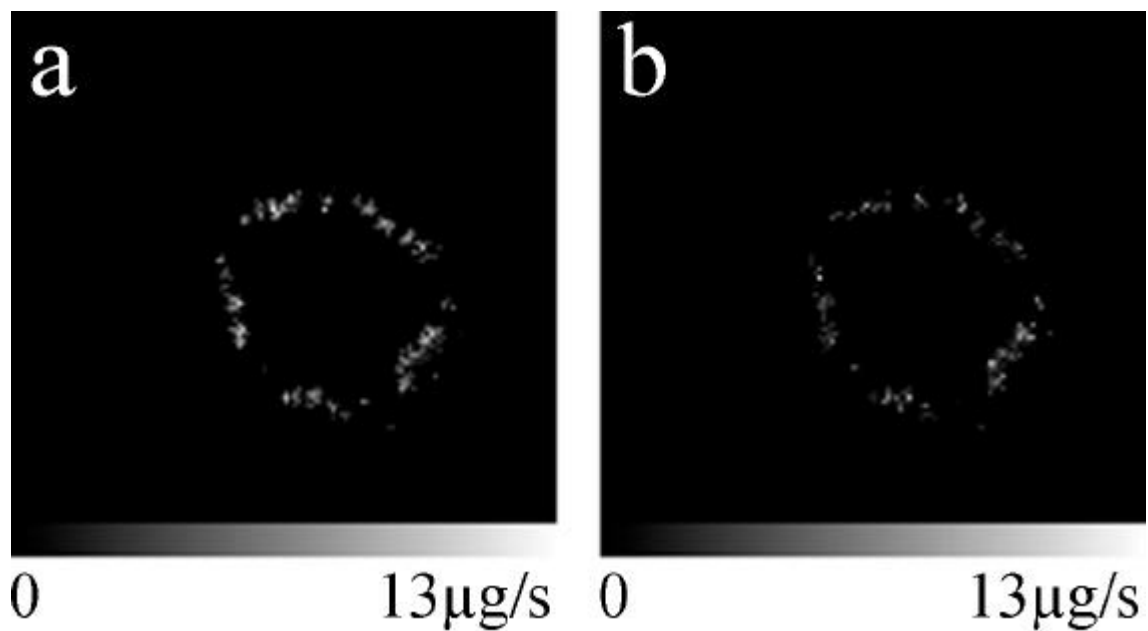
5. ^{1}H-NMR images of spatial resolved rates of water flow through a transverse section of a chrysanthemum stem segment. Transverse section was located 2 cm above the basal cut surface of the stem segment, which was cut at 28 cm height from the roots.
a) measured before air entrance at the basal cut surface
b) measured three hours after air entrance and reapplying water at the basal cut surface (the start of the recovering process from air embolisms).