AIR IN XYLEM VESSELS OF CUT FLOWERS

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Abstract

Until now all studies on the role of air emboli in the water uptake of cut flowers describe indirect methods to demonstrate the presence of air in the plant tissues. Using cut chrysanthemum flowers, this report is the first one that directly visualises both air and water in xylem ducts of cut flower stems, by observing transversal sections of frozen stalks in a cryo scanning electron microscope (cryo-SEM). After cryo-fixation frozen stems were sawed transversally at a desired height, polished with a cryo ultra mill, lightly etched, sputtered with platinum and investigated with the cryo-SEM. This method allows for detailed studies on the origin and fate of air emboli on water balance in cut flower stems.

1. Introduction

Water stress during vase life is often the cause of shortened vase life of cut flowers (Halevy and Mayak, 1981). Loss of turgidity results in wilting of flowers, leaves or stems. A high level of turgidity is necessary for the development of flower buds into full-bloom maturity, and for maintaining normal metabolic activity in the cut flower. The main reason for the occurrence of a water deficit, apparently is some kind of blockage to water flow that develops in the stem (Halevy and Mayak, 1981). The nature of this blockage can vary widely (Van Doorn, 1997). Factors such as micro-organisms (Marousky, 1969; Van Meeteren, 1978; Zagory and Reid, 1986; Put and Jansen, 1989), physiological responses to cutting of the stem (Marousky, 1971; Fujino and Reid, 1983; Van Doorn *et al.*, 1991), and the presence of gas bubbles in the xylem conduits (Durkin, 1979; Dixon *et al.*, 1988; Van Meeteren, 1992; Van Doorn and Otma, 1995) have been suggested as causes for the decline in hydraulic conductance. For cut roses, Dixon and Peterson (1989) proposed a combination of effects of wounding at the cut end along with increasing numbers of micro-organisms that induce an initial decline in stem water potential resulting in cavitation in the xylem vessels. Thereafter, further deline in stem conductance by xylem cavitation occurs. De Stigter and Broekhuysen (1989) also found cavitation in rose stems gradually developing during vase life when the cut surface of the

stem became clogged by microbial growth. However, there are conflicting reports about the relationship between bacterial numbers in the vase solution and the occurrence of cavitation. For example, Williamson and Milburn (1995) using Acacia found no clear relationship between the number of cavitation events as measured by audible acoustic emissions and the number of bacteria in different solutions.

Air entering the xylem vessels via the surface of the cut stem at the moment of harvesting and/or during dry storage of cut flowers can seriously block water uptake, as shown for chrysanthemums (Durkin, 1980; Van Meeteren, 1989, 1992) and roses (Durkin, 1979, 1980). Scholander *et al.* (1957), however, held liana stems in air for 15 minutes, during which 80 ml of air was taken up, and found that the subsequent rate of water uptake was as high as before. Considerable differences exist between rose cultivars in the degree of inhibition of water uptake upon exposure of the cut stems to air (Van Doorn and Reid, 1987). Van Doorn (1990) concluded that the presence of air in the lumen of the xylem elements in itself is not an obstacle to water uptake.

Only indirect methods have been used to establish the role of emboli on the water uptake of cut flowers: recutting under water (Durkin, 1979), removal of gas from water used for rehydration (Durkin, 1979; Slootweg, 1995), uptake of fluorescent dye or suspensions of macromolecular matter (Dixon and Peterson, 1989; Van Doorn and Otma, 1995), vacuum rehydration (Aarts, 1957; Van Meeteren, 1989), ice-cold vase water for a short period (Van Meeteren, 1989, 1992; Slootweg, 1995), detection of cavitation by acoustic emissions (Dixon *et al.*, 1988), the volume of air taken up at the cut stem end (Van Doorn, 1990), and the conductivity to air in short stem segments (De Stigter and Broekhuysen, 1989). Methods to directly observe emboli in ducts from the outside of the stem are scarce due to the woody status of most cut flower stems. The only really non-destructive method is nuclear magnetic resonance (NMR) imaging (Van As, 1992). However, the resolution of NMR imaging is limited. For optical or electron microscopy, sectioning is indispensable, which would replace water columns and thus emboli. To avoid this problem, the water has to be immobilised and this can be done by freezing. In this communication we report on the application of the cryo scanning electron microscope (cryo-SEM) to visualise the presence of air in the water conducting elements of cut flower stems. Initial attempts to cryofracture the stems were unsuccessful because the tissues tended to fracture longitudinally instead of transversely due to the arrangement of the wood fibres. Therefore we developed a technique to obtain transverse segments of the xylem tissue. We demonstrate the usefulness of the method with an experiment on rehydration after desiccation of cut chrysanthemums.

2. Materials and methods

2.1. Plant material

Chrysanthemum (*Dendranthema morifolium* Ramat cv. Cassa) cuttings were grown in pots with a commercial potting soil in a greenhouse at a day/night temperature of 16°C/18°C and a photoperiod of 16 hours during weeks 0-4 and 8 hours during weeks 5-11. The flowers were harvested at commercial maturity by cutting the stalks under water at soil level. To start the experiment with flowers of the same water status, the stalks were placed for 2 hours in a mixture of ice and water in dark in a cold room at 5°C. During this treatment the flowers got full turgidity. Thereafter, 13 cm of the stems was cut off under water, and leaves were removed till 12 cm above the cut plane. On these cut flowers the influence of dry storage after a desiccation treatment on vase life and vessel contents was studied.

2.2. Desiccation and storage

Dehydration of the flowers was accomplished by placing the individual flowers for 1 h horizontally on two wires in a conditioned room at 20°C, 60% relative humidity (RH) and a light intensity of 14 μmol $m^{-2}s^{-1}$. During this desiccation treatment, flowers

lost 4-6% of their initial fresh weight. Thereafter half of the flowers were stored dry for 24 hours under non-transpiring conditions in plastic bags at 20°C, cut off 2 cm, and placed for 2 hours in a mixture of ice and water at an air temperature of 20°C (rehydration treatment). The other half of the flowers were cut off 2 cm directly after desiccation, and placed for 2 hours in the ice water. After these pre-treatments the flowers were placed in vases to observe their fresh weight changes.

2.3. Vase period

During the vase period, each flower was individually placed in a vase with water of defined quality (demineralised water + 124.8 mg/l $NaHCO_3$ + 99.2 mg/l $CaCl_2$ + 1.2 mg/l $CuSO_4$) in a conditioned room at 20±1°C, 60%RH and a light intensity of 14 µmol $m^{-2}s^{-1}$ (Philips, TLD 50W/84HF) with a photoperiod of 12h.

2.4. Cryo Scanning Electron Microscopy

To investigate the contents of the xylem, two flower stalks were sampled before and after desiccation and after the rehydration treatment. They were cryo-fixed by plunging them into liquid nitrogen. Still under liquid nitrogen the lower 7 cm of the stems were sawed off with a diamond circle sawing device (Minicraft high precision drill, Black and Decker) and stored in a cryo vessel. Before investigation a selected stem part of 1 cm height was sawed out and mounted on a hollow messing stub with Tissue Tek (NR. 4583 Miles Inc., Elkhart, IN-USA.) with one of the transversal sawing surfaces up. This sawing surface was polished with a diamond cryo ultra mill (Reichert-Jung Polycut E, equipped with a cryostage). The surface was etched for 5 min at -89°C at 10^{-4} Pa in an Oxford CT 1500 HF cryo transfer unit and sputtercoated with 3 nm platinum. We studied the vessel contents at 2 cm above the stem base (after the recutting) in a JEOL 6300F field emission scanning electron microscope at -170°C at 2.5kV. After investigation samples could be stored and investigated for another time.

2.5. Fresh weight measurements

To observe changes in the water status of the cut flowers, stalks not used for cryo-SEM investigation (10 for both treatments) were weighed after each treatment (desiccation, storage, rehydration) and during several days of vase life at noon.

3. Results

3.1. Fresh weight measurements

Fig. 1 shows the changes of fresh weights after harvest for the two treatments. The flowers that were stored for 24 hours after the desiccation period showed a decrease of fresh weight, even when the flowers were placed in vase water. The leaves of these cut flowers were visibly wilted. During the 24 hours of storage the flowers did not lose weight as much, but apparently they lost the ability to restore the water uptake. The non-stored flowers totally regained their starting weight during the rehydration treatment. During vase life these flowers had turgid leaves, indicated by a positive water balance during the first days.

3.2. Cryo Scanning Electron Microscopy

The results of the cryo-SEM are illustrated in Fig. 2. An overview of a transverse section as seen in cryo-SEM is shown in fig 2a. The pith of the chrysanthemum stem consists of large dead cells of which only dry walls remain. Only the outer pith cells are still filled. The xylem consists of primary vessels, secondary vessels and libriform. The primary vessels can be discerned from the secondary vessels by their helical cell walls and their more centred position. The vessels lie embedded between libriform fibres. Most secondary vessels are situated within the former vessel bundles. Around the xylem the cambium, phloem and cortex are visible. The primary phloem has developed to strong

sklerenchymatous fibres (these are the fibres that can be peeled off from a fresh stalk). As a result of freezing and etching, the lumina of the xylem fibres show a lattice of solutes as a result of the crystallisation of the cytoplasm during freezing. Vessels contain less solutes and show therefore a flat ice-surface. Fig. 2b demonstrates at high magnification the distinction between fibres, empty vessels (air filled) and water filled vessels. Figs. 2c-f show the results of the different treatments at 2 cm above the cut plane. The situation before desiccation is shown in Fig 2c. All xylem vessels are filled. After the desiccation period numerous primary and secondary vessels are found empty (fig. 2d). After 24 hours storage followed by the rehydration treatment still a lot of vessels remain empty (fig 2e), while the xylem vessels of the non-stored stems have been refilled (fig 2f). However, approx. one fifth of the vessel bundles still contained empty vessels (not shown).

4. Discussion

The cryo-SEM technique presented here allows observation of undisturbed hydrated plant parts, and hence a precise detection of liquids and gases in plant tissues as earlier demonstrated by Canny (1997) in non-fibrous tissues (excised sunflower petioles). In a strict sense, only water is visible (as ice), whereas air that is present during the liquid nitrogen fixation is removed at the (usual) vacuum condition in the SEM. There may be argued that the freezing method will cause cavitation or embolism. Indeed this might be the case when the samples are thawed back. Figs. 2c and 2f show only filled vessels, thus proving that freezing itself does not induce empty vessels. The presented method allows polishing and investigating areas of even more than 1 cm^2. In this technique, the use of chemical fixatives is circumvented, thereby avoiding several artefacts. In cut flower research the appearance and disappearance of embolisms can be studied by comparing either cross fractures at different heights in the stem or by comparing different stages after harvesting and various treatments. The cryo-SEM method is destructive to the chrysanthemum flowers, so the vase life of the same flower cannot be tested. To compare xylem contents of flowers with the vase life of other, similar treated flowers, homogeneous plant material is required. Of course flowers can also be cryo-fixed after vase life.

Apart from water, the technique also allows for detection of other factors, such as micro-organisms that have been mentioned as a cause for changing water potentials (Marousky, 1969; Van Meeteren, 1978; Zagory and Reid, 1986; Put and Jansen, 1989). In this case water (ice) has to be removed by deep etching (Van Doorn *et al.*, 1991).

This is the first experiment where the presence of air embolism in xylem of cut chrysanthemum has been directly observed. Approximately 50% of the xylem vessels at the original cut plane will reach more than 2 cm higher up in the stem (data not shown). Therefore, at the height of sampling, 2cm above the cut plane, ±50% of the vessels is in open contact with the cut plane and may contain entered air. The 1 hour desiccated flowers (fig. 2d) and the non-restored flowers (fig. 2e) exhibited a percentage of empty vessels close to 50%, pointing that most of the cut open vessels contained air at 2cm above the cut surface. More sampling is needed to get reliable percentages of open vessels. The flowers that were stored were no longer able to restore their water balance. It was argued before that this absence of restoration was due to a lost ability to refill the embolised xylem vessels (Van Meeteren, 1992). Our cryo-SEM results agree very well with this hypothesis. In addition the non-stored flowers had a positive water balance during the first days of vase life and showed refilled xylem vessels. That some vessel bundles still contained some open vessels may be caused by the fact that de lowest leaves were taken off.

Our future work using this cryo-SEM technique on air embolism in cut chrysanthemum stems will focus on the following questions: 1) Under what conditions will air enter the stem? 2) To what height and how fast can air enter the stem? 3) What is the mechanism resulting in refilling xylem vessels? 4) Genetic and environmental conditions can influence early leaf wilting (Van Meeteren, 1989). Which of these

influences are directly related to the behaviour of air in the vessels?

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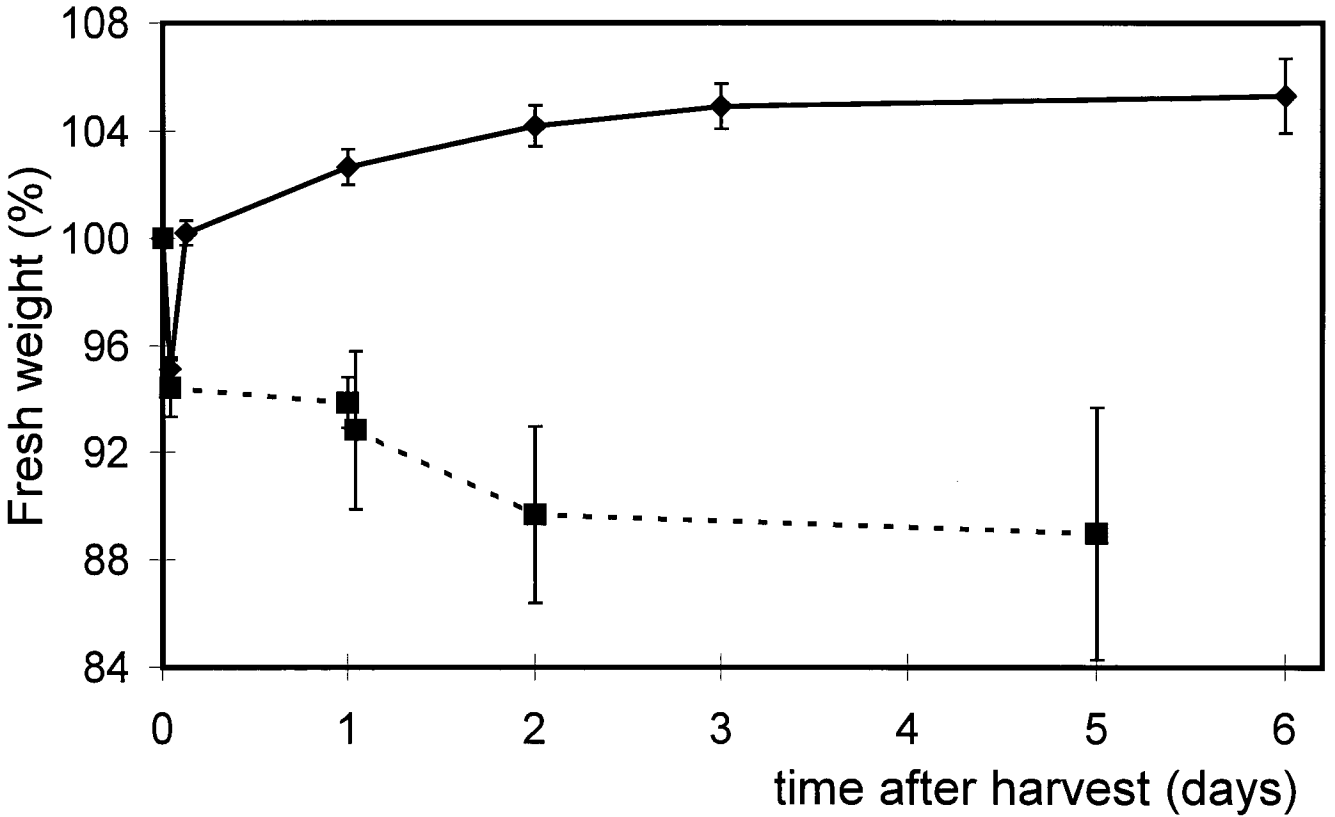
Figures

Figure 1: Time course during the vase period of the fresh weight of flowers given a rehydration treatment (2 hours in water of 0°C; air temperature: 20°C) after either 1 hour desiccation and 24 hours dry storage under non-transpiring conditions (■), or immediately after the 1 hour desiccation period (◆). Vertical bars represent the standard error of the mean.

Figure 2:
2a: schematic overview of a transversal section through a chrysanthemum stem.
2b: High magnification cryo-SEM image of the xylem showing two filled vessels (fv), one empty vessel (ev) and libriform fibres (lf). Note the different crystallisation patterns in the lumina of the fibres and the filled vessels
2c-f: Cryo-SEM transversal images of vessel bundles 2cm above the original cut plane of a cryo-fixed stalk after the following treatments:
2c: harvested and 2 hours in ice water.
2d: harvested, 2 hours in ice water and 1 hour desiccation.
2e: harvested, 2 hours in ice water, 1 hour desiccation, 24h storage and 2 hours in ice water
2f: harvested, 2 hours in ice water, 1 hour desiccation, and 2 hours in ice water
(co: cortex, sk: sklerenchyma, ph: phloem, sx: secondary xylem, px: primary xylem, pi: pith).

1.


108
104
100
96
92
88
84
Fresh weight (%)
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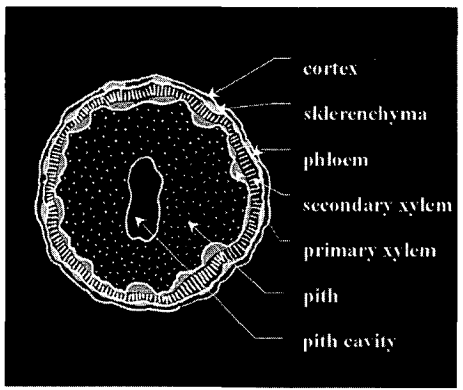
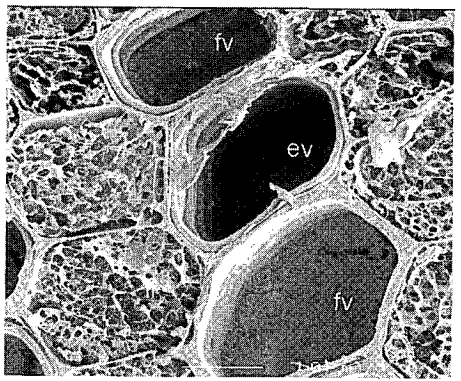
1
2
3
4
5
6
time after harvest (days)

2.

a)


cortex
sklerenchyma
phloem
secondary xylem
primary xylem
pith
pith cavity

b)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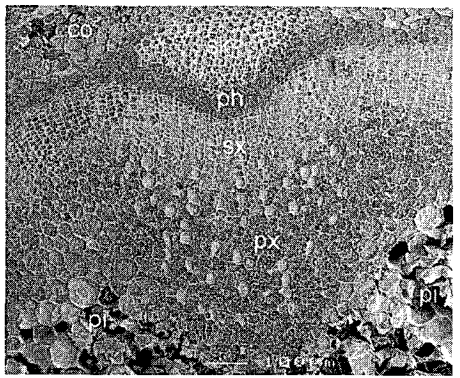
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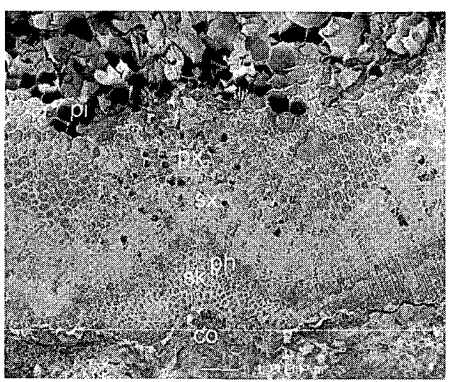
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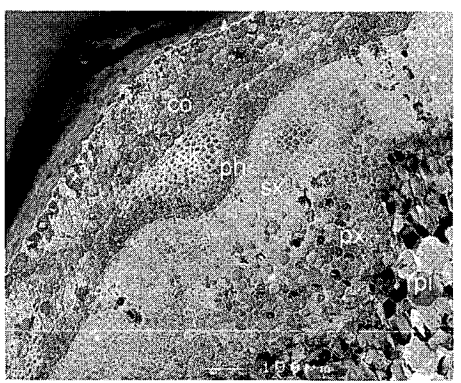
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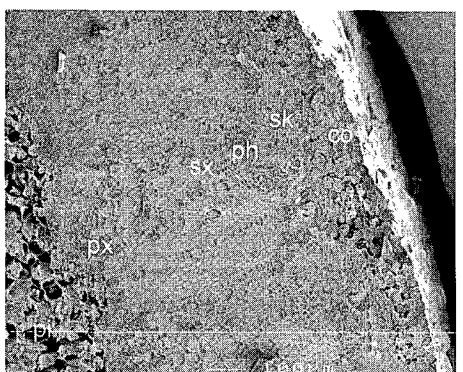
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