QUANTIFICATION OF EMBOLI BY VISUALIZATION OF AIR FILLED XYLEM VESSELS

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Abstract

Between harvest and vase life the cut surface of most cut flowers is exposed to air for a longer or shorter period. It was hypothesized that under normal harvest and transport conditions air only enters the cut open vessels and does not move to non-cut vessels. The vessel length distribution of chrysanthemum stems was analyzed with the latex particle method and compared to the distribution of air embolisms in 5% (w/w) desiccated stems, visualized using cryo-scanning electron microscopy. It was concluded that by moderate desiccation all cut open vessels are completely air-filled and that intact vessels are not embolized.

1. Introduction

In higher plants water transport from roots to leaves occurs mainly through the xylem vessel system. Xylem vessels consist of one to numerous tracheary elements, which are interconnected by inter-vessel pits. Xylem vessel lengths can vary from shorter than one millimeter to several meters. The diameter of xylem vessels varies from a few micrometers to a few hundreds of micrometers.

Between harvest and vase life the cut surface of most cut flowers is exposed to air over a shorter or longer period. In theory even a moderate water tension in the xylem vessel system is sufficient to allow air entrance into cut open vessels. The resulting embolisms can block the water transport system, which results in early leaf wilting or a shortened vase life (Van Meeteren, 1992). In theory, based on estimated pore-sizes in the inter-vessel pits, for induction of air embolism through an inter-vessel pit, a much higher water tension is required. Zimmermann (1983) estimated the tension needed for air entrance in non-cut vessels at -15 Atm for a maximal pit membrane pore size of 0.2 µm. Therefore it is generally hypothesized that under normal harvest and transport conditions only the cut open vessels should be air filled. If so, the vessel lengths will determine how far above the cut surface air-blockage can occur.

By visualizing the embolized vessels, it was tested in this study whether air enters the xylem vessels at moderate desiccation (5% w/w), and whether the entrance of air is restricted to the cut open vessels. In chrysanthemum cut flowers the vessel length distribution was characterized using the latex particle method and compared to the distribution of air embolisms in a moderately desiccated stem using cryo-scanning electron microscopy (cryo-SEM).

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2. Materials and methods

2.1. Plant material

Chrysanthemums (*Dendranthema x grandiflorum* Tzvelev 'Cassa') were grown in pots in a greenhouse of Wageningen University and investigated at the stage of commercial maturity. Early in the morning, the chrysanthemums were transported to the lab and cut under water at 25 cm above soil surface.

2.2. Vessel length determination

Stem segments of 30 cm length were cut out the chrysanthemum stem under water. The upper part of the segments was attached to a vacuum system, while the lower part was placed in an aqueous red latex solution (1% w/v). Latex particles can easily move through vessels, but are far too big to pass inter-vessel pits. After overnight uptake of the latex solution, the particles completely clogged (and thereby colored) the cut open vessels. Out of every internode two transverse sections (2 mm thick) were cut out and photographed with a digital camera attached to a microscope. Using a digital image analysis computer program (SCIL_Image 1.3, University of Amsterdam, Faculty of Mathematics and Computer Science, Amsterdam, The Netherlands) the amount of red-colored vessels was quantified as function of the distance to the cut surface (modification of the method of Zimmermann and Jeje; 1981).

2.3. Detection of embolized vessels

Stems were air-dried under 'vase life conditions' (20°C, 60% RH) until a weight decrease of 5%, which took around one hour. After this desiccation treatment, the stems were plunge-frozen in liquid nitrogen (-196°C) to immobilize the water in the vessels. As a control stems were frozen directly after cutting under water, without the desiccation treatment.

At different distances from the cut surface stem transversal sections were prepared for investigation in a cryo-scanning electron microscope (cryo-SEM) as described in Nijsse and Van Aelst (2000). In short, stem pieces of about 1 cm were sawed out under liquid nitrogen using a circular diamond saw. These stem pieces were cryo-planed at -90°C in an ultra-microtome (Reichert-Jung Ultracut E/FC4D) equipped with an 8 mm wide diamond knife (Histo no trough 45°, Drukker International, The Netherlands). The planed surfaces were freeze dried for 3 minutes at –89°C (to gain contrast) and photographed in a cryo-SEM (Jeol 6300F) at –190°C. The digital photographs were analyzed on the number of embolized vessels per transversal section.

3. Results

Figure 1a shows an overview of a cryo-planed section through a chrysanthemum stem. The vascular bundles are neatly arranged in a circle close to the cortex. In the center of the pith (consisting of dead, empty cells) a hole is made during preparation to avoid a rapid pressure increase of the boiling liquid nitrogen during cryo-planing. Figure 1b shows a detail of the xylem tissue of a desiccated stem. Xylem vessels can be distinguished from other cells by the solid gray appearance of their lumina, which is a result of the low solute contents of xylem sap. Embolized vessels are mostly filled with sawing debris and can easily be recognized. Figures 1d, 1f, and 1h show the distribution of embolized vessels in a desiccated stem at distances of respectively 1, 8, and 16 cm above the cut surface. The higher above the cut surface, the less embolized vessels are found. The control treatment (no desiccation) showed no embolized vessels. Figures 1c, 1e, and 1g show the results of red latex uptake at similar heights above the cut surface and show the same decrease of marked vessels as the cryo-planed sections do. The relative amount of vessels filled with red latex as function of the distance to the cut surface is shown in figure 2. In the same figure the decrease of embolized vessels is plotted. As found in other experiments (unpublished data) the amount of red vessels (open at cut surface) can be characterized by an exponential relation with an ever repeating distance called $X_{50\%}$ over which the amount of red vessels halves. The $X_{50\%}$ value of the latex treatment was on average 28.5 mm and corresponded very well with the $X_{50\%}$ of 27.8 mm of the embolized vessels in the desiccated stem.

In another group of stems the non-desiccated (control) stems contained air filled vessels. The desiccated stems of this group showed the exponential decrease of embolized vessels close to the cut surface, but higher up in the stem still a number of embolized vessels was visible. Both in the control and higher up in the desiccated stems the embolized vessels were clustered within one ore more vessel-bundles. This was in contrast to a random distribution in the desiccated stem of which the control did not contain embolized vessels.

4. Discussion

The close similarity between vessel length distribution and distribution of embolisms points out that air completely fills all cut open vessels. If any cavitation occurs, this must be rare, otherwise more embolized vessels should have been found higher in the stem. These results agree with the common hypothesis, based on estimated pit membrane pore sizes and physical laws, that the air-water meniscus will not pass inter-vessel pits at moderate water tension. In case the control (non-desiccated) already contained embolized vessels, the desiccated stems showed close to the cut surface a clear exponential decrease, but higher up still open vessels occurred in clusters in one or more vessel-bundles. These pre-harvest embolisms could be due to drought-stress, mechanical damage or due to insects. It can be questioned whether pre-harvest embolisms can cause vase life problems.

The used cryo-SEM technique is applicable for a wide range of embolism studies. For example the severity of desiccation at which cavitation starts to occur can be determined. Also the process of restoration of desiccated stems during subsequent vase life can be studied with this method. A disadvantage of the cryo-SEM method is the problem that not a great series of stems can be investigated due to the extensive preparation and investigation procedure.

In general, higher up in plant vessels are shorter (Zimmermann and Potter, 1982; own unpublished results). This can be one of the reasons that higher-cut chrysanthemum flowers have a better restoration of their water uptake after dry storage than lower-cut flowers (Van Meeteren and Van Gelder, 1999). At this time no data are available about variation of vessel length between cultivars in the same cut flower species. Neither the influence of growing conditions on vessel length is known.

If just before vase life the stem is re-cut under water (Van Meeteren and Van Gelder, 1999), the $X_{50\%}$ value of the vessel length distribution can be used to predict how much of the stem has to be cut off to restore a certain percentage of the vessels at the new cut surface. For example, to gain 75% of directly water containing vessels at the cut surface, a piece with a length of two times the $X_{50\%}$ value has to be cut off under water, i.e. about 6 cm in case of the plant stems studied here.

5. Conclusion

Xylem vessel length determines the height to which air, aspired via the cut surface, can block the xylem system in cut flowers. After moderate desiccation (5% w/w) of cut flowers, air embolizes all cut open vessels. Intact vessels get not air filled by moderate desiccation.

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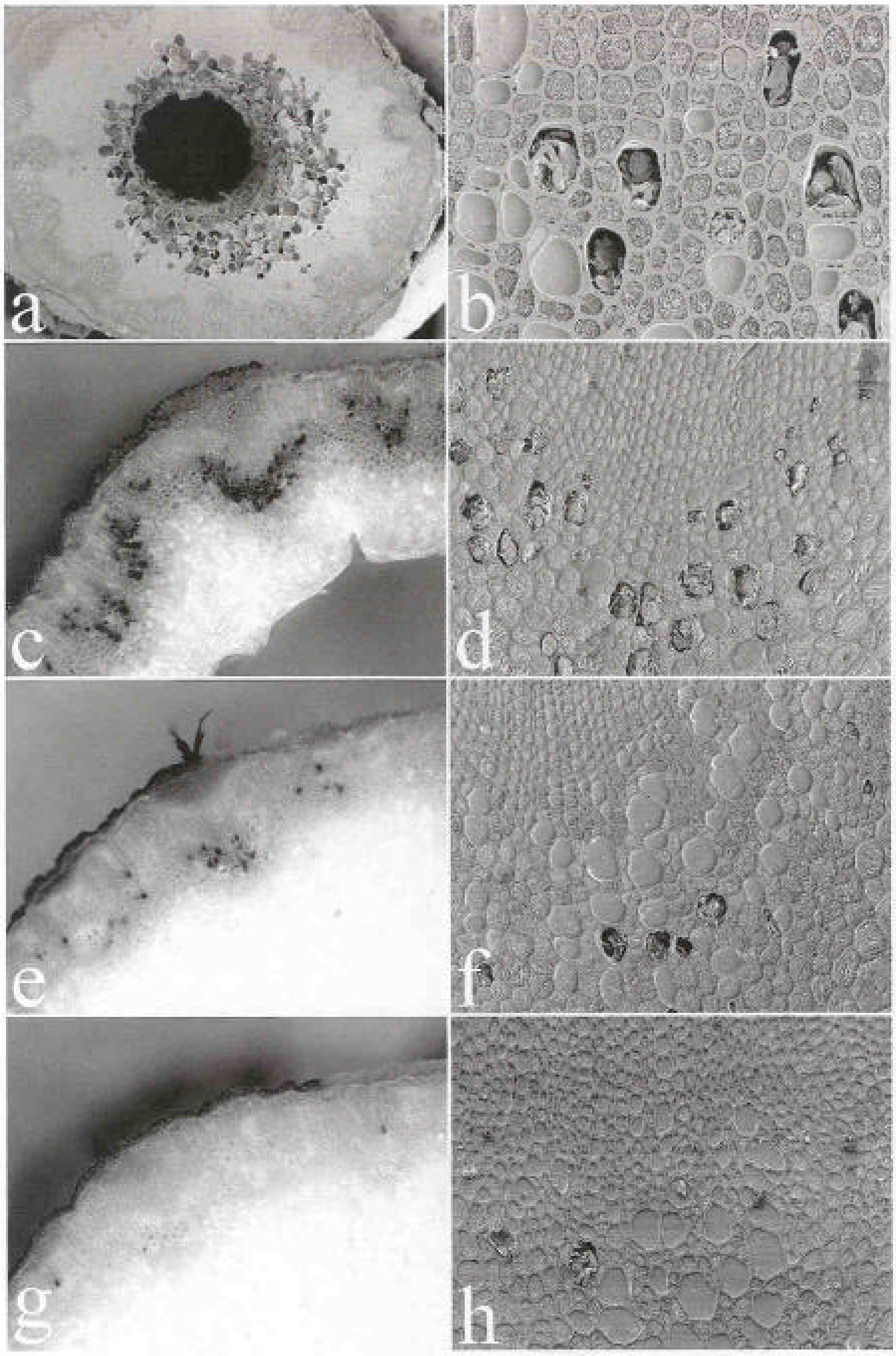
Figures

1. (see next page) Transverse sections through chrysanthemum stems.
   a): Overview of a cryo planed section. The vascular bundles are neatly arranged in a circle close to the cortex. The center consists of empty pith cells in which a hole is made during preparation.
   b): Detail of cryo-planed xylem tissue of a desiccated stem. Xylem vessel lumina can be distinguished from other cell lumina by their solid gray appearance, which is a result of the low solute contents of xylem sap. Embolized vessels are mostly filled with sawing debris and can easily be recognized.
   c-h): comparison of stems after red-latex uptake (left) and stems after desiccation and subsequent cryo-planing (right); at 1 cm (c,d), 8 cm (e,f) and 16 cm (g,h) above the cut surface.



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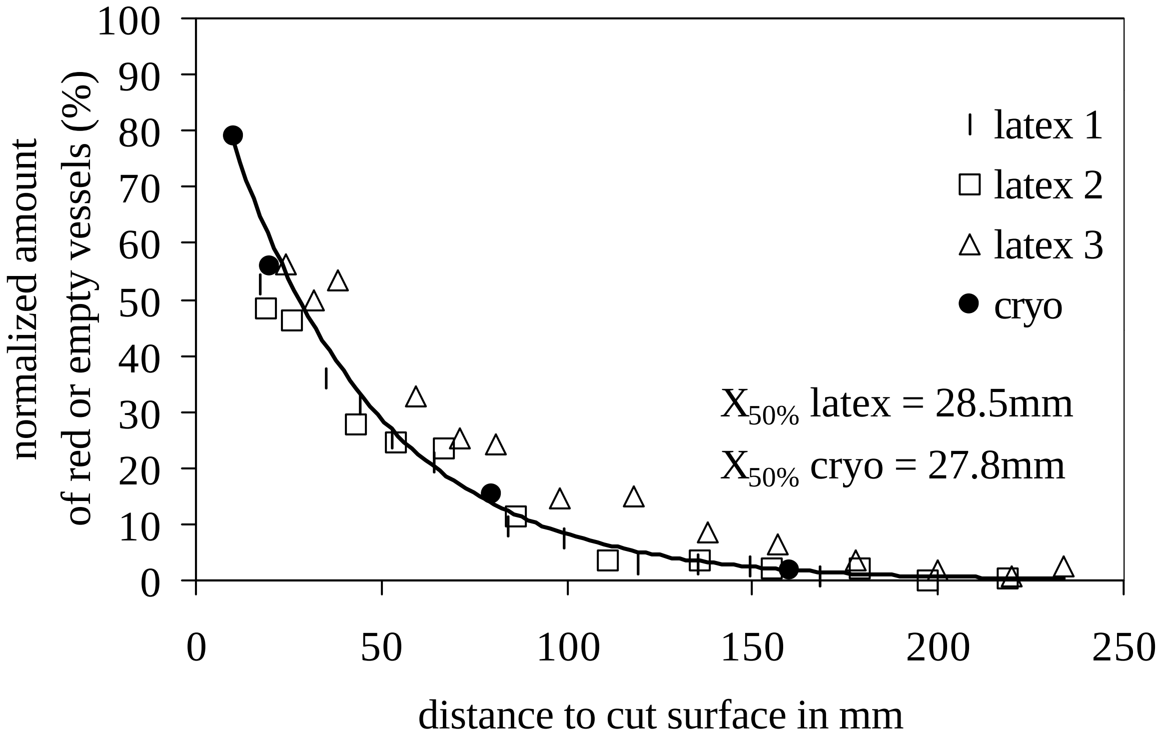

2. Comparison of red latex uptake (vessel length distribution) and embolism distribution after desiccation. Open symbols: percentage of vessels filled with red latex in three chrysanthemum stems versus distance to the cut surface. Closed symbols: percentage of embolized vessels in a 5% (w/w) desiccated stem versus distance to the cut surface.