

VASCULAR OCCLUSION IN CUT ROSE FLOWERS: A SURVEY

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Abstract

The causes of low water uptake and water stress symptoms in cut rose flowers are briefly reviewed. Low water uptake is due to an occlusion, mainly located in the basal stem end. No evidence has been found for a reaction of the plant after cutting, in the formation of this occlusion. The blockage has been found to be due to a combination of emboli and bacteria. In stems that are placed in water directly after harvest the blockage is initially due to the physical obstruction of the pores in the pit membranes, by living and dead bacteria, by bacterial exopolysaccharides and by degradation products from dead bacteria. The blockage by bacteria may be followed by cavitation, i.e. the sudden filling of xylem conduits with gas. When bacterial growth is excluded and stems are held dry, cavitation also occurs and is apparently the main factor leading to low water uptake, when such stems are subsequently placed in water.

1. Introduction

Many cut flowers show early wilting of the leaves and/or the flower. Such wilting is not observed when the flowers are left on the plant, hence it must relate to cutting. A sharp decrease in water uptake, within a few days of vase life, has been observed in flowers cut and placed immediately in water. When the flowers have been stored dry for some time they often already show low water uptake rates from the moment they are placed in water. Recutting under water usually reestablishes water flow into the stem. The cause of low water uptake, therefore, is apparently an occlusion in the stem, mainly located in the basal stem end.

In cut flowers three types of occlusion have been found:

- a. A physiological response to cutting of the stem
- b. A blockage related to microbial growth
- c. Occlusion due to the presence of gas bubbles (emboli) in the xylem conduits

The physiological blockages can themselves be divided into three groups (van Doorn, 1996):

1. Exudation of substances at the cut surface. These substances include latex (e.g. found in many genera of the Asteraceae, Euphorbiaceae, and Papaveraceae), of mucilage (for example Malvaceae, Tiliaceae, and many monocots such as Heliconia, Ginger, Canna), or gums/resins (many Gymnosperm families, and some Rosaceae, such as Prunus).
2. Deposition of material in the lumen of the water conducting elements, by living cells. Deposition of lignin and tannin has been found in cut Geranium petioles, and deposition of gums has been observed in many families, such as the Asteraceae, Malvaceae, Mimosaceae, Papilionaceae, Rutaceae, and Proteaceae. Some genera used as cut flowers include: Prunus, Acacia, and Amelanchier.
3. Formation of tyloses in the conduit lumen. Tyloses are balloon-like outgrowths of cells around a xylem conduit. The cell will dissolve the membrane in a pit between the cell and the lumen of a neighbouring xylem conduit, and the cell then protrudes into that lumen. In lilacs the number of tyloses found is too small to account for vascular blockage, but as tylose formation is accompanied by secretion of mucilage, the latter may be the main cause of the occlusion (van Doorn et al. 1991). Tyloses are observed in cut materials from families such as the Magnoliaceae, Oleaceae, Scrophulariaceae. Some genera which are used in the cut flower industry are (apart from lilacs): Eucalyptus and Prunus.

Cut roses are an important horticultural commodity, but the vase life of many rose cultivars is short due to early vascular occlusion. Since the related Prunus shows at least three types of physiological occlusion, we have determined whether the occlusion in rose stems would be based on similar mechanisms. Furthermore, we have analysed the role of various micro-organisms, and the role played by emboli.

This paper is a short survey of results that have been obtained to date, and *their interpretation*, with emphasis on results from our laboratory. A few of our papers are mentioned in the text and placed under References. Instead of quoting the remaining papers from our laboratory in full, those pertaining exclusively or partially to cut rose flowers have been included in a Literature list, which follows the References.

2. The role of physiological blockage in rose stems.

In cut rose flowers we found no exudation of blocking materials, such as latex, mucilage, or gum, at the cut surface. Furthermore, no deposition of tannin or suberin has been detected, nor any deposition of gums. Finally, no tyloses have been observed in the stems. Several other experiments have indicated that a physiological blockage, based on the mechanisms described above, is not involved in the occlusion found in rose stems. Importantly, when the occlusion due to microorganisms was excluded, by antimicrobial compounds or by keeping the vase solution and the stems sterile, no occlusion was found.

Others have noted the presence of amorphous material in conduit lumina of cut rose flowers. The number of vessels so blocked seemed too small to account for the blockage, but in other conduits the same material could be present in amounts which filled the whole lumen of the water conducting element. We found that the amorphous material in Sonia roses is bacterial in origin. It consists probably mainly of bacterial exopolysaccharides.

3. The role of micro-organisms

When the pH of the vase solution is not lowered to around 3, a role of fungi or yeasts was found to be minimal. In vase water of pH 4 and higher bacteria will rapidly grow. It has been concluded that the occlusion found in rose flowers that have not been stored dry is mainly due to bacteria, living and dead, bacterial exopolysaccharides, and to the products from decomposing bacteria. Particles smaller than bacteria but not smaller than the pores in the xylem pit membranes effectively block water flow in rose stems, even more effectively than bacteria.

The bacterial occlusion may be followed by cavitation in the stems (de Stigter and Broekhuysen, 1989; Dixon and Peterson, 1989; van Doorn and Suiro, 1995). Such cavitation in stems placed in water was also found in a *Thryptomene* species (van Doorn and Jones, 1994). Cavitation will exacerbate the occlusion when in a high number of water-conducting elements the sap is replaced by gas (see paragraph 4).

When flowers are transported or stored dry, the growth rate of bacteria is not clearly diminished compared to placement in water at the same temperature. Our measurements indicated that the xylem of intact rose stems does not contain bacteria. Bacteria are introduced by cutting and, mainly, by placement of the stems in water after harvest. After a period of dry handling, therefore, a blockage by bacteria may interact with the emboli which are formed during dry storage.

4. Cavitation

The xylem vessels and tracheids in intact plants can become cavitated by two mechanisms.

- I. Due to the instability of the water at low water potentials the water in a closed xylem vessel or tracheid may cavitate (suddenly evaporate), probably by a minute air bubble which suddenly expands. The water in the conduit will be pushed into the adjacent elements, and the cavitated elements will initially be filled with water vapour. Air, which is dissolved in the surrounding tissues, will rapidly diffuse into the lumen and replace the water vapour.

- II. Whenever a xylem element is opened, be it by a bite from an animal or breaking of the tissue, air will enter the opened conduit. Due to ongoing transpiration the water will be sucked out of the opened conduit, and air will replace this water. When the water potential across the pit membrane of the opened vessel and adjacent non-opened vessels becomes large enough, the water-air boundary will be pulled into the adjacent vessel, hence air will suddenly enter this vessel, resulting in cavitation. The water potential at which this occurs depends on the diameter of the largest pore in the pit membrane. The same mechanism applies to a vessel that has become cavitated without opening: the air in these vessels can also be pulled into adjacent ones, depending on the water potential gradient and the pore sizes.

In cut flowers both mechanism of cavitation may occur. Both forms, without being able to detect whether they belong to the first or second category, can be measured at the plant surface by the use of microphones, as every cavitation event gives a sound vibration, both in the sonic and ultrasonic range. In order to avoid background noise ultrasonic acoustic emissions are nowadays usually measured in the study of plant water relationships.

A potentially very interesting method to detect cavitation has been introduced by Dr. H.C.M. de Stigter, now retired from CABO-DLO at Wageningen. This method is based on the conductivity to air, in short stem segments. Air at very low pressure is used: when the xylem conduits in the segment contain only sap, no air will pass, but when the stem is more or less

cavitated the amount of air passing is a measure of cavitation. As it is a method based on conductivity, it will be sensitive especially to the cavitation of the vessels with the greatest diameter, which tend to cavitate first. Furthermore, the method provides information in addition to acoustic emission, as the repair of cavitation can also be followed. Using this method, de Stigter and Broekhuysen (1989) established cavitation in Sonia roses which were placed in water directly after harvest, at relatively high light levels.

Another pioneering study was also made by Dixon and Peterson (1989), who used a fluorescent dye, berberine hemisulphate, to qualitatively assess the extent of cavitation in Samantha rose stems that were placed in water without prior dry storage. They also concluded that cavitation occurred in these stems, which apparently partially accounted for the occlusion.

Cavitation in the xylem seems to be the main cause of the low rate of water uptake in rose stems that have been stored dry. In Samantha roses Dixon et al. (1988) established the onset of cavitation at relatively high water potentials, compared with other plants, and a loss of hydraulic conductance by about 70% concomitant with these initial cavitations. A further loss of hydraulic conductance was found when the frequency of cavitation increased, starting at about -2.0 MPa. In these tests the relationship between hydraulic conductance and water uptake was not assessed, and the hydraulic conductance readings may have been influenced by bacteria in the stems as the flowers had been placed in water prior to dry storage.

In experiments in which the effect of bacteria was excluded, various rose cultivars were found to be differentially sensitive to dry storage. Cara Mia roses, for example, already showed low water uptake when held dry (at 20°C) for a few hours. A similar low water uptake rate was found after some 14-21 h of dry storage in Madelon, and after 24-36 h in Sonia roses. We tested the hypotheses that these cultivars differ in the xylem wall pathway for water, and in the degree in which the air column can become compressed by water, in the conduits opened at the cut surface (which is related to drying of the conduit walls). No evidence in favour of these hypotheses was found. The time to the presence of a high number of acoustic emissions in these three cultivars, by contrast, was correlated with the time to a low rate of water uptake. It was concluded, therefore, that cavitation in a high number of conduits is a prerequisite for the occlusion (van Doorn and Suiro, 1995).

It is generally assumed that the conduits which cavitate first, during dry storage, are the widest vessels (Salleo and Lo Gullo, 1986; 1989). As the rate of water flow in cylindrical pipes increases with the fourth power of the radius, cavitation in the wide vessels will considerably reduce hydraulic conductance. Indeed, the hydraulic conductance in rose stems has been found to become

low already after a few cavitations (Dixon et al. 1988). Such a reduction in hydraulic conductance, however, is of little consequence for the rate of water uptake. The water potential of the flowering stems has become low, hence a high rate of water uptake will still be possible even when a relatively low number of water-conducting conduits is left. In Sonia roses we blocked about 66% of all vessels, using a razor blade. The rate of water flow in the remaining one third of the vessels and tracheids, measured with the heat conductance method, was considerably higher than before the insertion of the razor blade into the stem. Water uptake rates by these stems was the same, irrespective of blocking two thirds of their water conducting elements (van Doorn et al. 1989). Similarly, after dry storage a good number of the vessels and tracheids must apparently become inoperative, which mainly occurs by cavitation, before the rate of water uptake becomes reduced.

5. The role of air aspired into conduits opened by cutting.

Immediately after cutting air is aspired into the basal stem end. This air has been found not to impede water uptake. However, when Cara Mia roses are cut on a sunny day, they start to wilt and show bending of the pedicel within about 10 minutes. The uncut stems of Cara Mia roses have been found to show extensive cavitation on warm days. This does not normally lead to symptoms of water stress. Whenever such cavitated stems are cut, hence the effect of aspired air is added to that of cavitation, water uptake becomes severely blocked. In it assumed in this analysis that during the short period between cutting and wilting a few additional cavitations may have happened, but unless some unexpected runaway cavitation occurs in this very short time span, the effect is apparently mainly due to aspired air.

6. Interaction between effects of bacteria and emboli

The effect of bacteria, by themselves, may often not be enough to explain the low water uptake in cut rose flowers. Our experiments usually showed a low rate of water uptake only when including numbers of bacteria in the water that are too high to explain why roses wilt within a few days after placement in water. The effect of emboli as a result of air aspired into the conduits that are cut open is, by itself, also not enough to explain the low rates of water uptake found in rose flowers. Only after a prolonged period of dry storage, in the absence of bacteria in the stem, the water uptake can become fairly low, apparently due to a high percentage of conduits that have become cavitated.

When the effects of bacteria and dry storage are combined, however, the rate of water uptake is often very low. This occurs even when the separate effects of the bacterial treatment and the dry storage treatment are not leading to a clear problem. A clear non-linear interaction, therefore, exists between the effect of microbes and emboli. This interaction may be due to a threshold: the separate effects remain at or below the threshold, but the combined effect surpasses it. The threshold effect can be explained by the high number of conduits that have to become inoperative before water uptake is clearly inhibited.

7. Conclusions

Our research clearly indicates that the vascular occlusion in cut roses is not due to a cutting response. It is related, in stems that are not stored dry, to a physical blockage by living and dead bacteria, the bacterial exopolysaccharides, and the products from decomposed bacteria. When stems are stored dry, bacterial growth still proceeds, but an occlusion was still found in dry-stored stems that were kept free of bacteria. This latter occlusion was correlated with the presence of a high number of cavitated xylem conduits. In practice the low rate of water uptake in roses, the lack of flower opening, early flower wilting, and sometimes the bending of the pedicel, seems to be due to a combination of the effects of bacterial growth and of emboli formation. Due to this combination a threshold number of incapacitated conduits is surpassed.

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