VASCULAR OCCLUSION IN STEMS OF CUT ROSE FLOWERS

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Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 12 oktober 1993
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

ISN: 584371

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Doorn, Wouter G. van

Vascular occlusion in stems of cut rose flowers /
Wouter G. van Doorn. - [S.l.: s.n.]. - Ill.
With ref. - With summary in Dutch. - Thesis Wageningen.
ISBN 90-5485-139-2
Subject headings: cut flowers ; vascular occlusion.

INTERNATIONAL BIBLIOGRAPHIC REFERENCE

Van Doorn, W.G., 1993.

Vascular occlusion in stems of cut rose flowers.

Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands.

152 pp. ISBN 90-5485-139-2

The research described in this thesis was carried out at the Agrotechnological Research Institute (ATO-DLO), part of the Ministry of Agriculture, Nature Management and Fisheries of The Netherlands. It was partially supported by the Vereniging van Bloemenveilingen in Nederland (Association of Dutch Flower Auctions) and the Produktschap voor Siergewassen (Commodity Board for Ornamental and Horticultural Products).

Stellingen behorende bij het proefschrift van Wouter G. van Doorn.

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1. De vatverstopping in de stengels van snijrozen, voor zover deze wordt veroorzaakt door bacteriën, berust op een fysisch mechanisme (dit proefschrift).

2. In afgesneden rozestengels ontstaat tijdens droge bewaring ook een belemmering voor de wateropname. Deze vatverstopping wordt niet veroorzaakt door iets dat er in zit, maar door iets dat er niet meer in zit (dit proefschrift).

3. De conclusie van Marousky dat de vatverstopping in snijrozen berust op een fysiologische reactie van de stengel, is niet juist.

   Marousky, F.J., 1971. Inhibition of vascular blockage and increased moisture retention in cut roses induced by pH, 8-hydroxyquinoline citrate, and sucrose. J.Amer. Soc. Hort. Sci. 96: 38-41.

4. De conclusie dat de lage gevoeligheid van het anjerras 'Chinera' voor ethyleen het gevolg is van een lagere affiniteit van de ethyleenreceptor, is niet terecht.

   Wu, M.J., L. Zacarias and M.S. Reid, 1991. Variation in the senescence of carnation (Dianthus caryophyllus L.) cultivars. II. Comparison of sensitivity to exogenous ethylene and of ethylene binding. Sci. Hort. 48: 109-116.

5. De conclusie van de onderzoekers uit de groep van Thompson, dat de toegenomen ionenlek van cellen tijdens de veroudering het gevolg is van veranderingen in de samenstelling van de membranen, is onjuist.

   Fobel, M., D.V. Lynch and J.E. Thompson, 1987. Membrane deterioration in senescing carnation flowers. Coordinated effects of phospholipid degradation, and the activity of membranous lipoxygenase. Plant Physiol. 85: 204-211.

6. De uitspraak van Higinbotham, dat actiepotentialen in planten alleen voorkomen in soorten met zgn. motor-activiteit, berust op een verkeerde interpretatie van de door hem gerefereerde literatuur.

   Higinbotham, N., 1973. Electropotentials of plant cells. Annu. Rev. Plant Physiol. 24: 25-46.

7. De verwelking van planten, die optreedt als de bodem met water wordt verzadigd, is het gevolg van de verlaging van de permeabiliteit van het wortelstelsel voor water. De oorzaak van deze verlaging is nog geheel onduidelijk.

   Kozlowski, T.T., 1984. Flooding and Plant Growth. Academic Press, Orlando FL. 356 pp. ISBN 0-12-424120-4

8. De commissie Brandt is nog steeds actueel met de volgende constatering:

"De toename van de wereldbevolking verzwaart de taak voor het zorgen van voedsel, werk, onderdak, onderwijs en gezondheidszorg, en het verzachten van de armoede. De conclusie dringt zich op dat een wereld met 15 miljard mensen (rond 2010) kan worden verscheurd door ernstige economische, sociale en politieke conflicten. Of dit spookbeeld kan worden verdreven hangt in sterke mate af van wat nu wordt ondernomen om de omvang van de wereldbevolking te stabiliseren".

Report of the Independent Commission on International Development Issues. Nederlandse vertaling: Het Brandt Rapport: een Overlevingsprogramma. Staatsuitgeverij, 's-Gravenhage, 1980. ISBN 90-12-02976-7

9. De demografische en politieke ontwikkelingen in het Midden-Oosten en Noord-Afrika, de frustratie van de locale bevolking jegens het Westen, en de sterk toenemende wapenverkopen aan deze regio, zouden tot een militaire confrontatie tussen de islamitische en de westerse landen kunnen leiden.

10. De nederlandse en de europese landbouwpolitiek hebben geleid tot excessen in de behandeling van dieren, heeft het milieu ernstige schade berokkend, en de belastingbetaler veel geld gekost.

11. De invoering van een mineralenboekhouding gaat uit van de naïeve veronderstelling dat de boeren niet inventief zijn.

12. Het verdrag van Schengen is een grensgeval.

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The Chapters of this thesis are based on the following papers:

Chapter 2	J. Appl. Bacteriol. 70: 34-39, 1991. (with H.C.M. de Stigter, Y. de Witte and A. Boekestein) Scanning 12: 297-299, 1990. (with F. Thiel and A. Boekestein) Scanning 13: 37-40, 1991. (with F. Thiel and A. Boekestein)
Chapter 3	J. Plant Physiol. 134: 375-381, 1989. (with K. Schurer and Y. de Witte) J. Am. Soc. Hort. Sci. 115: 979-981, 1990. (with R.R.J. Perik and Y. de Witte)
Chapter 4	J. Appl. Bacteriol. 71: 119-123, 1991. (with Y. de Witte)
Chapter 5	J. Plant Physiol. 137: 160-164, 1990.
Chapter 7	Physiol. Plantarum (in press)
Chapters 6, 8, 9	Unpublished manuscripts

Abbreviations:

AE	= acoustic emission
cfu	= colony forming units,
DICA	= dichloroisocyanuric acid,
EPS	= extracellular polysaccharides
FW	= fresh weight
HQC	= 8-hydroxyquinoline citrate,
PAR	= photosynthetically active radiation,
SEM	= scanning electron microscope
STS	= silver thiosulphate
UAE	= ultrasonic acoustic emission

VOORWOORD

Dit proefschrift is de weerslag van onderzoek dat werd verricht binnen de Directie (later Dienst) Landbouwkundig Onderzoek (DLO) van het Ministerie van Landbouw, Natuurbeheer en Visserij, aanvankelijk in het toenmalige Sprenger Instituut, dat in 1989 is opgegaan in het Instituut voor Agrotechnologisch Onderzoek (ATO-DLO).

Het idee om aan dit onderwerp te werken is afkomstig van drs. O.L. Staden, destijds afdelingshoofd op het Sprenger Instituut, die me vervolgens de vrijheid gaf het werk zelf in te vullen. Zijn enthousiasme heeft bijgedragen aan de eerste resultaten. Nadat 'het Sprenger' opging in het ATO heeft de instituutsleiding me opnieuw de vrijheid gegeven om het onderzoek naar eigen goeddunken af te ronden, waarvoor ik bijzonder dankbaar ben.

Ik wil graag iedereen die heeft meegeholpen aan de totstandkoming van dit proefschrift van harte bedanken. Als eerste heeft Yke de Witte een heel belangrijke bijdrage geleverd. Zonder zijn inspanningen op microbiologisch gebied was er van het eerste deel van dit proefschrift weinig terecht gekomen. Zijn enthousiasme voor dit onderzoek, zelfs als de materie nogal weerbarstig bleek, was stimulerend.

Els Otma, Paul Struijlaart en Harmannus Harkema hebben als assistenten een belangrijke rol gespeeld in de laatste fase van het onderzoek. Rob Krutzen is door financiering van buiten gedurende een halfjaar bij het werk betrokken geweest. Het electronen-microscopisch onderzoek werd uitgevoerd in samenwerking met TFDL-DLO: Anke Clerkx, Felix Thiel en Bram Boekestein.

Dr. Peter van de Pol (Vakgroep Tuinbouwplantenteelt, Landbouwuniversiteit Wageningen) wil ik bedanken omdat hij een aantal van zijn studenten de mogelijkheid heeft gegeven in dit onderzoek mee te werken, voor zijn toestemming om materiaal uit zijn kassen te betrekken, en vanwege de opkweek van de enige echte Cara mia struiken in Nederland. Prof. D. Côme van de Université Pierre et Marie Curie in Parijs heeft eveneens bijgedragen door een aantal studenten enthousiast te maken voor een stage in Nederland.

De volgende studenten hebben aan het rozenprojekt meegewerkt: Henriëtte Buis, Katrien Hemmes, Ewald de Koning, René Perik, Frank Jonkhart, Vincent van der Kraan, Dennis Laverman, Geert Groenewegen, Benoît Lemont, en Véronique Suiro.

Bij het camera-ready maken van het manuscript hebben Rianne Maasen, Diana Oomen, Annie Bourquin, en Rinus Seijnaeve meegeholpen.

Tenslotte wil ik prof. dr. J. Bruinsma van harte dankzeggen dat hij wil optreden als promotor. De discussies die ik met hem mocht voeren hebben het niveau en de leesbaarheid van dit proefschrift niet onaanzienlijk verbeterd.

CHAPTER 1

INTRODUCTION

The cut rose flower is an important crop in the Netherlands. In 1992 the production value, as determined by the auction clock, was Dfl 833 million, more than for any other horticultural crop (Anonymous, 1992a and b; 1993). More than 90% of the production value of cut rose flowers is exported. The main importing country is Germany, followed (by a wide margin) by the other major countries in the EC, Switzerland, Sweden and Austria. Among 274 interviewed German retail florists, 39% mentioned that the vase life of roses imported from Holland was too short, while 38% of the florists indicated that the flowers showed bending of the stem just below the flower head. When compared to roses from Germany and Israel, the Dutch roses, in the opinion of the interviewed florists, showed more stem bending (Stroeken and Dierikx, 1990). In Holland 200 randomly selected retail florists, interviewed by telephone, also considered the quality of cut roses to be a problem (De Wit, 1990).

The two main factors affecting quality are the inadequate uptake of water, resulting in precocious wilting and stem bending (Durkin and Kuc, 1966), and infection of the flower head with the fungus *Botrytis cinerea* (Pie and de Leeuw, 1991). In a few cultivars the rate of flower opening is important. It may occur too rapidly, in cultivars such as Cocktail, or too slowly (if at all) in cultivars such as Madelon. Bud opening in cut Madelon roses was improved by inclusion of sucrose in the vase water and by cutting at a more advanced stage of development. The rate of opening was correlated with the level of corolla starch (Van Doorn et al., 1991). Poor bud opening may in some cultivars also relate to inadequate uptake of water, resulting in wilting already prior to opening (Zieslin et al., 1978).

The present thesis is confined to the water relations of cut flowering rose stems. It has been established by early workers that a water deficit often develops within 2-3 days of vase life (Durkin and Kuc, 1966; Marousky, 1969). Unsevered rose flowers had a longevity of 8-23 days, depending on the cultivar, whereas the cut flowers placed in water had a longevity of only 3.5-7 days. In cut stems an occlusion developed in the xylem prior to the development of the water deficit (Durkin and Kuc, 1966; Mayak et al., 1974).

The stem of flowering roses contains 15-25 vascular bundles, depending on the position along the stem. The bundles are in close juxtaposition forming a vascular ring (Fig. 1A). In general, the conduction of water in vascular plants occurs in three groups of elements: fibers, tracheids, and vessels. Fibers are less than 2 mm long and have a diameter of less than 50 μm. Tracheids are generally shorter than fibers and are less than 50 μm wide (Esau, 1960). Vessels consist of a number of members that are open at the upper and lower sides, except at the ends where the members are open at one side only. The length of vessel members varies from about 0.2 to more than 1 mm, and their diameter from less than 50 μm to more than 200 μm. The vessel length varies in one plant and between species, from less than a cm to over 10 m (Zimmermann, 1983). The xylem in rose stems also contains vessels, tracheids and fibers, the majority of the elements being vessels. As the water transport rate in conduits depends on the fourth power of diameter most water transport will occur in the wide vessels (Zimmermann, 1983). Water flow from conduit to conduit (Fig. 1B) is through pits which contain a (physical) membrane consisting of the primary wall. During development this primary wall has undergone several biochemical changes and mainly consists of a network of cellulose microfibrils (O'Brien and Thimann, 1969; Butterfield and Meylan, 1982). The pit membrane contains pores (Fig. 1B) the diameter of which depends on the plant species and has been found to vary from 60 to 840 nm (Stamm and Wagner, 1961). Such small pores may easily become occluded by material of a molecular size similar to the pore diameter. Van Alfen et al. (1983) found that the pores in the xylem pit membranes of alfalfa (*Medicago sativa* L.) petioles became occluded by molecules having a diameter of 200 to 800 nm. After uptake of only 150 picomoles of 2 x 10^3 kD dextran molecules the water flow in alfalfa petioles was greatly reduced (Van Alfen and Allard-Turner, 1979).

The nature of the occlusion found in stems of cut roses has not yet been elucidated. The literature suggests that there are several possible causes: a) micro-organisms, b) physiological responses to cutting, c) entrance of air at the cut end and cavitation in the xylem conduits (Aarts, 1957; Gilman and Steponkus, 1972; Durkin, 1980; Dixon et al. 1988).

A. Micro-organisms

Ultrastructural investigations in cut roses placed in water led to the conclusion that the region close to the cut surface contained bacteria (Lineberger and Steponkus, 1976). More distant from the cut surface, a substance was found which failed to react with nuclear stains. This material also did not react with stains for tannin or suberin, but showed a positive reaction with periodic acid-Schiff's stain and

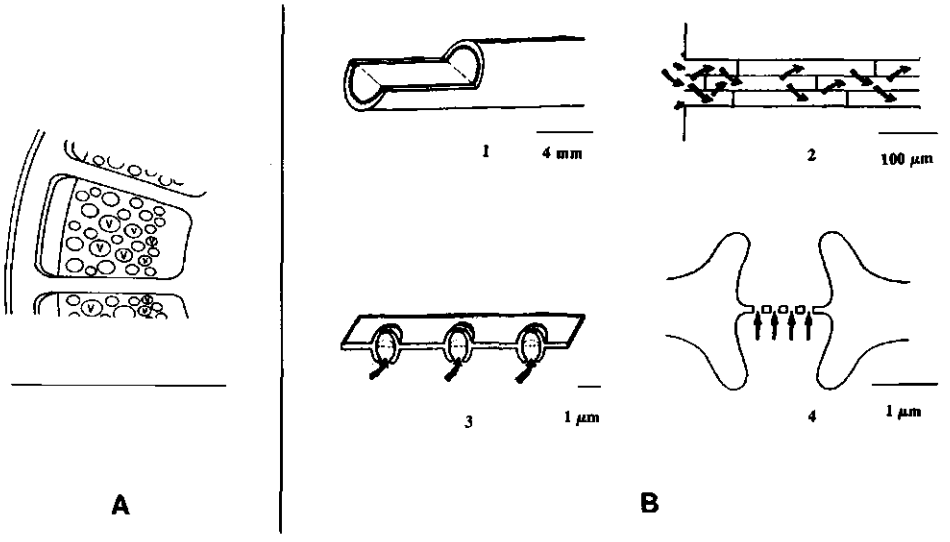

Fig. 1. Transverse section through vascular bundles in a stem of a cut rose flower, v = xylem vessel; bar = 1 mm (A), and a schematic representation of the flow of water through conduits, pits and pit membranes (B). 1: stem segment, 2: xylem vessels, 3: pits in a xylem vessel wall, 4: pit with pores. Sizes are indicated with bars.

ruthenium red, indicating it to be a carbohydrate (Burdett, 1970; Lineberger and Steponkus, 1976). When microbial growth was suppressed, using 8- hydroxyquinoline sulphate as an antimicrobial agent, these amorphous plugs were not observed (Burdett, 1970).

Inclusion of vase-water bacteria in the vase solution of cut Sonia roses at 10^9 colony-forming units (cfu) ml^{-1} resulted in immediate wilting and bending of the stem (Van Doorn et al. 1986; De Witte and Van Doorn, 1988; Put and Jansen 1989), indicating that bacteria may be responsible for the observed symptoms during vase life. De Witte and Van Doorn (1988) used several bacterial strains, isolated from the vase water of cut Sonia roses, and found no reduction of water uptake at 10^5 cfu ml^{-1} and a strong reduction at 10^7 cfu ml^{-1}, with all the bacterial strains. Some fungi known to cause wilting disease have been found to elicit the production of gum-like vascular plugs (Duniway, 1973; Hall and Busch, 1971;

VanderMolen et al., 1983). Even the culture filtrate of a pathogenic fungus caused vascular blockage and ethylene had the same effect (VanderMolen et al., 1983).

The above results indicate that micro-organisms may be important in the vascular blockage of cut roses. The role of bacteria as compared to fungi and yeasts, however, is not known. The mechanism of action of the micro-organisms has also not been established.

B. Physiological response of the stem

Several authors suggested that the occlusion in cut stems is part of a defense-mechanism inherent to the plant material (Aarts, 1957; Fujino and Reid, 1983; Marousky, 1969, 1971; VanderMolen et al., 1983). Cutting gives rise to a complex wound-reaction which involves ethylene synthesis and the activation of peroxidases and phenylammonia-lyase (Yang and Pratt, 1978). These enzymes seem mainly involved in the biosynthesis of monomers for lignin and other substances deposited in the cell walls and possibly in the vessel lumen (Rhodes and Wooltorton, 1978; Cline and Neely, 1983). Some authors noted the presence of amorphous plugs in the xylem, and suggested that these were due to the wound-reaction (Parups and Molnar, 1972). Tyloses are also known to be related to occlusion of xylem vessels (Zimmermann, 1983). Tyloses are outgrowths of paratracheal parenchyma cells which form a balloon-like structure in the vessel lumen. Although Parups and Molnar (1972) report absence of tyloses in cut flowering stems of Forever Yours roses, and Lineberger and Steponkus (1976) were not able to demonstrate tyloses in cut stems of Red American Beauty roses, it cannot be excluded that tylose formation occurs in other rose cultivars.

C. Occlusion due to exposure to air

Renner (1911) and Stocking (1948) observed that shoots of some plants which were cut in air, then allowed to wilt in air and subsequently placed in water, rapidly regained turgor. Shoots of other species, however, did not regain turgor when placed in water and showed a low rate of water uptake. Scholander et al. (1955, 1957) exposed liana stems to air and found aspiration of the air into the stem but no inhibition of water uptake. Stems of grapevine, however, showed inhibited water uptake after prolonged exposure to air, which was probably due to a layer of material exuded at the cut surface.

When stems of chrysanthemums and roses were held in air and then placed in water a reduction in the rate of water uptake was found (Durkin, 1979, 1980). This was attributed to the presence of air

bubbles (emboli) in the water-conducting elements opened by the cutting. The role of aspired air in vascular blockage has, however, not been fully addressed. It is known neither how much air is taken up after cutting, nor where the air resides, and whether water is able to bypass the emboli.

The theory of water cohesion to explain the ascent of sap in plants implies the existence of negative pressures in the xylem (Dixon, 1914). The negative pressures in cells were measured by early workers to be up to 20 MPa (Renner, 1911). Estimates using the pressure-chamber technique also indicated negative pressures as high as 20 MPa in some species (Scholander et al., 1965).

An objection against the cohesion theory is the possibility of cavitations in the xylem, i.e. the filling of the conduits with gas, causing a disruption of the water column continuity. In intact plants cavitation may occur spontaneously; the water column is thought to break because of a nucleus which gives rise to water vapour. The latter will immediately fill the lumen of the conduit, but the water vapour will be rapidly replaced by air diffusing into the lumen from the cell walls. Cavitation may also occur in xylem conduits which are adjacent to a conduit already filled with an embolus, when the gas is pulled through the pit membrane (Zimmermann, 1983). Although Stocking (1945) and Preston (1961) suggested that such cavitations would not occur before negative pressures reached -3.0 MPa, the measurements of cavitations using an acoustic detection technique revealed that they already occurred at -0.5 MPa in some plant species (Milburn and Johnson, 1966; Milburn, 1973a,b; Milburn and McLaughlin, 1974). The use of ultrasonic acoustic emissions to measure cavitations has been furthered by the groups of Sperry and Tyree (Tyree and Sperry, 1989).

Dixon et al. (1988) studied the acoustic emissions in cut Samantha roses. Stems were fully hydrated and then allowed to dehydrate in air. The flowers and leaves were removed in these measurements. Acoustic emissions were observed to start at a water potential of about -2.0 MPa. Eighty percent of the hydraulic conductance (measured using a gravitational suction head of 1 m of water) had been lost before cavitation began. The authors nevertheless suggested that the shelf life of cut Samantha roses would be reduced because of the cavitations. When stems are placed in water after exposure to air the cavitated conduits may become filled again as shown in isolated vascular bundles (Milburn and McLaughlin, 1974) and in wild-grapevine stems (Sperry et al., 1987). The role of cavitations in vascular blockage, therefore, is as yet unclear.

Outline of the thesis.

It follows from the above discussion that the development of micro-organisms in the vase water or in the stem, a response of the stem to cutting, and exposure to air, including the resulting cavitations, all may contribute to the vascular occlusion occurring in stems of cut rose flowers.

This thesis discusses the ultrastructure of the xylem at the cut surface and inside the stem of flowers placed in water, examining the presence of occluding material in the water-conducting elements and the possible role of bacteria, fungi and yeasts, and extracellular slime produced by micro-organisms (Chapter 2). The relationship between the vascular occlusion and the number of bacteria associated with the stems is described in Chapter 3. The role of physiological processes related to cutting has been examined by inhibition of wound-ethylene production, by blocking the activity of phenol-oxidases, by examination of the plugging material (including tyloses) in the xylem lumen, and by excluding microbial growth (Chapter 3). The mechanism by which bacteria induce occlusion in the xylem was investigated by inclusion of a suspension of bacteria in the vase solution, at 20°C and at 1°C (Chapter 4).

The uptake of air into the stem after cutting and its effect on water uptake when the stems are subsequently placed in water is described in Chapter 5. The variability between rose cultivars in the velocity of occlusion development during exposure to air has been assessed, and a preliminary investigation was started as to the possible reasons for the differences between the cultivars (Chapter 6). The localization of the blockage after exposing the flowering stems to air is described for Sonia and Cara mia roses, and the possibility of water flow in the xylem wall pathway after exposure to air is discussed (Chapter 7). Subsequently, the penetration of water into the lumen of the xylem elements opened by cutting is described as related to the vascular occlusion (Chapter 8). Finally, the kinetics by which the water-conducting elements cavitate when the stems of some cultivars are held in air was investigated (Chapter 9). A general discussion summarizes the findings and draws some conclusions (Chapter 10).

Throughout the following chapters the trade names of the rose cultivars will be used. The officially registered cultivar name is often different from the trade name. When the two names differ the cultivar

Table 1. Trade names and registered cultivar names of the cultivars mentioned.

Trade name	Cultivar name
Better Times	'Better Times'
Bettina	'Mepal'
Capella	'Meiriloca'
Cara mia	'Cara Mia'
Chantilly Lace	'Chantilly Lace'
Cocktail	'Meitakilor'
Forever Yours	'Forever Yours'
Frisco	'Korflapei'
Golden Phantasy	'Golden Phantasy'
Ilona	'Varlon'
Jack Frost	'Jack Frost'
Jacqueline	'Jacqueline'
Lavande	'Lavande'
Madelon	'Ruimeva'
Mercedes	'Merko'
Motrea	'Motrea'
Polka	'Meidia'
Prima Donna	'Tobone'
Privé	'Lifirane'
Red American Beauty	'Red American Beauty'
Royalty	'Hilroy'
Samantha	'Jacanth-PL'
Sonia	'Sweet Promise'
Town Crier	'Town Crier'

name is not well-known. According to international convention the trade names are neither placed between quotation marks nor indicated as cv. For example, Sonia (trade name) is equivalent to cv. Sweet Promise (which may be written as 'Sweet Promise'). The trade names and the registered cultivar

names of the cultivars mentioned in this thesis are given in Table 1 (T.M. Boerma, CPRO, Wageningen and R. Rader from E.G. Hill Company, Richmond, IN, USA, pers. comm. 1993).

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CHAPTER 2

MICRO-ORGANISMS AT THE CUT SURFACE AND IN XYLEM VESSELS OF CUT FLOWERING ROSE STEMS PLACED IN WATER

Summary

Stems of cut Sonia rose flowers (*Rosa hybrida* L.) were placed in water at 20°C to study the development of micro-organisms at the cut surface and in the xylem vessels. The cut surface became covered with bacteria within 2 d. The bacteria were accompanied by an amorphous substance which was apparently bacterial slime. After 7 d many fungal hyphae were also found at the cut surface. Inside the xylem vessels the bacteria were often clustered at the pits. After 4 d most of the vessels that had been opened by cutting contained bacteria. Only a few xylem elements, located several centimeters from the cut surface, contained an amorphous substance. A few fungal hyphae were observed inside the vessels. No yeasts were found, either at the cut surface or inside the xylem.

Pseudomonas species accounted for more than 70% of the total bacterial population of cut surface and the xylem conduits, and *Enterobacter* species (mainly *E. agglomerans*) for less than 10%. *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Citrobacter* and *Flavobacterium* species were occasionally observed.

Cryo-SEM allowed observation of a layer of extracellular polysaccharides that covered colonies of the bacterium *Pseudomonas aeruginosa*, growing on agar substrate. The layer was not found when using conventional preparation techniques and scanning electron microscopy. It disappeared partially as a result of glutaraldehyde fixation, but mainly during alcohol dehydration and critical point drying. When using cryo-SEM for the study of stems, the bacterial slime was more abundant than after conventional preparation. The ultramilling method resulted in smooth cross-sectioning of the xylem walls, and indicated that bacteria did not degrade the xylem walls.

INTRODUCTION

Aarts (1957) suggested that micro-organisms may be a cause of the vascular blockage in stems of cut flowers. Rasmussen and Carpenter (1974), however, made scanning electron microscope (SEM) photographs of the xylem of cut Forever Yours roses which had been held in water and found that only a few vessels contained visible occlusions. Even when the stems were placed in a 2% sucrose solution (without an antibacterial or antifungal compound) few bacteria and fungi were observed. Rasmussen and Carpenter (1974) therefore questioned the concept of vascular blockage in stems. We used SEM techniques to re-evaluate the presence and the spatial distribution of micro-organisms in stems of cut rose flowers, and investigated the species composition of the bacteria at the cut surface and inside the xylem.

The bacteria in the vase water of cut roses all produce slime when grown on agar (De Witte and Van Doorn, 1988). This bacterial slime mainly consists of polysaccharides, and is therefore often abbreviated as EPS (extracellular polysaccharides). It may also contain proteins as well as DNA and RNA (Eagon, 1962; Pier et al. 1978). In bacterial colonies that are grown on an agar substrate, the slime is present mainly as a layer on top of the colonies (Sutherland, 1977). The layer that covers bacterial colonies growing on agar apparently has a high water content as it shrinks considerably when the colonies are exposed to dry air. Since cryo-SEM allows observation of materials with a high water content, the slime layer of bacterial colonies and the role of bacterial slime in vascular occlusion were investigated by using cryo-techniques for observations on the xylem conduits.

MATERIALS AND METHODS

Plant material

Flowering shoots of Sonia roses (*Rosa hybrida* L.) with a stem length of about 40 cm were obtained from commercial growers or by growing rooted stems in a nutrient solution (De Stigter, 1980). Commercially-grown shoots were held dry during transportation for at most 2 h, after which stems were recut under water and individually placed in the vase solution (tap water).

Rooted shoots were cut under water and immediately individually placed in tap water. Vases were

of glass, and were washed, not sterilized prior to the experiments. Experiments were at 20°C, 60% RH, and a photon flux (PAR) of 15 μmol m^{-2} s^{-1} from fluorescent tubes, for 12 h per day.

Scanning electron microscopy of stems

The basal 9-10 mm of the stems were cut off and prepared for conventional SEM or cryo-SEM. For conventional SEM stem segments were fixed overnight in 3% glutaraldehyde (in cacodylate buffer 0.1 M; pH 7.2), washed twice in the buffer, and dehydrated through a graded series of ethanol/water (10 to 100% v/v) leaving the specimens 10 min in each solution. After freezing in liquid nitrogen the stems were freeze-fractured longitudinally and the fractured segments were placed in 100% ethanol. The specimens were subsequently critical point dried, mounted on a brass stub using carbon cement and silver paint (Demetron, Hanau, FRG), sputtered with gold, and examined using a JEOL 35 C scanning electron microscope at 293 K and 15 kV accelerating voltage.

For cryo-SEM, a 9-10 mm segment was cut from the basal end of the stem, mounted on a brass stub using Tissue-Tek (Miles Laboratories, Elkhart, IN) and immersed in nitrogen slush (about 60 K), using a Hexland CT1000/CP2000 cryo-system. The samples were placed in liquid nitrogen and milled with a Reichert-Jung Polycut E microtome equipped with a cryostage (about 100 K) and an ultramilling device. During milling cooled nitrogen gas (about 120K) was blown on the milling device and the specimen surface, to prevent contamination with water particles. The milled samples were transferred to liquid nitrogen under a continuous flow of nitrogen gas, placed in the Hexland cryo-system where they were etched (8 min at 20 Pa and 190 K), and sputtered with gold (2 min at 6.5 Pa and 100 K). The specimens were then transferred under vacuum to a Philips SEM 535 equipped with cold stage (120 K) and examined at 15 kV accelerating voltage. During etching, sputtering, and examination, the temperature of the anticontaminator was held at 90 K.

Scanning electron microscopy of bacterial colonies

A pure culture of *Pseudomonas aeruginosa*, originating from the vase water of cut roses, was grown on Plate Count Agar (Oxoid) at 30°C for 48 h. The colonies were cut from the agar substrate, mounted on brass stubs as described above and cryofixated in nitrogen slush in an Emscope SP 2000 cryostage. The samples were etched for 8 min at 190 K and gold-sputtered at 27 Pa and 100 K prior to examination in a JEOL 35C scanning electron microscope equipped with a cold stage (100 K), in which the vacuum was held at 0.13 mPa. The accelerating voltage was 15 kV.

Fig. 1. Cut surface of a freshly cut stem of a Sonia rose. Transverse section. Bar = 100 µm.

Fig. 2. Cut surface of a Sonia rose stem held in water for 2 d. Bar = 100 µm.

Fig. 3. Detail of the cut surface of a Sonia rose stem held in water for 2 d. Bar = 10 µm.

Fig. 4. Wall of a xylem vessel in a Sonia rose stem, showing bacteria at the pit membranes, in a stem held in water for 2 d. Longitudinal section. Bar = 10 µm.

Fig. 5. Wall of a xylem vessel in a Sonia rose stem, containing bacteria and an amorphous substance, in a stem held in water for 2 d. Bar = 10 µm.

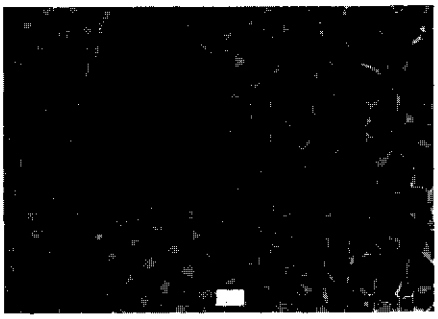
Fig. 6. Bacteria in a xylem vessel of a Sonia rose stem held in water for 2 d. Longitudinal section. Bar = 10 µm.

Bacterial colonies were also chemically fixated as described above, using an alcohol series. A third series of colonies was fixed overnight in 3% glutaraldehyde as described, then rinsed with distilled water, mounted on stubs, prepared for cryo-SEM as described above, and examined in the JEOL cryo-SEM.

Identification of bacteria

Flowering stems were placed in water in vases which were either sterilized or not before adding tap water. After various periods stems were randomly selected and the basal 5 cm of the stem were cut with standard sterile equipment. The bark was removed and the segment was cut into 1-2 mm parts using standard sterile equipment. The parts were placed in sterile bags and weighed. A sterile 0.85% NaCl solution was added, the weight of the added solution being ten times the fresh weigth of the stems. The bacteria were removed from the stem parts using a Colworth Stomacher-400 for one min. Bacteria were grown on selective media as described by De Witte and Van Doorn (1988).

For each determination of the species composition 80 isolated bacterial colonies growing on Plate Count Agar (Oxoid) were randomly selected, and characterized with the ENTEROTUBE and the OXI/FERM systems (Oberhofer, 1979). Additional tests were made according to the descriptions given in Bergey's Manual (Buchanan and Gibbons, 1974).



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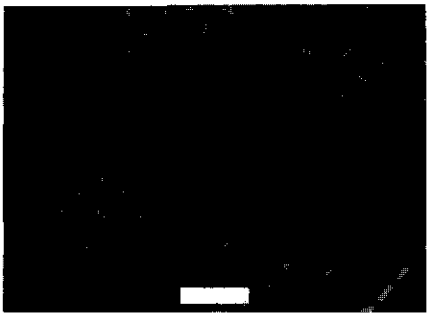

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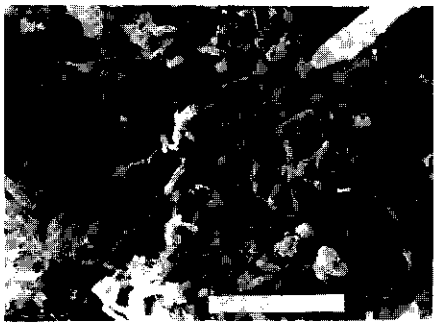

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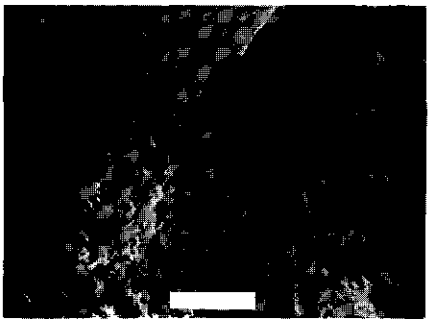

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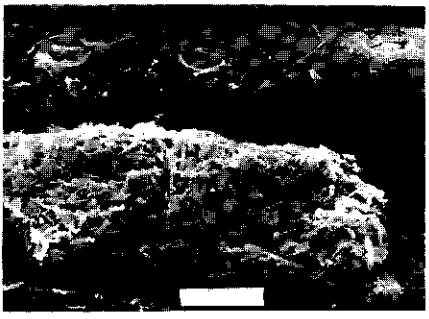

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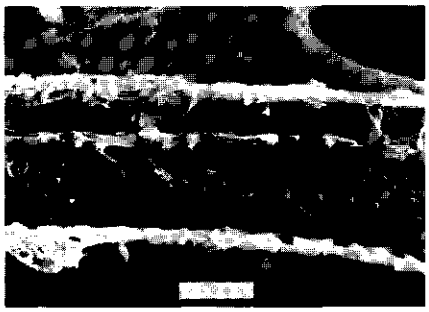

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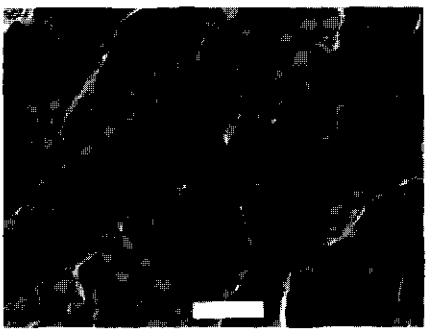

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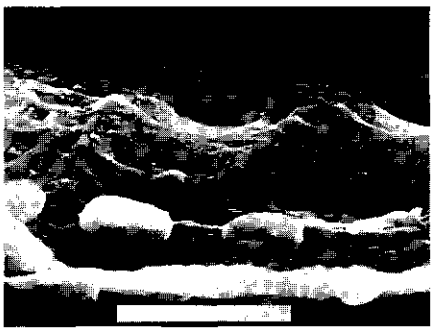

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Fig. 7. Bacteria embedded in an amorphous substance, in a xylem vessel of a Sonia rose stem held in water for 4 d. Longitudinal section. Bar = 10 μm.

Fig. 8. Detail of the surface structure of a colony of *Pseudomonas aeruginosa*, grown on agar, after glutalaldehyde fixation, alcohol dehydration, and critical point drying, observed with conventional SEM. Bar = 1 μm.

Fig. 9. As figure 8, but without chemical fixation, examined using cryo-SEM. Bar = 1 μm.

Fig. 10. Individual bacteria adhering to a vessel wall in a Sonia rose stem, observed without chemical fixation, using cryo-SEM. Bar = 10 μm.

Fig. 11. Accumulation of bacteria in a xylem vessel of a Sonia rose stem, observed without chemical fixation, using cryo-SEM. Bar = 10 μm.

Fig. 12. Longitudinal section of a wall of a xylem vessel in a Sonia rose stem held in water for 4 d, using cryo-ultramilling cross-sectioning and examination in cryo-SEM. Bar = 10 μm.

RESULTS

Development of a microbial population at the cut surface

Transverse sections of freshly-cut rose stems showed no micro-organisms (Fig. 1). When stems were held in water for 2 d, a layer of micro-organisms was present on the cut surface, both on the phloem and on the xylem, but not on the central pith tissue (Fig. 2). This layer consisted of bacteria embedded in a layer containing granular material and thin filaments (Fig. 3). A few fungal hyphae were observed in some of the stem samples after 4 d of vase life (not shown).

When stems were held in water for 7 d most of the xylem vessels at the cut surface were covered with a layer of bacteria and a matrix substance. Fungi were present on the xylem and after more than 7 d of vase life fungal hyphae occasionally entered the xylem. Throughout the vase life no yeasts were observed at the cut surface.

Development of a microbial population inside xylem vessels

Xylem vessels of freshly cut rose stems contained numerous pits, generally elliptical but sometimes having a round shape. Their diameter was about 5 μm. Bacteria or fungi were not observed in xylem conduits of freshly cut stems, nor any other material. Two days after the onset of vase life, some bacteria were present in the conduits. These bacteria were mainly localized close to the pits (Fig. 4). An amorphous substance was often associated with the bacterial cells (Fig. 5). Within 4 d most of the vessels which were opened by cutting contained bacteria. Some were filled with bacterial cells (Fig. 6), others contained both bacteria and amorphous substance (Fig. 7). The relative amount of bacterial cells decreased from the stem base towards the flower head. A few vessels, several centimetres from the cut surface, contained the amorphous substance only. Throughout vase life no yeast cells were observed inside the xylem. Fungal hyphae were only occasionally found inside the xylem, after more than 7 d vase life.

Cryo-SEM and cryo-ultramilling

Using conventional SEM the slime on bacterial colonies disappeared (Fig. 8), but with the cryo-SEM method the slime was still present after fixation (Fig. 9). Using only glutaraldehyde fixation followed by cryo-SEM the slime layer was also present (results not shown). In cryo-SEM observations of the cut surface and of the xylem interior of rose stems held in water the bacteria were found to be surrounded by slime (Fig. 10) and also adhered together rather than forming a loose stucture (Fig. 11). Cryo-ultramilling techniques showed no visible degradation of the xylem cell walls (Fig. 12).

Identification of bacteria associated with the stems

The bacteria found in stems and at the cut surface of rose stems placed in water are listed in Table 1. No difference was found between the flora from the basal 0.1 cm, which included the cut surface, and the flora from inside the xylem (next 4.9 cm). *Pseudomonas* spp. accounted for more than 70% of the total population, and their frequency increased from about 74% on day 1 to about 83% on day 7 (Table 2).

Enterobacter species (mainly *E. agglomerans*) decreased from 8% on day 1 to 0% on day 7 (Table 2). Up to 20% of the bacteria could not be identified as they belonged to poorly defined genera. Of these genera about one-third was oxidase-positive and two-thirds oxidase-negative.

Table 1. Composition of the bacterial flora in the basal 5 cm stem segment of cut Sonia rose flowers placed in tap water for 7 d.

Aeromonas sp.	*Pseudomonas* sp.
Acinetobacter sp.	*P. aeruginosa*
Alcaligenes sp.	*P. cepacia*
A. faecalis	*P. fluorescens*
Bacillus sp.	*P. maltophilia*
Citrobacter sp.	*P. mendocina*
C. freundii	*P. pikettii*
Enterobacter sp.	*P. putida*
E. agglomerans	*P. stutzeri*
Flavobacterium sp.	*P. vesicularis*

In repeat experiments *Pseudomonas* spp. always predominated and *Enterobacter* spp. were always found, while genera such as *Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Citrobacter* (mainly *C. freundii*) and *Flavobacterium* were occasionally observed.

Table 2. Frequency of *Enterobacter* spp. and *Pseudomonas* spp. in the basal 5 cm of Sonia roses placed in tap water for 1-7 d. The remaining species were *Citrobacter* (0-3%) or belonged to poorly defined genera (16-20%).

Day	*Enterobacter* spp. (%)	*Pseudomonas* spp. (%)
1	8	74
4	4	78
7	0	83

DISCUSSION

Within 2 d after the rose stems had been placed in water, most of the xylem elements at the cut surface were covered with a layer of bacteria embedded in a matrix substance, which most probably was bacterial slime. In the xylem interior bacteria were mainly found close to the cut end. The bacteria were apparently unable to pass through the pits, and therefore remained localized in the conduits that had been opened by cutting. These results agree with the SEM study of Put and Clerkx (1988) in which rose stems were placed in diluted pure cultures of bacteria, fungi, and yeasts. Also in this study the micro-organisms remained confined to the xylem elements close to the cut surface.

The abundant development, starting within 2 d, of a population of bacteria both at the cut surface and inside the vessels, correlated with the development of a blockage to water uptake, which occurs within 2-3 d after the onset of vase life (Durkin and Kuc, 1966; De Stigter, 1980). In their SEM study on stems of cut rose flowers, Rasmussen and Carpenter (1974) found only a few vessels which were visibly filled with occluding material and concluded that vascular blockage could not be the cause of the observed reduction of water uptake in cut rose flowers. These authors, however, paid little attention to the development of a population of micro-organisms at the cut surface. Furthermore, they did not take into account the possibility of blockage of the pores in the pits by macromolecular matter, such as bacterial slime.

The layer found on the cut surface and the presence of occluding material inside the xylem may both contribute to the restriction of water-uptake. Their relative contribution, however, is not evident from our results. Although a layer of jelly was observed on the cut surface after 11 days of vase life, it may not fully explain the blockage to water uptake. As to the situation inside the stem, the opposite reasoning may apply: the fact that only few vessels were observed to be massively blocked by bacteria does not prove that there was no blockage to uptake of water. Even if no visible occlusion occurs, macromolecules of bacterial origin may block the pores in the pits, and reduce the rate of water uptake (Van Alfen et al., 1983; De Stigter and Broekhuysen 1986).

Several authors noted an amorphous substance in xylem vessels of cut rose flowers. Vessels with amorphous plugs were observed several centimetres from the cut surface (Lineberger and Steponkus, 1976). We found that many vessels close to the cut surface contained both bacteria and an amorphous

substance. Several centimetres away from the cut end a few vessels contained the amorphous substance only. The amorphous plugs in the xylem were reported to stain with ruthenium red, indicating the presence of pectinaceous compounds (Burdett, 1970; Parups and Molnar, 1972). We found that the slime covering colonies of bacteria that were isolated from vase water, the cut surface, or the xylem interior, also stained with ruthenium red (unpublished results). We therefore assume that the amorphous substance in the xylem vessels was a fraction of the slime that was able to pass the pores in the pits.

When using cryo-SEM, in contrast with conventional SEM, colonies of *Pseudomonas aeruginosa*, isolated from rose stems, were found to be covered with a layer of amorphous material. Alcohol dehydration removed most of the water in the slime layer, but apparently also dissolved most of the dry matter constituent. When using cryo-SEM for examination of the micro-organisms in stems of cut rose flowers, the bacteria inside the xylem were generally associated with amorphous material, which was more abundant and less dehydrated than after conventional SEM. The latter method, however, did not result in the disappearance of all of the dry matter constituent. The ultra-milling technique showed no apparent degradation of the cell walls in the xylem. This is evidence against the hypothesis of Burdett (1970) that bacteria cause blockage by degrading the xylem walls.

As no yeast cells were observed on the cut surface or inside the xylem, our results indicate that yeasts play no role in vascular blockage of cut rose flowers that are placed in water. The presence of a population of fungi on the cut surface started after the development of vascular blockage. We therefore assume that fungi are not a primary cause of the blockage either.

On day 6 of vase life, De Witte and Van Doorn (1988) found that five *Pseudomonas* spp. and one *Alcaligenes* sp. belonged to the predominant strains in vase water of cut Sonia roses. The composition of the bacterial flora at the cut end and inside the xylem of cut Sonia roses (Tables 1 and 2) was similar to that found in vase water. During vase life the decline in the frequency of *Enterobacter* sp. and the increase of *Pseudomonas* spp. may depend on the relatively more demanding growth requirements of the former group whereas *Pseudomonas* spp. can rapidly multiply in tap water containing only trace amounts of a single organic compound (Van der Kooy et al., 1982a,b; Konings and Veldkamp 1980). With time, the flow of sap from the phloem may decline and the contents of cut cells as a source of nutrition may become depleted. As in repeat experiments *Pseudomonas* and *Enterobacter* spp. were always found, there was apparently no great variety in the bacterial flora at the cut surface and

in the xylem. Genera other than *Pseudomonas* and *Enterobacter* were only occasionally found, in any combination, and these may therefore represent chance contaminants. As their frequency in the total population was low, these contaminants were apparently unable to compete with the *Pseudomonas* and *Enterobacter* species.

It is concluded that a population of bacteria, mainly *Pseudomonas*, at the cut surface and inside the xylem vessels preceeds vascular occlusion. The blockage may partially be due to the copious slime produced by the bacteria. No evidence was found for a role of fungi and yeasts in the occlusion.

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CHAPTER 3

ROLE OF BACTERIA AND STEM-INDUCED RESPONSES IN VASCULAR BLOCKAGE OF CUT FLOWERING ROSE STEMS PLACED IN WATER

Summary

When cut flowers of five rose varieties (Sonia, Ilona, Motrea, Jack Frost and Mercedes) were held in water for 7 days, the lowest hydraulic conductance was found in the basal 5 cm stem segment. After artificially blocking about two thirds of the xylem in cut Sonia roses, the uptake of water by the stem was not reduced, indicating that vascular occlusion involves a majority of the xylem conduits. Hydraulic conductance was related to the number of bacteria inside the lowermost 5 cm stem segment and at the cut surface, but was only significantly lowered when the number of bacteria exceeded 10^6 cfu per gram fresh weight. Bacteriostatic chemicals ($AgNO_3$, benzalkone, HQC, DICA) inhibited growth of bacteria and prevented the decrease of hydraulic conductance. The effect of $AgNO_3$ (also an anti-ethylene agent) on hydraulic conductance was related to the number of bacteria associated with the stems. HQC inhibited production of ethylene by the cut surface of rose stems, but had the same effect on hydraulic conductance as DICA and benzalkone, which stimulated ethylene production with respect to controls. The data indicate that vascular blockage was due neither to ethylene production by the cut surface nor to other physiological processes occurring after cutting of the xylem. When stems of four cultivars (Sonia, Ilona, Polka, and Frisco) were held in a sodium hypochlorite solution and placed in sterile water a high number of bacteria was found associated with the stems even when the number of bacteria in the water was below the detection limit. Inclusion of hydroxyquinoline citrate (HQC) in the water limited the number of bacteria in the stems. HQC prevented vascular blockage by reducing the number of bacteria in the stems and not, as suggested in the literature, by preventing a stem-induced response leading to the occlusion.

It is concluded that vascular blockage in these experiments was mainly due to the presence of bacteria, and their extracellular slime, in the xylem conduits and at the cut surface, and not related to a physiological reaction by the stem.

INTRODUCTION

The previous chapter showed that bacteria, mainly from the genus *Pseudomonas*, and their EPS accumulate at the cut surface and inside the xylem vessels of flowering rose stems placed in water. It was concluded that the bacteria may play a role in vascular blockage in the stems, whereas fungi developed too slowly and yeasts were not observed.

A role of bacteria in vascular blockage has also been inferred from the positive effect of bacteriostatic chemicals on water relations of flowers (Aarts, 1957) and the negative effect of aqueous suspensions of bacteria (Van Doorn et al., 1986). Cutting generally gives rise to the production of wound-ethylene (Yang and Pratt, 1978), and in *Ricinus communis* exogenous ethylene induced vascular blockage (VanderMolen et al., 1983).

Some authors noted the presence of amorphous plugs in vessels of cut rose flowers, at about 15-30 cm from the cut surface. These plugs did not show staining reactions typical for bacterial cells, but were found to be mainly carbohydrate in nature (Burdett, 1970; Lineberger and Steponkus, 1976). Formation of such vascular plugs could possibly be induced by ethylene, produced either by the cut surface or by bacteria. Zagory and Reid (1986) found that some bacteria from flower vase water produce ethylene.

Blockage of water flow might also be due to an impermeable layer which may become deposited as a response to cutting. Enhanced extracellular deposition of lignin and suberin has been reported in various tissues after cutting (Rhodes and Wooltorton, 1978). Within two days after cutting an increased amount of suberin was found at the cut surface of geranium stems, and some suberin plugs were present in xylem vessels (Cline and Neely, 1983). The biosynthesis of monomers for lignin and suberin is generally enhanced after cutting, and oxidative enzymes are involved in the polymerization processes (Rhodes and Wooltorton, 1978). Ethylene is known to play a role in the induction of these stress enzymes (Yang and Pratt, 1978). Some authors suggested that this phenomenon may also be important in the vascular blockage of cut roses (Durkin and Kuc, 1966; Buys, 1969).

Although the literature often refers to a role for physiological processes in vascular blockage of cut roses, the evidence supporting this concept is scarce. The most direct evidence for a role of physiological processes in the xylem blockage of cut rose flowers was given by Marousky (1969, 1971). Cut Better Times rose stems were placed in 3-5% chlorine bleach (sodium hypochlorite) and then in sterile water. After two days no bacteria were found in the vase water. Nevertheless, when

hydroxyquinoline citrate (HQC) was added to the vase water, hydraulic conductance of stem segments was higher than in controls. Stems that were held in a buffer at pH 3.0 also had higher hydraulic conductance than stems held in a buffer at pH 6.0. As no bacteria were present in the water, a bacterial effect was thought to be excluded. It was therefore inferred that the differences between the treatments indicated a physiological blockage in the stems.

In the present experiments the effect of aspired air was excluded. We investigated the localization of the blockage in the stems and the possible role of stem-induced processes and of bacteria.

MATERIALS AND METHODS

Plants

Sonia, Polka and Frisco roses (*Rosa hybrida* L.) were obtained from a commercial grower or (Sonia, Ilona, Jack Frost, Mercedes and Motrea) from the greenhouse of the Dept. of Horticulture, Agricultural University, Wageningen. When obtained from a commercial grower, the stems were kept dry in a refrigerated room (5°C) for some hours, before transport to the laboratory. When obtained from the university greenhouse, the stems were only held dry for about 30 min. In the laboratory, leaves were removed until the uppermost five remained. About 5 cm was cut from the basal end of stems, in air. Water uptake into these stems was as in stems of which more than 25 cm was recut under water. The possible effect of aspired air was therefore excluded in the present experiments.

The flowering stems were held in about 200 ml vase solutions at 20°C, 50% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1} at leaf level from 7 a.m. to 7 p.m. Photon flux was measured with a Li-cor (Lincoln, Nebraska) quantum meter, mounted on a Li-cor model 1600 steady state porometer.

All experiments were repeated at least two times.

Artificial blockage of the stem

A razor blade was introduced into about one third or two thirds of the horizontal stem area. The efficiency of the occlusion was checked by placing stems in a 1% aqueous acid fuchsin solution. The stem was cut at various distances above the razor blade. The stained area was determined with a microscope. Peeling off the bark also showed the xylem area stained by the dye.

The rate of water uptake per unit fresh weight of the flowering stems was determined by daily weighing of the vases and the flowering stems.

Rate of water flow in the xylem

The rate of water flow in stems of rose flowers was determined with the heat pulse method described by Schurer et al. (1979) and Schurer (1986). The output in mV was proportional to the rate of water uptake in the rose stems.

After introduction of a razor blade into the xylem, the flow rate was measured at about 10 cm above the site of insertion of the blade.

Hydraulic conductance of stem segments

Stems were cut under water into segments of 5 cm length, which were inserted into tygon tubes to which 130 cm head pressure of water (13 kPa) was connected. The water used for conductance measurements was replenished daily and was checked for concentration of bacteria (lower than 10^2 cfu per ml) at the end of the day. The flow direction of water in the stem segments was as in the intact stem. Water flowing through the segments was collected in plastic tubes. The plastic lids of the tubes had a hole in the middle and could therefore be snapped onto the segments. The tubes were connected to the segments after two hours of equilibration. The flow rate was then determined by weighing the tubes after thirty minutes. Six stem segments were used for each treatment. When the conductance was measured in stem sections at various positions from the base (0-5, 5-10, 10-15, 15-20, 20-25 cm), six sections were measured at each position.

Bacteria

Solutions were regularly tested for the numbers of bacteria. Water samples were diluted and placed on Plate Count Agar (Oxoid, Basingstoke, Hants., U.K.) using the spiral plate machine model C (Spiral Plate System, Cincinatti, Ohio). Petri dishes were kept at 30°C for 48 hours before enumeration.

The number of bacteria was also determined in stem segments. These were surface-sterilized with 95% ethanol and cut into 0.5 cm parts using standard sterile equipment. The stem parts were placed in sterilized bags and weighed. A sterile 0.85% NaCl solution was added; the weight of the added solution was ten times the fresh weight of stems. The bacteria were removed from the stem parts using a Colworth Stomacher-400 (Sharpe and Jackson, 1972) for one minute. Preliminary experiments had shown that recovery of bacteria by the Stomacher method was as good as by grinding the tissue (results not shown). Samples of the solution were then placed on Plate Count Agar and numbers of cfu were enumerated as described above. A 5 cm stem segment was used for each determination of the number of bacteria, and measurements were replicated once.

Chemicals

Various concentrations of HQC (La Quinoléine, Oissel, France), benzalkone, i.e. n-alkyl dimethyl-benzyl ammonium chloride (Merck), and DICA (BDH) were tested. Some stems were pulse-treated with 4mM $AgNO_3$ (Analar) for 40 min, or (for 4 h) with the anionic silver thiosulfate complex (STS), prepared by adding 0.2 mM $AgNO_3$ to 1.2 mM sodium thiosulfate (Merck). After the pulse-treatment the stems were placed in water. All solutions were renewed daily, unless otherwise indicated.

Ethylene measurements

Fresh rose stems were cut into 2-3 mm segments, which were immersed in water. Solutions contained HQC (250 g l^{-1}), benzalkone (1000 mg l^{-1}), DICA (250 mg l^{-1}), or no chemical. Solutions were renewed daily. Some segments were treated with 4mM $AgNO_3$ for 40 min and then transferred to water. After various periods of time nine stem segments were taken out of the solution, weighed and placed in a 10 ml hypodermic syringe. Syringes were kept in the dark at 20°C for 1 hour, and the gas phase (10 ml) was injected into an Intersmat GC-120 gas chromatograph, equipped with a flame ionization detector.

Vessel length

The length of the longest vessels was measured according to the method of Peresse (1974). In short, low pressure (0.01 MPa) was exerted at the lower stem end, using the Scholander pressure chamber. A 5 cm tygon tube filled with water was attached to the upper stem end. The upper end was repeatedly recut until a flow of gas bubbles through the water was observed.

pH of the solution

The pH was determined with a Philips PW 9410 meter. Since solutions were renewed daily, pH changes remained small during the course of the experiments.

Use of sterile water and sterile bottles, inclusion of HQC

After arrival of the cut flowering stems from a commercial grower, the stems were recut with a non-sterile knife and placed in sterilized bottles containing sterilized water. Experiments on the effect of HQC were according to the methods described by Marousky (1971). In short, glass bottles (containing 150 ml water) were autoclaved for 20 min at 120°C. Sucrose (30 g l^{-1}) was added before autoclaving, and HQC (200 mg l^{-1}, la Quinoléine, Oissel, France) was included after autoclaving. The

basal 5 cm of stems was removed and stems were placed in 3% sodium hypochlorite (commercial bleach) solution for 5 min. Stems were placed with the lower 15 cm immersed in the hypochlorite solution, and were then placed in autoclaved bottles with the lower 7 cm immersed. A cotton plug was placed on the bottle opening to reduce the entry of bacteria from the surrounding air.

RESULTS

Water uptake after artificial blockage

The razor blade method for blocking water uptake was tested by placing stems in a 1% aqueous fuchsin solution. After one day, the xylem area above the razor blade that remained unstained was 38 ± 5% and 64 ± 5% (n=6), when the cut covered about one third or two thirds of the stem area, respectively. Vascular bundles that had been cut by the razor blade did not contain the dye. After one week of dye uptake, the area above the blade became more stained especially at the margins of the area that was previously unstained and particularly towards the flower head, but still most vascular bundles above the blade contained no dye.

Mechanical blockage of up to two thirds of all xylem vessels by the razor blade did not affect water uptake (Fig. 1), neither under conditions that are standard for vase-life evaluation of cut flowers, where bent neck and vascular blockage in cut roses are often observed, nor under conditions in which the rate of water uptake was doubled, i.e at higher temperatures and a higher photon flux. In these expermiments, a bacteriostatic compound (250 mg l^{-1} HQC) was added to the vase solution and solutions were renewed daily. After seven days the number of bacteria in the stems was below the detection limit (data not shown).

Flow rate in the xylem

After mechanical blockage of two thirds of the horizontal stem area (using a razor blade), the rate of water flow in the blocked vessels was zero, but the rate of water flow in the remaining open vessels had increased (Fig. 2). The flow rate remained high when HQC was present in the solution, but decreased to a low level without HQC (Fig. 2).

When cut roses were held in water without HQC and without artificial xylem blockage by a razor

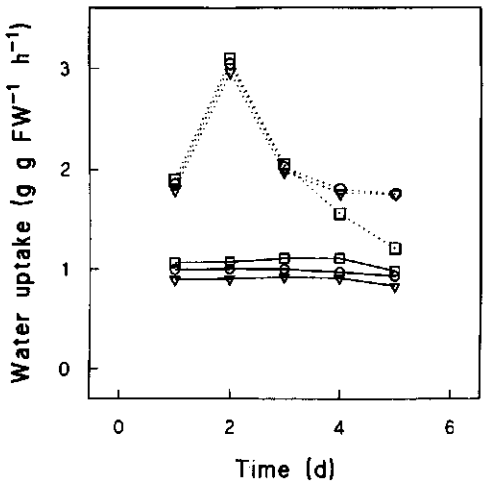

Fig. 1. Effect of mechanical blockage on water uptake of cut Sonia roses. A razor blade was inserted into zero percent (○), 38 percent (△) or 64 percent (□) of the xylem. The flowering stems were held at 20°C and a photon flux density of 15 µmol m^{-2}s^{-1} (means connected with a solid line) or at 27°C and 190 µmol m^{-2} s^{-1} (means connected with a dotted line). Solutions contained 250 mg l^{-1} HQC and were renewed daily. Data plotted on day 1 refer to day 0-1. The data are means of five replications; LSD ($P<0.05$) was 0.6.

blade, the rate of water flow did not increase, but became low by the third day. When the lowest 5 cm of the stem was cut off under water, the flow rate of water was restored to the levels found during the first days.

The number of bacteria in the HQC-treated water was $<10^2$ per ml after 7 days, whereas it was $1.3x10^7$ per ml in water without HQC.

Hydraulic conductance of isolated stem segments

When cut Sonia, Motrea, Jack Frost and Ilona roses were held in water for seven days, the hydraulic conductance of isolated stem segments was lower than shortly after harvest (Fig. 3). Lowest conductance was found in the 5 cm stem segment close to (and including) the cut surface (Fig. 3). The same was found in Mercedes roses (not shown). Depending on the cultivar tested, 250 mg l^{-1} HQC in the water prevented or reduced the decrease of conductance (Fig. 3). When stems of these cultivars were held in HQC solution for seven days, the number of bacteria associated with the basal 5 cm of

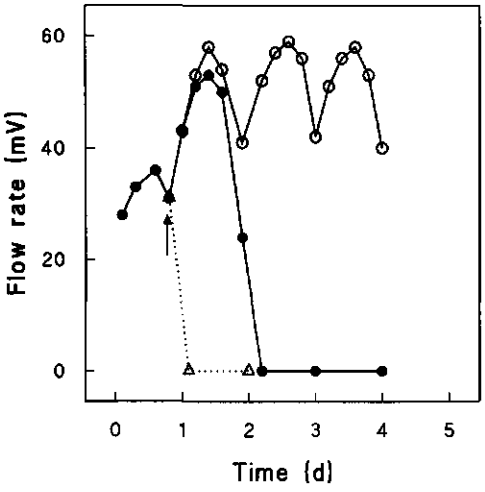

Fig. 2. The rate of xylem flow, determined with the heat pulse method, after insertion of a razor blade into about two thirds of the stem of Sonia roses (arrow). Flowering stems were held in water (●) or in a 200 mg l^{-1} HQC solution (o). Flow rates were only measured from 10 a.m. to 6 p.m. The light period was from 7 a.m. until 7 p.m. The circles show the rate of xylem flow opposite to the site of insertion of the razor blade, the triangle gives the rate of flow above the site of insertion. The data represent single determinations.

the stems varied between 1.1 x 10^3 and 4.6x10^4 cfu per gram fresh weight, whereas in controls the range was 2.1x10^7 to 3.6x10^8 cfu per gram fresh weight.

Number of bacteria in stem segments

In cut Sonia roses held in water for seven days, the hydraulic conductance was lowest in the basal 5 cm segment (Fig. 3). This was correlated with the highest number of bacteria (Fig. 4). When followed through time, the basal stem segment showed a decline of conductance after 2-3 days. This coincided with about 10^6 cfu of bacteria per gram fresh weight, also determined in the basal 5 cm segment (Fig. 5)

Effects of bacteriostatic chemicals

Various concentrations of bacteriostatic compounds were used to obtain a range of endogenous bacterial concentrations in stems of Sonia roses. In these experiments the roses were held in the sol-

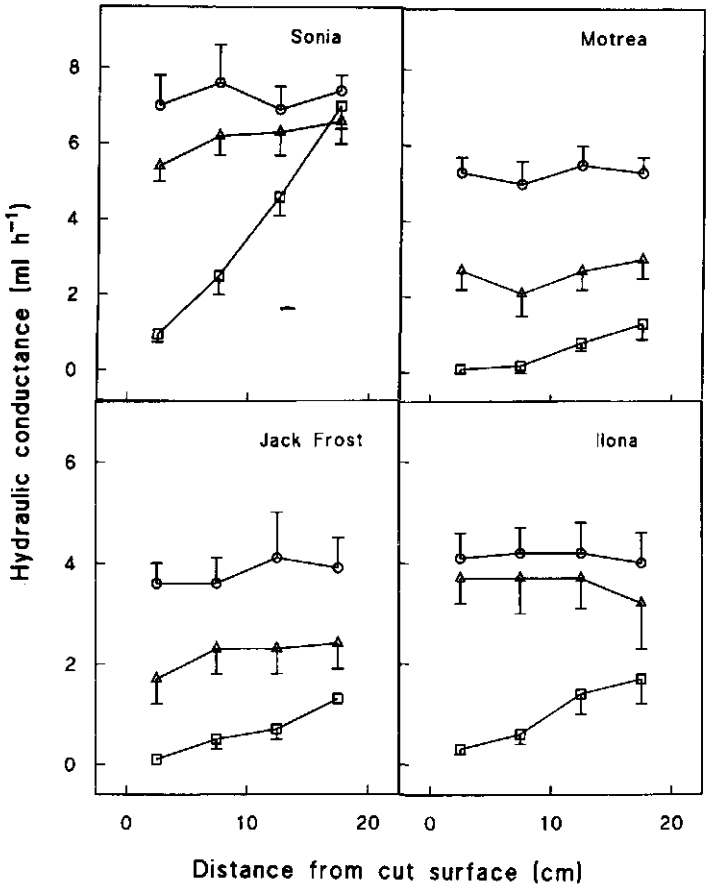

Fig. 3. Hydraulic conductance (ml per hour) of 5 cm stem segments from four rose cultivars. Stems were freshly cut (○) or held in water for seven days without (□) or with (△) 250 mg l^{-1} HQC. Solutions were renewed daily. Data are the means of 6 replications, ± SD.

utions for seven days. When the number of bacteria was lower than 10^6 cfu per gram fresh weight, the hydraulic conductance of the basal 5 cm stem part did not significantly differ (at $P<0.05$) from the conductance in 5 cm segments of freshly cut stems (Fig. 6). The effect of a 40 min pulse treatment of

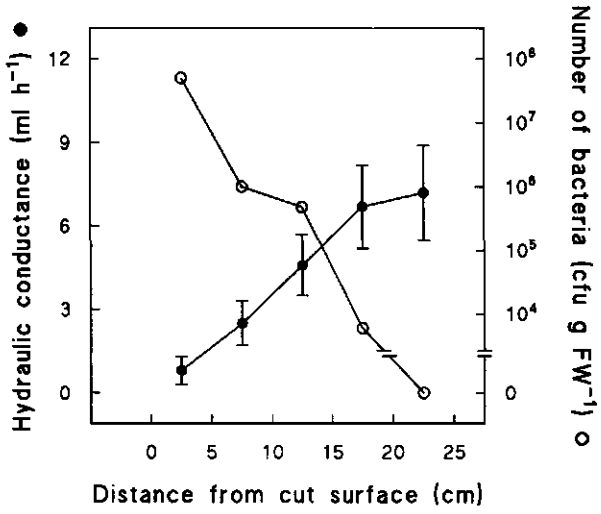

Fig. 4. Hydraulic conductance (left axis) and the number of bacteria (right axis), in 5 cm segments of cut Sonia roses held in water for seven days. Data are the means of 6 replications, ± SD.

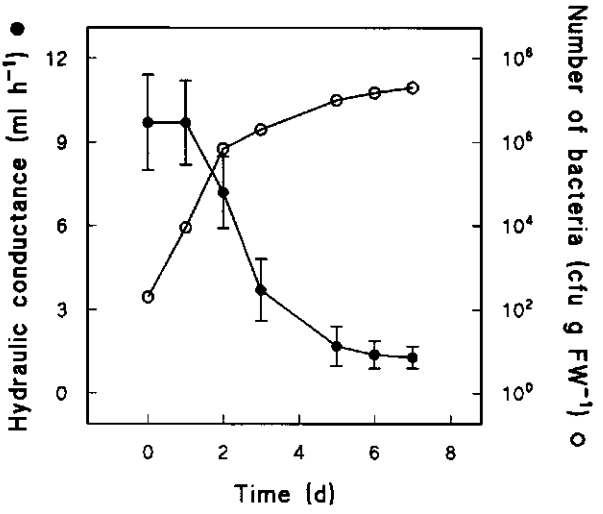

Fig. 5. Hydraulic conductance (left axis) and the number of bacteria (right axis) in the basal 5 cm segment of cut Sonia roses held in water for various periods of time. Data are the means of 6 replications, ± SD.

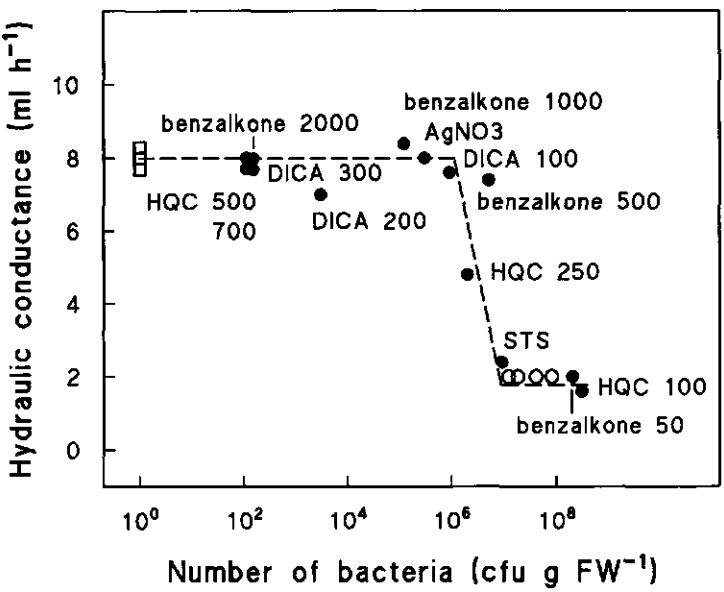

Fig. 6. Relationship between hydraulic conductance and the number of bacteria in 5 cm segments from the base of cut Sonia roses held for 7 days in aqueous solutions of various compounds (•, concentrations in mg l^{-1}). $AgNO_3$ treatment was at 4 mM, for 40 min prior to placement in water. STS treatment was at 2 mM for 4 h prior to placement in water. Controls were held in water for seven days (○) or were freshly harvested (□). Data are the means of 10 replications. The standard deviation varied between 0.31 and 2.46.

4 mM $AgNO_3$ on conductance was the same as that of the other tested bacteriostatic chemicals (HQC, benzalkone, DICA). A 4 h pulse treatment of 0.2 mM STS had no effect, either on the number of bacteria or on conductance (Fig. 6).

Production of ethylene by cut stems

Segments cut from rose stems were found to produce ethylene, which peaked at about 24 hours after cutting. At the tested concentrations, the production of ethylene was increased by DICA and benzalkone, and inhibited by HQC (Table 1). In this experiment $AgNO_3$ had no effect on ethylene production; in a second experiment it increased ethylene production by 27%.

Table 1. Effect of some antimicrobial compounds on ethylene production of 2-3 mm stem segments of Sonia rose flowers. Measurements were made 24 h after cutting. Data are the means of six replications ± SD.

	Ethylene production ($nl\ g^{-1}\ h^{-1}$)
control	1.68 ± 0.30
HQC 250 mg l^{-1}	0.14 ± 0.10
DICA 200 mg l^{-1}	3.83 ± 0.55
Benzalkone 1000 mg l^{-1}	3.60 ± 0.76
$AgNO_3$ 4 mM 40 min	1.82 ± 0.49

pH of the solutions

In order to test the hypothesis that polyphenol oxidase plays a role in the vascular blockage of roses, bacteriostatic agents with a wide range of pH were used. The pH of the HQC solutions varied between 4.2 (highest conc.) to 6.4 (lowest conc.). The DICA solutions had a pH of 7.4 and the benzalkone solutions ranged from pH 8.0 (lowest conc.) to 8.4 (highest conc.). These solutions were renewed daily. After seven days of vase life, there was no relationship between hydraulic conductance (Fig. 6) and the acidity of the solutions.

Vessel length and tylose formation

The maximum length of xylem vessels in cut Sonia roses was 25-30 cm. No tyloses were observed in the vessels, either when using light microscopy or scanning electron microscopy.

Relationship between the number of bacteria in vase water and in stems

Sonia roses were cut with a non-sterile knife and were then placed in sterilized vases with sterilized water. For several days no bacteria were found in the water but there was a growing population of bacteria in the basal 5 cm segment of rose stems. The population associated with the stems was as high as 3×10^6 cfu per gram fresh weight before bacteria were found in the water (Table 2).

Effect of hydroxyquinoline citrate in sterilized water

In a repeat of the experiments by Marousky (1969, 1971), we found that the number of bacteria in the vase water generally remained below the level of detection. When bacteria were present in the water the stems were not used for determination of hydraulic conductance. Even when no bacteria were

Table 2. Number of bacteria in vase water and in the basal 5 cm segment of stems of cut Sonia roses, placed in sterilized water and sterilized bottles. *Measurements* were made in duplicate (vase 1 and 2). *cfu*: colony forming units; n.d.: not determined

Day	Number of bacteria in vase water (cfu ml^{-1})		Number of bacteria in the basal 5 cm of stems (cfu per g FW)	
	1	2	1	2
0	0	0	$<1.2 \times 10^2$	$<1.2 \times 10^2$
1	0	0	1.2×10^3	2.2×10^3
4	0	0	n.d.	n.d.
7	0	7×10^3	4.8×10^4	3.0×10^6

present in the water a high number of bacteria (up to 10^6 cfu per gram FW) was found in the basal 5 cm stem segment of control flowers or flowers placed in a sucrose solution. Hydraulic conductance of the basal 5 cm stem segment of these stems was lower than in stems of freshly harvested flowers (Table 3). The number of bacteria in stems of flowers held in solutions containing HQC was below the detection limit and hydraulic conductance in these stems was the same as in stems of freshly harvested flowers (Table 3).

Experiments were repeated with the cultivars Ilona, Polka and Frisco, with results similar to those obtained with Sonia roses (results not shown).

DISCUSSION

When cut roses were held in water for several days, removal of the basal stem segment under water resulted in a (temporary) resumption of water uptake. This indicates that a resistance developed at the base of the cut stem. This was confirmed by our measurements of conductance in 5 cm stem segments (Fig. 3). We found the lowest conductance in the basal stem segment in all of the five rose cultivars investigated. This was also found by De Stigter and Broekhuysen (1986) and Put and Jansen (1989) who worked with Sonia roses, by Durkin and Kuc (1966) who investigated Better Times roses, and by Dixon and Peterson (1989) in Samantha roses. Burdett (1970), however, showed that in Forever

Table 3. The number of bacteria and hydraulic conductance in 5 cm segments of stems from cut Sonia roses held in various solutions for two days. No bacteria were present in the vase water. cfu: colony forming units.

Stem segment	Number of bacteria (cfu per gram fresh weight)			
	Control	HQC	Sucrose	HQC + sucrose
0-5 cm	8.4×10^5	$<1.2 \times 10^2$	9.2×10^5	$<1.2 \times 10^2$
5-10 cm	5.4×10^4	$<1.2 \times 10^2$	4.1×10^4	$<1.2 \times 10^2$
Stem segment	Hydraulic conductance (ml per 30 min.)[a]			
	Control	HQC	Sucrose	HQC + sucrose
0-5 cm	2.6 ± 0.5 a	3.5 ± 0.4 b	2.0 ± 0.2 a	3.4 ± 0.6 b
5-10 cm	3.6 ± 0.4 b	3.2 ± 0.6 b	3.4 ± 1.0 b	3.8 ± 0.4 b

[a] Means of six replications (± SD) followed by the same letter are not significantly different ($P < 0.05$). The mean (± SD) hydraulic conductance of freshly harvested stems was 3.4 ± 0.5 and 3.6 ± 0.4 ml per 30 min. at 0-5 cm and 5-10 cm from the cut surface, respectively.

Yours roses the area of lowest hydraulic conductance was located 15-20 cm from the cut surface, at the water table.

In the presence of a bacteriostatic compound, occlusion of two thirds of the xylem conduits by a razor blade did not reduce water uptake, even under high transpirational demand (Fig. 1), apparently because the loss of conduits was counteracted by an increased flow rate in the remaining non-occluded conduits (Fig. 2). It is concluded that the observed reduction of water uptake in cut roses that are held in water without a bacteriostatic compound must be due to blockage of at least two thirds of all conduits at some horizontal cross section of the stem. Since we found no effect on water uptake even when the transpiration was more than doubled (Fig. 1), probably even a much larger percentage of the conduits must become blocked before water uptake is inhibited.

In our experiments hydraulic conductance was decreased whenever the number of bacteria associated with the 5 cm segment exceeded about 10^6 cfu per gram fresh weight (Figs. 4, 5, and 6). This shows that bacteria at least contribute to the vascular blockage in cut roses.

The distribution of bacteria in stems reflected the length of the vessels that were cut open. Maximum vessel length was 25-30 cm in cut Sonia roses, whereas the number of bacteria in stems sections 20-25 cm from the cut surface was virtually zero (Fig. 4). Zimmermann (1983) reported that a majority of the vessels in plant stems are very short, and that the relationship between length and number of vessels is exponentially declining. The exponential decline in numbers of bacteria in stems of cut rose flowers (Fig. 4), starting at the cut surface, therefore indicates that bacteria are too big to pass the pores in the pit membranes and that bacteria do not digest pit membranes to an extent which would allow for the movement into the next conduits.

The production of ethylene by the cut surface of stems has been implicated in the development of vascular blockage (Olien and Bukovac, 1982; Fujino and Reid, 1983). Rose stems cut into 2-3 mm segments produced considerable amounts of ethylene (Table 1). Inhibition of ethylene production (by HQC) or stimulation of ethylene production (by DICA or benzalkone) had no effect on vascular blockage (Fig. 6). Silver nitrate prevented the development of blockage and also reduced the number of bacteria (Fig. 6). Silver ions are also known to counteract the effects of ethylene. The anionic silver thiosulfate complex (STS) is more mobile in the xylem than $AgNO_3$ but allows only a low concentration of free Ag^+ (Veen and Van de Geijn, 1978) and hence its bacteriostatic effect is low. In our system STS did not reduce the number of bacteria and neither did it affect hydraulic conductance. The above data indicate that ethylene production near the cut surface had no effect on the development of vascular blockage. The data also indicate that ethylene production by bacteria (if any occurred) did not trigger vascular blockage. This conclusion is substantiated by the absence of any visible symptoms such as leaf wilting which occur when supplying exogenous ethylene in aqueous solution to cut rose stems (Woltering, pers.comm. 1987).

Some authors hypothesized that physiological blockage involves oxidation of phenolic substances, e.g., by ethylene-induced enzymes (Durkin and Kuc, 1966; Buys, 1969). The activity of the enzyme system involved in oxidation of polyphenols is completely inhibited by pH around 4.0 (Vámos-Vigyázó, 1981). However, the effect on hydraulic conductance was the same after inclusion of benzalkone (high pH of the solution), DICA (neutral pH) or HQC (pH down to 4.2) in the vase solution (Fig. 6). This is evidence against the above hypothesis.

Physiological plugging of xylem vessels, either through tyloses or gum deposits, has been found in many plant species (Klein, 1923; Zimmermann, 1983). According to Chattaway (1949) tyloses are generally found in plants with relatively large pit apertures (> 10 μm) between the vessels and the tylosis-producing cells around them, while gums are usually found in species with smaller pit apertures. In the *Rosaceae* she found gums only, except in the subfamily *Chrysobalanoidaea*, which is now considered to be a separate family (Heywood, 1978). We never detected any tylose-formation. Also in Forever Yours and Red American Beauty roses no tyloses were found in the xylem vessels (Parups and Molnar, 1972; Gilman and Steponkus, 1972), but a substance described as gums was observed (Parups and Molnar, 1972; Lineberger and Steponkus, 1976). More than 66% of all vessels have to become blocked, however, in order to result in a reduction of water uptake (Fig. 1). Such high numbers of vessels occluded with gums are not reported in the literature on cut roses. When examining transverse sections, Lineberger and Steponkus (1976) reported a maximum of 20% of the vessels to contain carbohydrate-type plugs, Burdett (1970) found such deposits in 3-11% of all vessels, and Dixon and Peterson (1989) in 8-23%.

We found that whenever the number of bacteria remained lower than 10^6 cfu per gram fresh weight, the hydraulic conductance was the same as in freshly cut roses, even after stems had been held in water for seven days (Fig. 6). This means that a detectable physiological blockage did not develop during the seven day period. Theoretically, it cannot be excluded that the added bacteriostatic chemicals had an inhibiting effect on physiological blockage. However, the mode of action of the tested chemicals is very different. HQC introduces lethal amounts of Fe and/or Cu into the cell (Albert, 1979). DICA is a slow-release chlorine compound, and chlorine acts as the undissociated hypochlorous acid which enters the cell and probably oxidizes enzymes (Albert, 1979). Benzalkone is a quaternary ammonium compound, which desintegrates membranes (Albert, 1979). It is not likely that chemicals with such a diverse mode of action would all counteract physiological blockage to a degree correlated with their bacteriostatic effect.

Our results indicate that the vase water of cut rose flowers may be sterile (a number of bacteria below the detection limit) while a considerable number of bacteria is present in the xylem and on the cut surface. In the experiments of Marousky (1969) on cut Better Times roses the vase water was held sterile and yet an occlusion was found, which was therefore interpreted as caused by a physiological process. We repeated his experiments, using other cultivars, and conclude that, at least for the cultivars

we investigated, the above conclusion is not correct. The blockage was correlated with the number of bacteria associated with the stems.

From these experiments (in which the effect of air embolism was excluded) we conclude that, whereas gum deposits or other mechanisms of physiological blockage may possibly occur, their contribution to the blockage of the vascular system is negligible. The high resistance to flow of water in rose stems placed in water is related to the number of bacteria developing within the xylem and on the cut surface.

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CHAPTER 4

MODE OF ACTION OF BACTERIA IN THE XYLEM OCCLUSION OF CUT FLOWERING ROSE STEMS

Summary

A suspension of *Pseudomonas aeruginosa* at 5 x 10^9 cfu ml^{-1} was either left untreated, pasteurized (15 min, 70°C), or autoclaved (15 min, 121°C). Stems of cut Sonia rose flowers (*Rosa hybrida* L.) placed in these solutions showed decreased water uptake and a reduction of hydraulic conductance of the basal 5 cm stem segment. No difference was found between the living or dead bacteria. When the pasteurized or autoclaved solutions were left at 4°C for 7 days, bacterial cells had autolysed, and stems placed in these suspensions showed lower water uptake and hydraulic conductance than stems in freshly prepared suspensions. The results show that living and non-living bacteria have the same effect on vascular occlusion, and indicate that hydraulic conductance was more reduced when the average size of the particles was smaller than that of bacterial cells.

Experiments at 1°C with suspensions of 5 x 10^9 cfu ml^{-1} of *P. aeruginosa*, either living or sterilized, showed the same reduction of hydraulic conductance as experiments at 20°C; the same was found when comparing the effects of a suspension of 2 x 10^7 cfu ml^{-1} at 20°C and 1°C. Isolated extracellular polysaccharides from *Pseudomonas aeruginosa* and 2 x 10^3 kD dextran from *Leuconostoc*, consisting of relatively linear molecules, also resulted in blockage. Cellulase had the same occluding effect as ovalbumin (both at 1 g l^{-1}), showing that occlusion may also occur by relatively small globular molecules. The experiments indicate that the occlusion induced by bacteria is due to a physical effect only.

INTRODUCTION

The onset of vascular blockage in stems of cut rose flowers was delayed by including antimicrobial compounds in the vase solution (De Stigter, 1981) and advanced by including bacteria in the water (Van Doorn et al., 1986; Zagory and Reid, 1986). Antimicrobial compounds in the vase solution

reduced the number of bacteria both in the solution (Chapter 3; Marousky, 1976) and in the stems of the rose flowers (Chapter 3; Van Doorn et al., 1990).

The blockage of water uptake in cut rose stems placed in water was mainly located in the basal 5 cm of the stem. A correlation was found between a decrease in hydraulic conductance and a high number of bacteria per gram FW, in this segment (Chapter 3). These results suggest that bacteria are a factor in vascular occlusion of cut rose flowers. The mechanism by which bacteria cause vascular occlusion, however, is not known. One of the possibilities is the induction of a defense mechanism in the stems. Zagory and Reid (1986) noted that some bacteria from vase water produced ethylene, which induced vascular blockage in *Ricinus communis* (VanderMolen et al., 1983). Some bacteria may furthermore elicit phytoalexin production which could lead to a defense reaction in the xylem (VanderMolen et al., 1977). Although we were unable to detect cell wall breakdown (Chapter 2) or to find any clue for another, stem-induced factor for vascular occlusion (Chapter 3), even small molecules originating from cell wall breakdown may elicit a reaction in living cells (Albersheim et al., 1983) and such an effect could also be exerted by extracellular products from bacteria.

In order to further test the hypothesis that vascular occlusion induced by bacteria depends on the action, direct or indirect, of living bacteria, the effect of a suspension containing living bacteria was compared with the effect of the same suspension after a pasteurisation or autoclaving treatment. Vascular blockage was measured at short term intervals after treatment in order to test the hypothesis that vascular occlusion is a physiological response rather than a physical mechanism.

MATERIALS AND METHODS

Plant material

Cut flowering Sonia rose stems of 60 cm length were obtained from a commercial grower. After harvest the stems were held dry at 4°C for 3-4 h, and then transported dry to the laboratory within 1 h. There all but the five uppermost leaves were removed. The stems were individually placed in various aqueous solutions or suspensions, after recutting of the stems with a sterile blade (removing about 10 cm). Experiments occurred at 20°C, 60% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}, or at 1°C, 85% RH and a photon flux (PAR) of 5 μmol m^{-2} s^{-1}. Lights were on for 12 h per day.

Suspensions of bacteria

Cut Sonia roses were held in non-sterile water in a non-sterilized bottle. After seven days of vase life, the bacteria from the basal 5 cm of the stems were extracted and grown on Plate Count Agar (Oxoid, Basingstoke, Hants., U.K.) as described in Chapter 3. The bacteria were identified using methods as described in Chapter 2. Pure cultures of *Pseudomonas aeruginosa* were isolated and grown on Plate Count Agar. After 24 h of growth the bacteria were scraped off the agar, suspended in sterilized distilled water and the concentration was adjusted to 5×10^9 or 2×10^7 cfu ml^{-1}. Ten rose stems were individually placed in part of this solution for various periods of time, at 20°C or at 1°C. A second part of the solution containing 5×10^9 cfu ml^{-1} was held at 75°C for 15 min (pasteurization treatment), and a third one at 121°C for 15 min (autoclave treatment). Part of the suspension at 2×10^7 was also autoclaved.

Bacterial counts

The numbers of bacteria in vase water and in the basal 5 cm of rose stems were determined as described in the Chapters 2 and 3. The total count was determined with a Hawksley counting chamber (A70 Helber, Hawksley Ltd, Lansing, U.K.) using the method described by Gerhardt et al. (1981).

Suspensions of proteins and extracellular polysaccharides

Stems were placed in aqueous suspensions of cellulase (Merck) and ovalbumin (Merck), both at 1 g l^{-1}, for 3 h at 20°C. The extracellular polysaccharides from *Pseudomonas aeruginosa* were obtained by growing the isolated strain on GM medium (Kaplan et al. 1987). The medium was centrifuged at 10,000 g and the retentate filtered through a sterile Millipak-60 filter unit (22-45 µm pore diameter) to remove the bacteria. The aqueous suspension was then placed in a Millipore PT/NMWL separator, with a nominal cut-off level of molecules of 10 kD. This system, however, allowed the passage of most of the bacterial polysaccharides. Only the use of a filter with a nominal cut-off level of 0.5-2.0 kD prevented the passage of most of these linear molecules, but did allow the passage of the salts and sugars from the medium.

The effect of the isolated polysaccharides from *P. aeruginosa* was compared with the effect of dextran, an extracellular polysaccharide from *Leuconostoc mesenteroides* (obtained from Sigma), with molecular masses of 75 kD and 2000 kD.

Table 1. The number of bacteria in the basal 5.0 cm or the basal 0.1 cm of the stems of cut Sonia roses, placed in a solution containing no bacteria or in a suspension of *P. aeruginosa* at 5 x 10^9 cfu ml^{-1}, which was not treated, pasteurized (15 min 70°C) or autoclaved (15 min 121°C). The number of bacteria is the means of three replicate stems. Statistical differences ($P < 0.5$) are indicated by a different letter, separately for the basal 5 cm and the basal 0.1 cm.

Period of exposure (h)	Number of bacteria in the basal 5 cm of stems (cfu per g FW)			
	Controls, no bacteria	Suspension of living bacteria	Pasteurized suspension	Autoclaved suspension
0.5	0	2.7 x 10^9 a	0	0
1.0	0	3.8 x 10^9 b	0	0
2.0	0	5.6 x 10^9 c	0	0
4.0	0	8.8 x 10^9 d	0	0
	Number of bacteria in the basal 0.1 cm of stems (cfu per g FW)			
0.5	0	9.0 x 10^8 a	0	0
1.0	0	1.4 x 10^9 a	0	0
2.0	0	2.4 x 10^9 b	0	0
4.0	0	4.6 x 10^9 c	0	0

Water relations of the stems

The amount of water absorbed by the stems of cut rose flowers was determined by weighing of the vials, after correction for evaporation which was determined in vials containing the same suspension without stems. Hydraulic conductance in the basal 5 cm of the stems was determined as described in Chapter 3.

Statistical analysis

Analysis of variance was performed using the GENSTAT V Statistical Package. Experiments were at least once repeated.

Table 2. Hydraulic conductance of the basal 5 cm of the stems of cut Sonia roses, and rate of water uptake by the stems, after placement in water containing no bacteria or in a suspension of *P. aeruginosa* at 5 x 10^9 cfu ml^{-1}, which was not treated, pasteurized (15 min 70°C) or autoclaved (15 min 121°C). Data are the means of ten replications, ± SD. Statistical differences ($P < 0.05$) in hydraulic conductance and in water uptake rate are indicated by a different letter.

Period of exposure (h)	Hydraulic conductance (ml/30 min)			
	Controls, no bacteria	Suspension of living bacteria	Pasteurized suspension	Autoclaved suspension
0.5	5.2 ± 2.6 a	1.3 ± 0.4 b	1.4 ± 0.7 b	1.7 ± 0.7 b
1.0	5.5 ± 1.5 a	0.7 ± 0.6 c	0.7 ± 0.6 c	1.2 ± 0.7 c
2.0	4.7 ± 1.7 a	0.5 ± 0.2 d	0.2 ± 0.2 d	0.3 ± 0,1 d
4.0	4.8 ± 2.8 a	0.3 ± 0.2 d	0.2 ± 0.1 d	0.2 ± 0.1 d
	Rate of water uptake (ml/h)			
0.5	8.8 ± 1.7 a	3.6 ± 1.5 b	3.4 ± 1.3 b	4.2 ± 1.6 b
1.0	8.8 ± 1.8 a	3.6 ± 1.4 b	4.1 ± 1.6 b	4.4 ± 1.5 b
2.0	8.4 ± 1.5 a	3.8 ± 1.6 b	3.4 ± 1.5 b	4.3 ± 1.7 b
4.0	8.5 ± 1.6 a	3.8 ± 1.3 b	3.7 ± 1.3 b	4.0 ± 1.6 b

RESULTS

Numbers of living and dead bacteria in suspensions

When *P. aeruginosa* was suspended in water after growth on Plate Count Agar for 48 h at 30°C, the number of living bacteria (in cfu ml^{-1}) was only 13% of the total number determined with the Hawksley counting chamber, indicating that the suspension contained numerous dead bacteria. Only when the isolated strain was grown for 24 h the number of living bacteria was equal to the total count. In this study, therefore, the bacterium was grown on agar for 24 h only. The untreated suspensions of *P. aeruginosa* contained 5 x 10^9 viable bacterial cells and no cell fragments. After pasteurization or

Table 3. Number of bacteria in the basal 5.0 cm and 0.1 cm of the stems of cut Sonia roses, and hydraulic conductance of the basal 5.0 cm, after 4 and 6 days in non-sterile water at 20°C. The number of bacteria is the means of three replicate stems, hydraulic conductance is the means of six replicate stems, ± SD.

Period of exposure (d)	Number of bacteria (cfu per g FW)		Hydraulic conductance (ml per 30 min)
	5.0 cm	0.1 cm	
0	$< 1.6 \times 10^2$	$< 1.6 \times 10^2$	4.6 ± 2.3
4	9.4×10^7	9.1×10^7	0.2 ± 0.1
6	4.5×10^8	4.0×10^8	0.3 ± 0.2

autoclaving the suspensions contained 5×10^9 cells and no cell fragments within 1 day after the heat treatment. Plating the heat-treated solutions on Plate Count Agar did not result in bacterial growth.

Effect of living bacteria

Flowering stems placed in sterile water for up to 4 h contained no bacteria in the basal 5 cm (Table 1) while their hydraulic conductance remained high (Table 2). Cut rose flowers placed in a suspension containing 5×10^9 cfu of *P. aeruginosa* cells ml^{-1} showed reduced hydraulic conductance in the basal 5.0 cm already after 0.5 h (Table 2). The number of bacteria in the basal 5.0 cm of the stems was then 2.7×10^9 cfu per gram fresh weight, while the basal 0.1 cm contained 9.0×10^8 cfu per gram fresh weight (Table 1). Upon further exposure the hydraulic conductance diminished more while the number of bacteria in the basal stem segment increased (Tables 1 and 2).

The number of bacteria in the stems was about one order of magnitude higher than the number in controls held in non-sterile water for 4-6 days, while the hydraulic conductance of the latter was similar (Table 3).

Effect of dead bacteria

When flowering rose stems were placed in the pasteurized or autoclaved bacterial suspensions of *P. aeruginosa* the hydraulic conductance declined as drastically as in stems placed in a suspension of living bacteria (Table 2). No living bacteria were detected in the basal 5.0 cm of the stems after 4.0

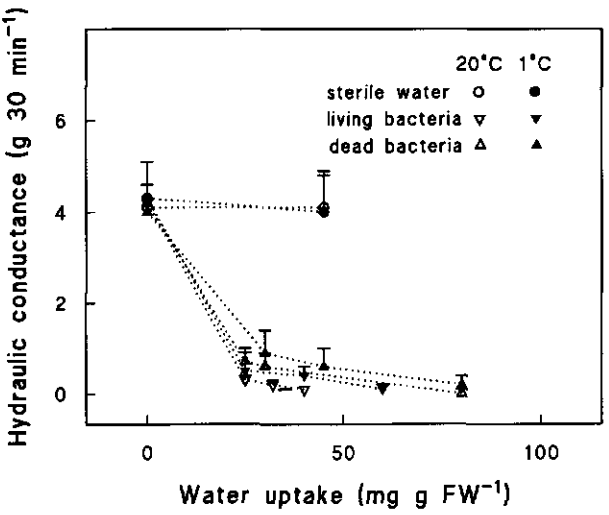

Fig. 1. Relationship between the accumulated uptake of water and hydraulic conductance in the basal 5 cm of the stems of cut Sonia roses, placed in sterile water and in aqueous suspensions containing 5 x 10^9 ml^{-1} of living or dead *Pseudomonas aeruginosa*, at 20° and 1°C. Data are the means of 8 replications, ± SD.

h (Table 1). In these experiments the rate of water uptake was reduced to about half the value of sterile controls with suspensions of living bacteria as well as with heat-treated suspensions (Table 2).

Effect of autolysed bacteria

The heat-shocked and autoclave suspensions were left at 5°C for 7 days in order to obtain a high number of lysed cells. Plating the solutions on Plate Count Agar did not result in bacterial growth. Placing of flowering rose stems in these solutions for 4 h resulted in hydraulic conductance of 0.01 ± 0.01 ml per 30 min. The rate of water uptake was 0.37 ± 0.06 and 0.34 ± 0.05 ml per hour, in the 7-day-old pasteurized and 7-day-old autoclaved suspension, respectively.

Comparison of effects at 20 C and 1 C

At 20°C the rate of water uptake was of the sterile control was about five times higher than at 1°C, but at both temperatures it was strongly reduced by the presence of living or dead bacteria. When aqueous suspensions containing 5 x 10^9 cfu of living *P. aeruginosa* ml^{-1} were given at 20°C and at

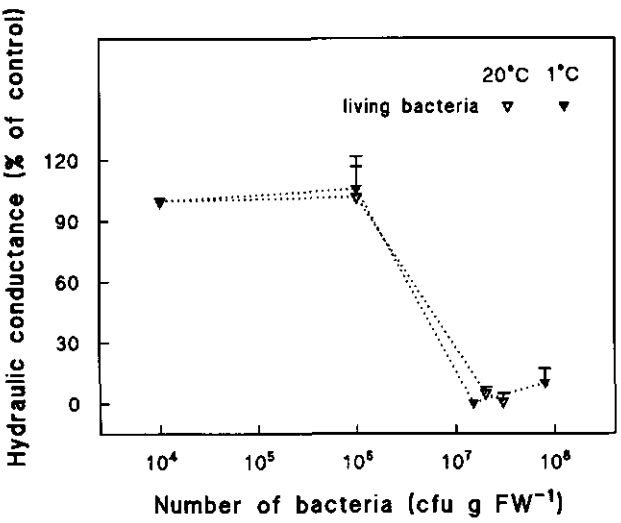

Fig. 2. Relationship between the number of bacteria in the basal 5 cm of the stems of cut Sonia roses, and hydraulic conductance of these stem segments. The cut flowers were placed in aqueous suspensions containing 2 x 10^7 ml^{-1} of living *Pseudomonas aeruginosa*, at 20° and 1°C. Results are from two experiments. Data on bacterial counts are the means of five replications; data on hydraulic conductance the means of ten replications, ± SD.

1°C, provided that the same amount of water was taken up, the hydraulic conductance was reduced to the same extent at both temperatures (Fig. 1). This also occurred when a suspension of dead bacteria, at the same concentration, was given (Fig. 1). Experiments with a suspension of 2 x 10^7 cfu ml^{-1} of living *P. aeruginosa* also showed that the effects on hydraulic conductance were similar at both temperatures (Fig. 2).

Effect of extracellular polysaccharides

Stems placed in suspensions of 1 g l^{-1} cellulase or ovalbumin (both about 50 kD) at 20°C for 3 h showed low hydraulic conductance in the basal 5 cm of the stems, measured 30 min, 60 min and 120 min after the stem segments were connected to the head of water (Fig. 3).

When the stems were placed in a sterile flask containing a sterile suspension of extracellular polysaccharides, they rapidly contained a high number of bacteria. After several unsuccessful attempts

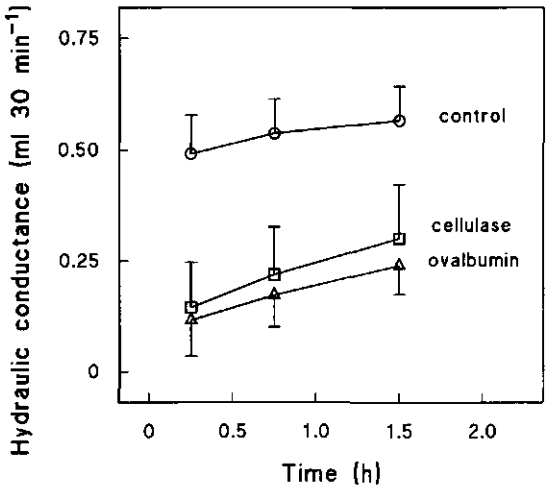

Fig. 3. Hydraulic conductance in the basal 5 cm of cut Sonia roses, after placing the stems in aqueous suspensions of 1 g l^{-1} cellulase or ovalbumin for 3 h at 20°C. Hydraulic conductance was measured after 30, 60 and 120 min. Data are the means of ten replications, ± SD.

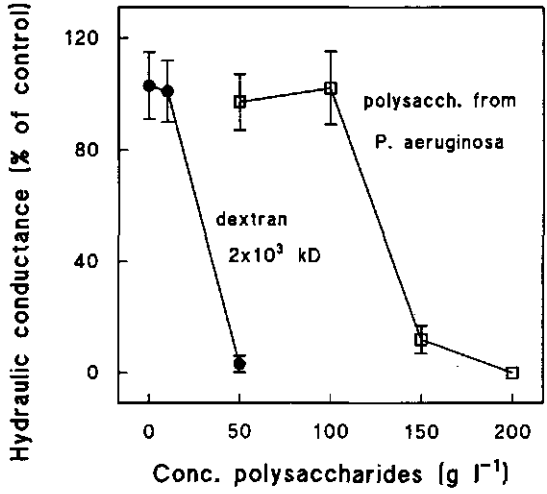

Fig. 4. Hydraulic conductance in the basal 5 cm of cut Sonia roses, after placing the stems in an aqueous suspension of extracellular polysaccharide from *Pseudomonas aeruginosa*, or dextran (2 x 10^3 kD) from *Leuconostoc*, for 5 d at 20°C. Microbial growth in the stems was controlled (< 10^4 cfu per 5 cm) by the inclusion of 350 mg l^{-1} of HQC and 24 mM of a potassium citrate/ potassium phosphate buffer (pH 3.1) in the suspension.

using streptomycin or penicillin as bacteriostatic compounds, we succeeded in controlling bacterial growth by including a relatively high concentration of HQC (350 mg l^{-1}) and a potassium citrate/ potassium phosphate buffer at pH 3.1 in the suspension. The polysaccharide isolated from *Pseudomonas aeruginosa* resulted in low hydraulic conductance of the basal 5 cm of the stem (Fig. 4). The dextran from *Leuconostoc mesenteroides* with a molecular mass of 75 kD had no effect (results not shown), but the dextran with molecular mass of 2000 kD also resulted in low hydraulic conductance (Fig. 4).

DISCUSSION

In previous experiments the stems of cut Sonia roses were placed in a solution containing a mixed population of bacteria originating from its vase water. At 3 x 10^9 cfu ml^{-1} the flowering stems showed bending just below the flower head, within a few hours (Van Doorn et al., 1986). In the present experiments with 5 x 10^9 cfu ml^{-1} of *P. aeruginosa*, the most predominant bacterium in the vase water of cut roses (De Witte and Van Doorn, 1988) and in the basal 5 cm of the stems (Chapter 2), bending of the stem was also observed and found to be correlated with a decrease in hydraulic conductance in the basal part of the stem. Hydraulic conductance was already reduced within 30 min after treatment. The hypothesis that a physiological reaction in the stem tissue is a prominent cause of occlusion during vase life is, therefore, less likely as the blockage developed more rapidly than expected in a physiological response, at least at this relatively high exogenous concentration of bacteria.

The present results show that vascular blockage can be induced by dead bacteria. Blockage, therefore, does not depend on the metabolic activity of the bacteria nor on the response of the plant cells to living bacteria. When the solutions had the same titre, and stems had taken up the same bacterial mass, from suspensions of either living or dead bacteria, the level of blockage was the same.

When stems of cut rose flowers were held in non-sterile water, a detectable decrease in hydraulic conductance was correlated with a number of bacteria (in the basal 5.0 cm) exceeding about 10^6 cfu per gram FW (Chapter 3, Table 3). The present study shows that after 4-6 days of vase-life in a non-sterile solution 90% of the bacteria in the basal 5.0 cm segment were present in the lowermost 0.1 cm of the stem. After a shorter exposure to a high concentration of bacteria, however, only 50% of the number of bacteria in the basal 5.0 cm was found in the lowermost 0.1 cm. This indicates that under

vase life conditions the bacteria proliferate predominantly at the cut surface, possibly because of the available nutrients. When stems were subjected to 5 x 10^9 cfu ml^{-1} of *P. aeruginosa* for 30 min the number of bacteria in the basal 0.1 cm stem segment was comparable to the number of bacteria after standing stems in vase water for 6 days, but hydraulic conductance was much higher in the former treatment. In the next 4.9 cm of the stems the number of bacteria was 1.6 x 10^9 and 5.2 x 10^7 cfu per gram FW, respectively, which was inversely correlated with hydraulic conductance. The relatively low values of hydraulic conductance observed in stems which had been held in vase water may relate to high numbers of particles of smaller size than bacteria, such as the constituents of bacterial slime, cell fragments, and the contents of lysed cells.

In the pasteurized or autoclaved suspensions no lysed cells were observed. The heat-treated suspensions and the suspension of living bacteria had only the presence of cells and their extracellular products in common. Vascular blockage may have been due to either of these, or to their combination. Suspensions of lysed cells were even more effective in reducing hydraulic conductance than those of non-lysed bacteria, suggesting an effect of particles of a size smaller than bacteria.

Blockage may be partly due to extracellular products. *P. aeruginosa* grown on Plate Count Agar produced a visible layer of extra-cellular slime on the colonies (Chapter 2). Such a layer has been found to consist mainly of polysaccharides, in addition to proteins, RNA and DNA (Eagon, 1962). The isolated polysaccharides resulted in low hydraulic conductance in the stems of Sonia roses (Fig. 4). This confirms the results of Put and Klop (1988) who found decreased hydraulic conductance in cut Sonia roses by a fraction of the EPS from *P. aeruginosa*. Dextran, a polysaccharide isolated from the extracellular slime of *Leuconostoc*, has been found to completely block water flow in cut stems and in petioles of alfalfa plants (*Medicago sativa* L.). The 2000 kD dextran already blocked at picomole concentrations (Van Alfen et al., 1983). Such dextrans were also found to effectively reduce water uptake into stems of cut Sonia roses (De Stigter and Broekhuysen, 1986) and this was related to a low hydraulic conductance in the stems (Fig. 4). In our experiments only the 2000 kD dextran resulted in blockage; the 75 kD dextran had no effect. As most of the polysaccharides from *P. aeruginosa* passed a molecular sieve with a nominal cut-off value of 10 kD (as described in the Materials and Methods section), the fraction of high molecular mass polysaccharides that actually results in blockage may be relatively small compared to the total amount of polysaccharides produced.

Both cellulase and ovalbumin, an inert protein of similar molecular weight as cellulase, resulted in rapid blockage. These experiments, too, indicate that the effect of bacteria does not depend on the action of a cell-wall degrading enzyme such as cellulase, and also show that relatively small particles such as proteins, which are released by living bacteria as well as upon the lysis of dead bacteria, rapidly result in stem occlusion.

The experiments comparing the effects of living and dead bacteria at 20°C and 1°C indicate that the effect of the bacteria is the same at both temperatures, provided the same amount of suspension is taken up by the stems, i.e. when the same amount of bacterial mass is taken up. These results also indicate that bacterial action is not dependent on physiological activity, either from the part of the bacteria or from the part of the stem. This was not only found at relatively high concentrations of bacteria, but preliminary experiments indicated that this also occurred at concentrations found in vase water, after a few days.

Throughout our experiments with a number of isolated bacterial strains, given at both relatively low and relatively high concentrations (De Witte and Van Doorn, 1988; and this Chapter), and experiments with isolated bacterial polysaccharides placed in the vase solution, we never noted a visible toxic effect on the flowers. Visible symptoms of water stress were always accompanied by hydraulic conductance in the basal 5 cm of the stem being close to zero. Although some products from bacteria and fungi found in vase water may increase membrane permeability in the beetroot test (Put and Klop, 1990), the decrease in turgor observed in cut roses is apparently not the result of a direct effect on the plasmalemma.

It is concluded that the results strongly suggest that the mode of action of bacteria in vascular blockage is a purely physical one.

The present results have implications in practice. At commercial growers we found that the water used for rehydration of rose flowers generally contained about as many living as non-living bacteria. Both living and non-living bacteria, as well as macromolecules from bacterial origin, may contribute to vascular blockage.

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CHAPTER 5

ASPIRATION OF AIR AT THE CUT SURFACE OF ROSE STEMS AND ITS EFFECT ON THE UPTAKE OF WATER

Summary

Flowering stems of Sonia roses were cut by methods which precluded air uptake. When the cut surface was exposed to air the volume of aspired air depended on the size of the leaf within 6 to 10 cm from the cut surface, and was up to about 1.2 ml. When the lowermost leaf was removed prior to measurement and the leaf scar covered with laboratory grease, the average uptake (37 ± 8 µl $stem^{-1}$) corresponded with the calculated lumen of the xylem vessels opened by cutting, indicating that the boundary of water/air did not pass the pores in the pit-membranes. Uptake of air was maximal after 20-30 min, even when flowers were held dry for 6 d. Flowers left in air for three hours and then placed in water showed a high rate of water uptake, which indicated that the presence of air in the xylem conduits did not impair the subsequent uptake of water. However, when flowers were left in air for 24 or 36 h, the rate of water uptake became progressively inhibited.

INTRODUCTION

When plant parts are cut in air, transpiration continues and it is likely that air is taken up in the xylem elements that are opened by cutting (Scholander et al., 1957; Zimmermann, 1983). Durkin (1980) showed that water uptake was inhibited when cut rose stems had been kept in air overnight, and it was inferred that the presence of air in the xylem was the cause of impeded water uptake. Scholander et al. (1957), however, held liana (*Tetracera* sp.) stems in air for 15 min, during which 80 ml of air was taken up, and found that the subsequent rate of water uptake was as high as before aspiration of air.

A considerable pressure gradient is necessary to transfer the air-water boundary through the pores in the vessel-to-vessel pits (Milburn, 1979; Zimmermann, 1983). The water potential at which the air-water boundary is pulled into the adjacent xylem vessels depends on the size of the pores (Zimmermann 1983). In wild grapevine species the air-water boundary was displaced at a stem water potential of about -2.4 MPa (Sperry et al., 1987) and similar values were recorded for stems of grape and sugar maple (Sperry and Tyree, 1988).

Since uptake of air was suggested to result in vascular blockage in cut rose flowers (Durkin, 1980), we studied the kinetics of air uptake in rose stems as related to leaf water potential and stomatal conductance, as well as the subsequent uptake of water.

In this study we eliminated the effect of bacteria. It was previously found that the number of bacteria in the stems increases during exposure to air. Whenever the stems are placed in water after harvest, the cut surface and the stem interior will contain a population of bacteria. This population increases, which may partially account for the vascular blockage observed after exposure to air (Van Doorn and De Witte, 1991). When the stems are not held in water before exposure to air, they do not contain bacteria. If in the present experiments cutting under water was necessary the experiments lasted too short to give rise to a population of bacteria high enough to cause blockage, and in the prolonged experiments population growth was controlled by using antimicrobial compounds in the water.

MATERIALS AND METHODS

Plants

Flowering Sonia rose stems were cut at the commercial stage, from the greenhouse of the Horticulture Department of the Wageningen Agricultural University. Stem length was about 40 cm and stem diameter 4 mm, unless otherwise specified. Stems carried one leaf at the lowermost 10 cm.

Uptake of air

In order to obtain rose stems without air, stems were cut using three different techniques. The first was based on the results from Chapter 3 showing that the longest vessels are less than 30 cm. In order to remove the initially aspired air, stems of 90 cm length were cut in the greenhouse and in the laboratory the lower 50 cm was cut under water. The second method prevented the initial aspiration of air: in the greenhouse stems of 40 cm length were cut under water in a wide bucket using long

shoots which were bent into the water. Alternatively, stems were cut according to the method described by De Stigter (1981): a plastic bag wrapped around the stem of intact plants was filled with water and the stem was cut through the plastic bag. The clippers used for cutting had walls mounted on top of the blades, which precluded draining of the water at the moment of cutting and water was therefore introduced at the cut surface at the very moment of cutting. The three methods gave identical results with respect to the subsequent aspiration of air.

Stems cut by either of these methods were connected to a 1 ml capillary pipette graded into 0.01 ml. The pipette contained air and a drop of 1% acid fuchsin at the lower end. Flowering rose stems were taken out of their solution, the water attached to the cut surface was swept off, and the stem was rapidly introduced into a piece of tygon tubing connected to the upper end of the pipette. The pipette and the flowering stem were placed horizontally on the laboratory bench in a climate room at 20°C, 60% RH, and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}.

Leaves remained attached to the flowering stems or leaves were removed, either prior to measurement or during measurement of air uptake, and the scars were immediately covered with vaseline. The effectiveness of the seal was checked by placing the stem in a Scholander pressure chamber and increasing the pressure to 0.01 MPa. When leaf scars were not sealed the pressure resulted in movement of a drop of mercury in an air-filled pipette connected to the cut surface. When sealed no such movement occurred.

In several experiments the effects of removal of leaves at different positions along the stem were determined.

Uptake of water

Flowering stems were held dry as described above, and the subsequent uptake of water was measured by attaching the cut surface to a pipette filled with distilled water, carefully avoiding any inclusion of air in the tygon tubing connecting the stem with the pipette. The movement of the air-water interface at the lower end of the pipette was followed through time.

In order to assess the effect of air uptake on water uptake some flowering rose stems (controls), 80 cm long, were cut in the greenhouse. Before exposure to air, the leaves were removed from the basal 40 cm of these stems, allowing transpiration similar to the stems of 40 cm length. After exposure to air and prior to assessment of the rate of water uptake 40 cm was cut from the basal end of the stems, essentially removing the aspired air.

Water potential and stomatal opening

The water potential of the third leaf from the flower head was measured with a Scholander pressure chamber, before and during exposure to air. Measurements were made in sixfold, at about noon.

Stomatal conductance was measured with a Licor-1600 porometer (Licor, Logan, Utah), in the middle of the abaxial side of the top leaflet in the third leaf from the flower head. Conductance values were measured in the intact plant, and at various intervals after cutting. After cutting the flowering stems were held dry and placed individually on a bench in the climate room as described above. Measurements were made in fivefold, around 11 a.m.

Bacteria and their control

The number of bacteria in the lowermost 5 cm of the stems was enumerated after various periods of exposure to air, using the method described in Chapter 2. The number of bacteria in the stems were kept at low levels by inclusion of 200 mg l^{-1} 8-hydroxyquinoline (HQC, from La Quinoléine, Oissel, France) in the water.

Estimate of the total volume of the lumen of the conduits opened by cutting

The number of xylem vessels in the stems was calculated by counting the vessels in cross sections under the microscope. The diameter of the vessels was determined from scanning electron micrographs. Vessel length was measured with the india-ink method according to Wiebe et al. (1984). The distribution of vessel length was similar to that of *Acer rubrum* L., for which detailed data are available (Zimmermann, 1983). The length categories used for *A. rubrum* were the basis for the calculation of the vessel lumen opened by cutting.

RESULTS

Aspiration of air

After cutting of flowering rose stems, air was aspired at the cut surface until the leaves had desiccated (Fig. 1). When the leaf close to the cut surface was removed after ca. 20 hours of air uptake, further uptake immediately halted (Fig. 1). The volume of aspired air depended on the size of that leaf (Fig. 1). Removal of the other leaves failed to affect the amount of aspiration.

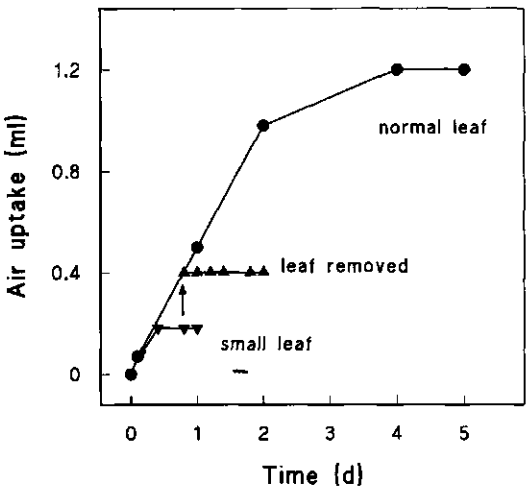

Fig. 1. Aspiration of air at the cut surface of leafy Sonia rose stems. The stem segment within 10 cm from the cut surface carried one seven-leaflet leaf, which was either of average size (normal leaf), left on the stem or removed after ca. 20 h (arrow), or with small leaflets (small leaf). Data from typical repeat experiments (single flowering stems).

When no leaf was present within 10 cm from the cut surface (or when the leaf was removed prior to exposure to air) the total uptake of air was only 30-60 µl, depending on the stem diameter (Fig. 2). In stems of uniform diameter (3.9 ± 0.3 mm) the average uptake was 37 ± 8 µl (n = 14). Uptake ceased after 20-30 min (Fig. 2), even when the stems were held dry for 6 d (not shown).

The rate of air uptake was reduced by decreasing the rate of transpiration, e.g., by covering the cut flowering stem with a polyethylene bag or by removal of the upper leaves (Fig. 3).

Water uptake

When flowering rose stems were cut in the greenhouse and then left in air (at 20°C and 60% RH) for 3 h, the subsequent rate of water uptake into the stems rapidly increased and then declined (Fig. 4), and the flowers became turgid within an hour.

Cut flowering rose stems left in air for 24 or 36 h, on the contrary, showed lower rates of water uptake than flowering stems left in air for 3 h (Fig. 4). After being held dry for 36 h the stems did not regain full turgidity within 12 h, and became turgid only after several hours when held dry for 24 h.

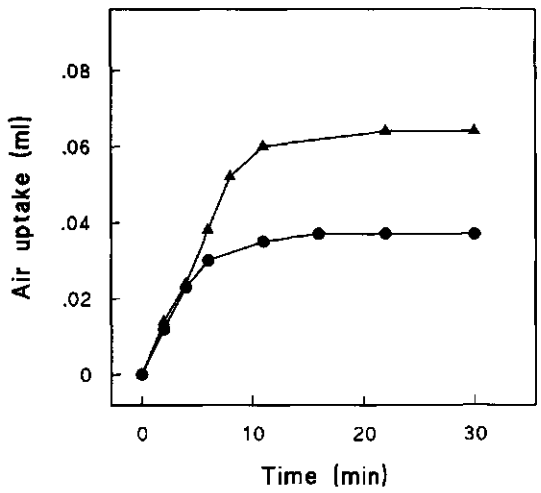

Fig. 2. Aspiration of air at the cut surface of leafy Sonia rose stems. The lowermost leaf was removed prior to the onset of the experiment and the leaf scar covered with laboratory grease. The lowermost line represents a stem of 3.9 mm diameter, the upper line a stem of 5.4 mm diameter.

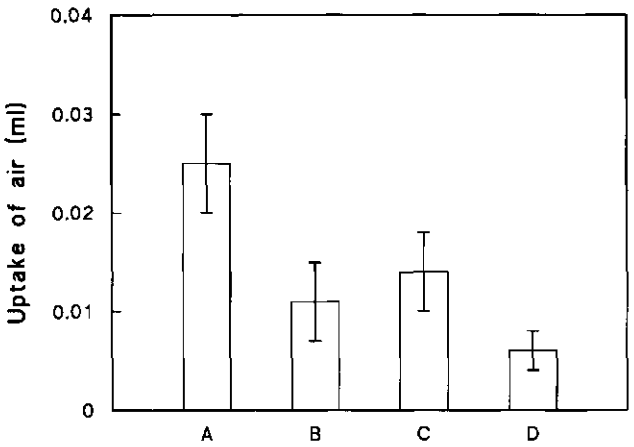

Fig. 3. Air aspiration at the cut surface of leafy Sonia rose stems during the first 5 min of the experiment. The stems were either controls of which the leaf at the lowermost 10 cm was removed and the leaf scar covered with laboratory grease (A), or covered with polyethylene bags (B), or uncovered stems from which half (C) or all (D) leaves were removed and the leaf scars covered with laboratory grease, prior to measurement. Data are means of six replications, ± SD.

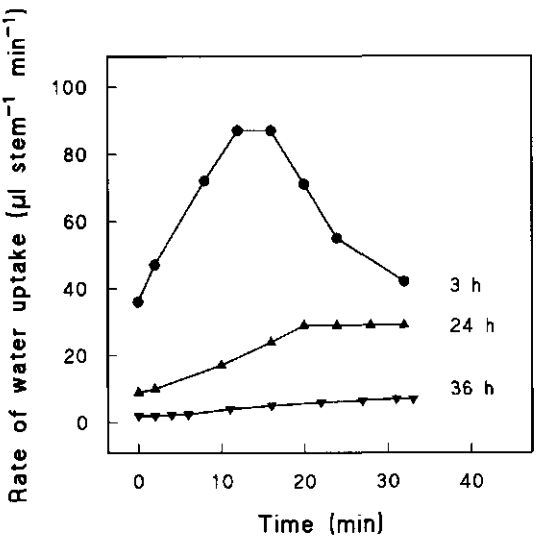

Fig. 4. Rate of water uptake into stems of cut Sonia roses which had been exposed to air in the light at 20°C and 60% RH for 3, 24, or 36 h. The rate of transpiration was about 10 µl min^{-1} stem^{-1} throughout. Data from typical repeat experiments (single flowering stems).

Water potential and stomatal conductace

Flowering rose stems cut under water and held in water at 20°C and 60% RH for several hours, had a leaf water potential of about -0.4 MPa. In stems that were held dry for one day (at 20°C and 60% RH) the leaf water potential was about -2.0 MPa and by the fourth day it was lower than -3.9 MPa (Table 1).

In flowering rose stems held dry at 20°C and 60% RH leaf stomatal conductance fell to low values within 3 h after cutting and remained at this value when held dry for 24 or 36 h (Table 2).

Estimate of the volume of the xylem lumen opened by cutting

The volume of the lumen of all xylem vessels at any transverse section of the stem was calculated to be about 77 µl (Table 3). When stems were cut, about half of this volume (about 39 µl) remained present in the basal end of the stem.

Table 1. Water potential of leaves from cut Sonia roses that were held dry in the light, at 20°C and 60% RH, for 6 days. Data are means of 6 replications, ± SD.

Time after cutting (d)	Water potential (MPa)
0	-0.43 ± 0.05
1	-2.19 ± 0.10
2	-3.18 ± 0.25
4	> -3.90
6	> -3.90

Table 2. Stomatal conductance in leaves of cut Sonia roses that were held dry in the light, at 20°C and 60% RH. Data are means of 5 replications, ± SD.

Time after cutting (h)	Stomatal conductance ($\mu mol\ cm^{-2}\ s^{-1}$)
0	280 ± 52
1	116 ± 47
3	22 ± 4
24	24 ± 4
36	20 ± 5

Bacterial numbers

In the experiments in which the stems were exposed to air for several hours only, no precautions as to bacterial control were taken, and the number of bacteria in the lowermost 5 cm stem segment did not exceed 10^4 cfu per gram FW. Experiments with a longer period of exposure to air were carried out with and without 200 mg l^{-1} HQC in the water used for cutting or recutting the stems. This had no effect on the aspiration of air. The number of bacteria in the stems shown in Fig. 4 were also kept below the above limit.

Table 3. Estimate of the total lumen of xylem vessels opened by cutting, in stems of Sonia roses. Vessel diameter was derived from (unpublished) SEM photographs of Sonia stems. The total number of vessels was about 1600.

Vessel length category (cm)	Average vessel diameter per category (μm)	% of total	Total number	Total volume per category (μl)
2	10	70	1120	1.77
6	20	10	160	3.04
10	30	5	80	5.70
14	40	5	80	14.20
18	50	5	80	25.53
22	50	3	48	19.20
26	50	1	16	8.00

				77.44

DISCUSSION

Immediately after cutting, air was found to be taken up by the cut surface of the flowering rose stems, as was previously demonstrated after cutting the stems of liana species (Scholander et al., 1955, 1957). Our measurements do not show where the air was absorbed at the cut surface but the correlation between the rates of transpiration and air aspiration (Fig. 3) indicates uptake into the xylem elements.

When the lowermost leaf was removed, the volume of air aspired at the cut surface (37 ± 8 μl) corresponded with the estimated volume of the lumen of the vessels opened by cutting (Table 3). The air was apparently taken up only by the xylem elements that were opened by cutting, hence did not pass the pores in the pit-membranes.

When the lowermost leaf was present, the volume of aspired air depended on the size of that leaf (Fig. 1). The other leaves had no effect on the amount of aspiration. Apparently, when the lowermost leaf was present, the aspired air passed this leaf only. This indicates a connection for transport of air between the cut surface of this proximate leaf, and no such connection to the distal leaves. The length of the connection apparently is smaller than the length of the longest vessels in the stem, which was

about 25-30 cm (Chapter 3). These results are in agreement with studies of stems in other plant species (Salleo et al., 1974; Wiebe et al., 1984), showing that only a few vessels enter the petiole, and that these vessels are shorter than the vessels in the stem.

Although uptake of air ceased after about 20-30 min (Fig. 2) the rate of water uptake was not inhibited when stems were placed in water, after being held in air for as much as 3 h (Fig. 4). The presence of air in the xylem vessels apparently posed no major obstacle to the water flow. Scholander et al. (1957) exposed stems of *Tetracera* lianas to air for 15 min and found that the rate of water uptake was not inhibited. They presumed that air was present in the lumen of the vessels and that water would therefore not be able to flow through the lumen. They suggested that the rapid uptake of water might be due to flow in a second pathway, viz. the interstices (tracheids and cell walls). Scholander et al. (1955) also exposed *Vitis labrusca* L. (wild grapevine) to air for 30 min, after which the uptake of water was clearly inhibited. In this species it was not possible to relate the inhibition of water uptake to the presence of air in the stems as the cut surface of the stems exuded a gummy substance which could be responsible for the inhibition of water uptake. We found that stems of cut flowering roses did not exude material at the cut surface, but also noted a progressive inhibition of water uptake after stems had been held in air for 24 or 36 h.

The decreased rate of water uptake after 24 or 36 h of exposure to air was not related to stomatal closure, which was already complete 3 h after cutting. Accumulation of bacteria at the cut surface or in the stem was not the cause of the blockage either: a decrease in hydraulic conductance of 5 cm stem segments of Sonia roses was correlated with the presence of more than 10^6 bacteria per gram fresh weight (Chapter 3). In the present experiments bacteria were found to enter the stems with the water in which the stems were cut, before dry storage. When taking adequate precautions, however, the number of bacteria in 5 cm segments was kept below the detection limit, thereby excluding a possible effect of bacteria on blockage.

The inhibition of water uptake after 24 h or 36 h of dry storage was apparently not due to movement of the air-water interface from the opened vessels to the adjacent vessels. The data of Sperry et al. (1987) on grapevine species indicate that the air-water boundary passed the pores in the vessel-to-vessel pits when the vines were dehydrated below a leaf water potential of -2.4 MPa. The average water potential in leaves of Sonia roses, after being held dry for six days, was lower than -3.9 MPa (Table 1), and the water potential of the stems of cut rose flowers show a 1:1 relationship with the

water potential of the leaves (Dixon, 1987). Since no detectable uptake of air occurred after the initial 30 min exposure of Sonia roses to air even when the flowering stems were held dry for 6 d, the water potential at which the air-water boundary moves from vessel to vessel was apparently not reached even at a water potential of -3.9 MPa.

From these experiments we conclude that the aspiration of air into the cut surface of rose stems is restricted to the xylem elements that are cut open, while the air apparently does not move to the adjacent vessels even at a water potential of -3.9 MPa. The presence of air in the stem posed no immediate barrier to the subsequent uptake of water, whereas prolonged exposure to air did inhibit water uptake. This is further investigated in the following chapters. Chapter 6 gives an account of the susceptibility of a range of rose cultivars to dry storage, in relation to transpiration and water potential. Three hypotheses about the cause of the occlusion are investigated in Chapters 7-9, respectively.

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CHAPTER 6

INTERVARIETAL DIFFERENCES IN WATER UPTAKE AFTER EXPOSURE TO AIR OF CUT FLOWERING ROSE STEMS

Summary

A 3 h period of exposure to air (at 20°C and 60% RH) did not inhibit subsequent water uptake in cut flowering rose stems of such cultivars as Bettina, Capella, Frisco, Lavande, Privé, and Sonia. Flowering stems of these cultivars rapidly regained turgidity after placement in water. Other cultivars showed a lower rate of water uptake and more slowly regained turgidity (Golden Phantasy, Madelon, Royalty, Samantha, Prima Donna). A few cultivars had a still lower rate of uptake and remained wilted after placement in water (Cara mia, Chantilly Lace). Recutting the stems of Cara mia flowers under water resulted in water uptake as in Sonia, indicating that an occlusion had developed in the basal stem segment.

In representatives of the above three groups (Sonia, Madelon, Cara mia) the patterns of transpiration, stomatal closure and leaf water potential during 3 h exposure to air were the same. Ultrastructural studies on these three cultivars also showed no difference in the number and length of the conducting xylem elements or in the lumen diameters of these elements. No deposition of hydrophobic materials was observed in the xylem conduits.

After prolonged exposure to air (48 h at 20°C) the rate of water uptake of cut Sonia roses did not exceed the rate of transpiration, and these flowering stems did not regain turgidity, but Frisco roses recovered with a rate of water uptake which was much higher than the rate of transpiration. The latter cultivar lost less water during dry storage. Under some conditions the rate of stomatal closure was more rapid in Frisco roses than in Sonia roses. The cuticular transpiration was always lower in Frisco roses than in Sonia.

INTRODUCTION

In Chapter 5 it was reported that 3 h of exposure to air of cut Sonia rose stems resulted in aspiration of air at the cut surface but did not impair subsequent water uptake. After 24 or 36 h exposure to air, however, no more air was aspired but the rate of water uptake was progressively inhibited. The literature indicates differences among the various rose cultivars in the capacity for water uptake after exposure to air. In experiments where flowers were cut in the greenhouse on a warm day, held dry for some time, and then placed in water in the greenhouse, flowers of the cultivar Cara mia showed more bending of the stem than those of the cultivars Jacqueline, Town Crier and Forever Yours (Zieslin et al., 1978). Cara mia roses also did not regain turgidity when placed in water after holding overnight in air at 2°C (Durkin, 1979, 1980).

When intact plants become water-stressed a wide range of responses have been observed. Some species survive drought by properties such as deep rooting, water storage tissue, rapid stomatal closure, and low cuticular transpiration (Levitt, 1980). Several species of resurrection plants can be dried to extremely low water potentials but nevertheless reabsorb water as soon as it becomes available and then still show meristem activity (Milburn, 1979). Stocking (1948) observed considerable differences between cut shoots of various species which had been exposed to air. Some species readily absorbed water when the stems were placed in an aqueous solution, other species did not absorb water and did not regain turgidity. The reasons of these interspecific differences have not been elucidated.

The aim of the present investigation is to further study the differences between various rose cultivars for exposure of their stems to air, and to investigate possible causes of these differences.

MATERIALS AND METHODS

Plants

Flowering rose stems with a length of 50-55 cm were cut at commercial maturity, from the greenhouses at the Dept. of Environmental Horticulture, Davis, USA, and the Dept. of Horticulture, Wageningen Agricultural University, and brought to the laboratory within 10 min of cutting. Stems were then recut in air, to a length of 40 cm. The lowermost leaves were removed, leaving 6 leaves.

The flowering stems were held dry by placing them horizontally on a bench, about 5 cm apart, in a climatized room at 20°C, 60% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}.

Water uptake

After the period of dry storage, the cut surface of the stems was connected to a horizontal 10 ml pipette, which was filled with deionized water. Tygon tubing was used to connect the stem with the pipette. The occurrence of air bubbles in the tygon tubing was carefully avoided. The rate of water uptake was followed by measuring the movement of the air-water interface at the lower end of the pipette. In control flowers the lowermost 5 cm of stems was recut under water before attaching the cut surface to the pipette. Water uptake was studied in the climatized room, at the above conditions.

Transpiration

Flowering stems were exposed to air at the conditions specified above, and then individually placed in water at 20°C, 60% RH and a photon flux of 15 μmol m^{-2} s^{-1}. Transpiration was determined for the first 60 min by measuring the weight of the vial and flower. Control flowering stems were placed in water immediately after harvest. Measurements were made using 6 replications. Leaf area was measured with a Li-cor 3000 area meter (Li-cor, Lincoln, Nebraska).

Stomatal conductance

Conductance was measured abaxially in the terminal leaflet of the first leaf with seven leaflets from the flower head, using a Li-cor 1600 porometer (Li-cor, Lincoln, Nebraska), in 5-10 replications. Conductance was measured in the greenhouse, before cutting, and at intervals after cutting. Stems were brought into the climatized laboratory within minutes after cutting, or were wrapped in paper and transported by car. In the latter case, conductance was measured 2 h after cutting.

Water potential

The water potential was measured in the first seven-leaflet leaf from the flowering head, using a Scholander pressure chamber, in 6 replicate flowering stems.

Xylem anatomy

The number of water-conducting elements was counted in transverse stem sections taken 40 cm from the flower head, using light microscopy and scanning electron microscopy (SEM).

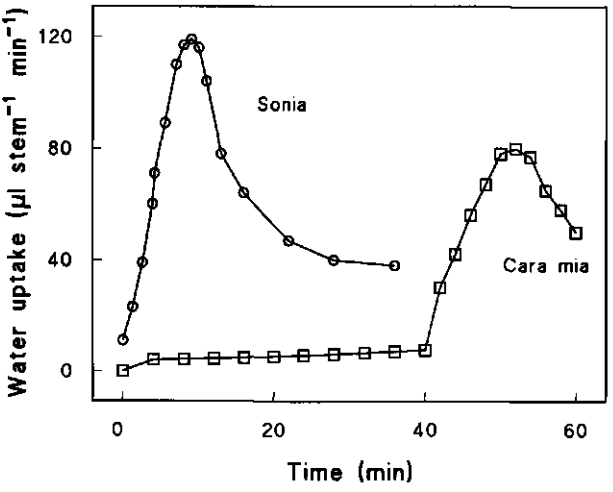

Fig. 1. Rate of water uptake by a single representative cut stem of Sonia (o) and Cara mia (□) roses held in air for 3 h after cutting, then placed in water. After 40 min of water uptake five cm of the basal stem part of the Cara mia rose was cut under water.

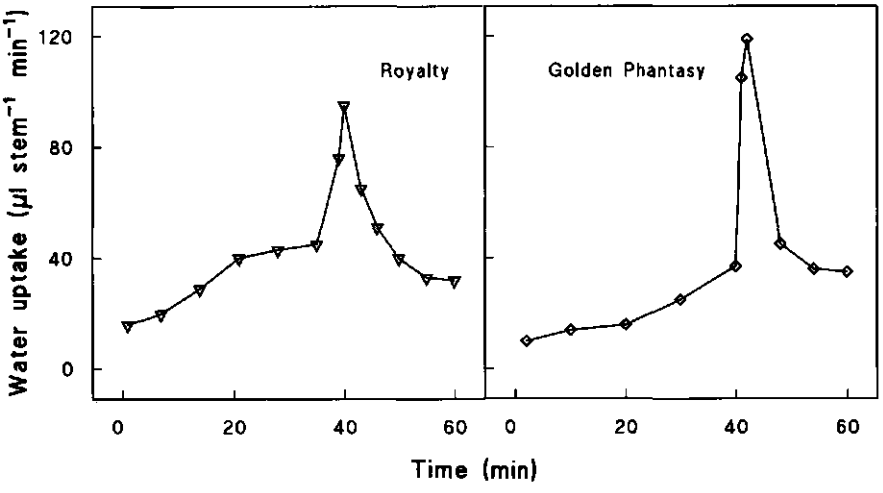

Fig. 2. Rates of water uptake occasionally observed in some individual rose stems after holding in air for 3 h after cutting. Data refer to Royalty and Golden Phantasy roses.

Three randomly selected SEM photographs of transverse sections from 5 stems each were used to determine xylem wall thickness. The methods used for SEM have been described in Chapter 2. The distribution of vessel length was studied by placing the stems in a suspension of india ink, latex paint or water colour, using the methods of Zimmermann and Jeje (1981) and Salleo et al. (1984). Freehand transverse sections of fresh stems and stems held dry for 3 h as described above were stained with Oil-red 0, Sudan III or Sudan IV (Fluka), and examined using light microscopy.

RESULTS

Water uptake

Cut flowering rose stems of most cultivars exposed to air for 3 h and then placed in water showed an increasing rate of water uptake followed by a gradual decrease, whereas some cultivars hardly showed any uptake of water (Fig. 1). Occasionally some flowers of various cultivars showed a period of relatively low rates of uptake followed by a short period of rapid uptake (Fig. 2).

The maximum rate of water uptake was dependent on the cultivar (Table 1). In several cultivars no appreciable differences were found between the dry-stored stems and controls recut under water. The rate of water uptake in these flowering stems was higher than 40 mg H_2O $stem^{-1}$ min^{-1}, generally leading to full turgidity within 20 minutes (Group 1). In other cultivars the rate of water uptake was initially lower than in controls. These flowering stems had a rate of water uptake varying from 20 to 40 mg H_2O $stem^{-1}$ min^{-1}, and slowly regained full turgidity (Group 2). In Cara mia and Chantilly Lace the rate of uptake was lower than 20 mg H_2O $stem^{-1}$ min^{-1} and remained below the rate of transpiration. These stems severely wilted after placing them in water (Group 3). Recutting the stem of flowering Cara mia roses under water, after 3 h of dry storage, restored water uptake (Fig. 1) and turgidity.

Comparison between Frisco, Sonia, Madelon, and Cara mia roses

At the same photon flux density, stomatal opening in leaves of intact Sonia and Cara mia roses, measured as conductance, was similar (Table 2). During exposure of the cut flowering stems to air, the changes in stomatal opening (Table 2), rate of transpiration, total fresh weight and water potential (Fig. 3) were also similar in both cultivars. The experiments were repeated comparing Sonia and Madelon roses. The rate of stomatal closure (Table 2), the rate of transpiration, the fresh weight and the water potential of Madelon roses was as in Sonia roses (results not shown).

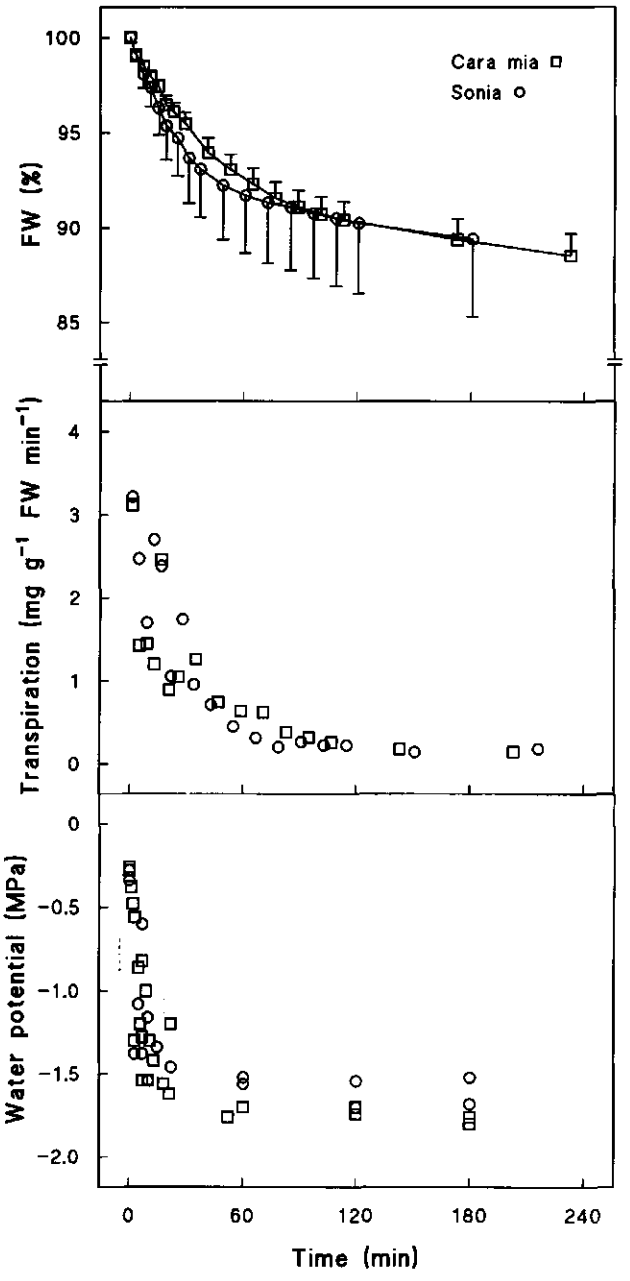

Fig. 3.

Effects of exposure to air of Sonia and Cara mia roses, on fresh weight of the flowering stems (upper figure), transpiration rate (middle figure), and water potential in the first seven-leaflet leaf (lower figure), during exposure to air.

Table 1. Maximum rates of water uptake in cut flowering rose stems, after 3 h of exposure to air at 20°C, 60% RH, and a photosynthetically active photon flux of 15 μmol m^{-2} s^{-1}. The rate of water uptake was measured during the first hour. Stems were not recut. Data represent the range found in 6 replications.

	Cultivar (trade name)	Maximum rate of uptake (mg $stem^{-1}$ min^{-1})
Group 1	Lavande	170 - 280
	Bettina	120 - 190
	Frisco	110 - 180
	Sonia	90 - 180
	Privé	80 - 175
	Ilona	70 - 130
	Motrea	70 - 120
	Jack Frost	70 - 110
	Capella	50 - 70
	Mercedes	40 - 50
Group 2	Madelon	25 - 40
	Golden Phantasy	20 - 40
	Royalty	20 - 40
	Samantha	20 - 40
	Prima Donna	20 - 30
Group 3	Cara mia	15 - 20
	Chantilly Lace	10 - 20

The number of xylem vessels, the distribution of their length, their wall thickness and lumen diameter were all similar in Sonia, Madelon and Cara mia roses. Staining with Oil-red 0, Sudan-III or Sudan-IV did not show deposition of hydrophobic substances in the conducting elements or at the pit membranes (Table 3).

Cut flowering stems of Sonia and Frisco with the same leaf area, exposed to air for 48 h, showed the same average rate of transpiration during the first day the flowering stems were placed in water

Table 2. Stomatal conductance of the terminal leaflet of the first seven-leaflet of cut flowering stems of Cara mia, Madelon, Sonia and Frisco roses, before and after cutting. After cutting the flowering stems were held in air at 20°C, 60% RH, and a photon flux of 15 μmol m^{-2} s^{-1}, for 2 h. Data are the means of 10-14 replications, and represent repeat experiments.

Stomatal conductance (cm s^{-1})			
Cara mia		Madelon	
Intact	2 h dry	Intact	2 h dry
196	173 (88%)	163	161 (99%)
205	188 (92%)	185	152 (82%)
218	167 (77%)	190	163 (86%)
Sonia		Frisco	
Intact	2 h dry	Intact	2 h dry
167	142 (85%)	158	55 (35%)
172	86 (50%)	192	61 (32%)
180	164 (91%)	184	85 (46%)
220	225 (102%)		

(about 100 mg H_2O $stem^{-1}$ h^{-1}). The rate of water uptake, however, was lower in Sonia (about 100 mg H_2O $stem^{-1}$ h^{-1}) than in Frisco (250 mg H_2O $stem^{-1}$ h^{-1}). Sonia roses showed no increase in fresh weight (Fig. 4) and remained wilted, whereas the fresh weight of Frisco increased and the flowers regained turgidity. Stomatal opening measured before cutting was similar in both cultivars. When stems were cut stomatal closure occurred within 2 h in Frisco roses, and was variable in Sonia roses: in three out of four repeat experiments the stomatal conductance in leaves of Sonia roses obtained from different greenhouses remained higher during the first two h after cutting (Table 2). Sonia roses obtained from the greenhouse in which their stomatal conductance was the same as in Frisco were held dry for 36 and 48 h. The number of stems with visible symptoms of low turgidity was much higher in Sonia (Table 4).

Table 3. The number of conduits with a diameter >20 µm, in stems of cut Cara mia, Madelon, and Sonia roses, determined at 20 cm from the flower head; the distribution of the length of the conduits, as indicated by the penetration of india ink to 2, 10 and 20 cm from the cut surface, the wall thickness (measured from lumen to lumen) of the conduits with a diameter >20 µm, the lumen diameter of these conduits, and presence of hydrophobic substances in their lumina after staining with Oil-red 0, Sudan-III or Sudan-IV. Measurements were made in summer. Data are the means of 5 stems, ± SD.

	Cara mia	Madelon	Sonia
Number of conduits	1615 ± 211	1403 ± 185	1561 ± 133
Number of filled conduits at			
2 cm	103 ± 25	117 ± 34	101 ± 37
10 cm	13 ± 4	15 ± 6	11 ± 5
20 cm	4 ± 2	3 ± 2	5 ± 3
Wall thickness (µm)	2.4 ± 1.9	2.2 ± 2.0	2.7 ± 1.8
Lumen diameter (µm)	41 ± 34	46 ± 39	45 ± 37
Staining	none	none	none

The amount of water loss from the leaves during dry storage of Frisco and Sonia roses is shown in Fig. 4. Apart from a lower loss during 0-24 h in Frisco, which may partially be attributed to more rapid stomatal closure, the water loss was also considerably lower during the 24-36 h period.

DISCUSSION

Based on their recovery after dry storage, we classified the tested rose cultivars into three categories. The first comprised the flowers that showed rapid recovery of turgor after 3 h of dry storage at 20°C; in the second category this recovery occurred slowly and in the third group it was absent. Within the first category, however, clear differences were found between Sonia and Frisco roses, which came to the fore only after prolonged dry storage (48 h at 20°C). Frisco roses then still showed a high

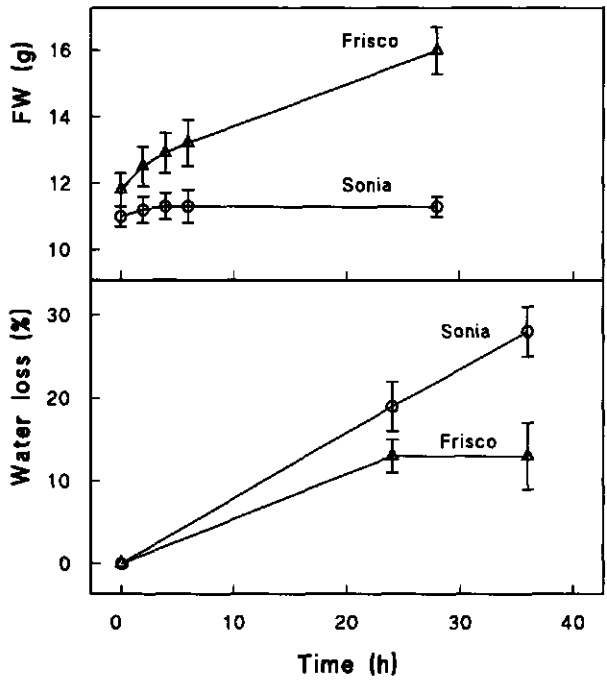

Fig. 4. Effects of exposure to air on Frisco and Sonia roses. Upper figure: Water loss of the leaves during 36 h of dry storage. Lower figure: Fresh weight of the flowering stems during the first 28 h in water, after 48 h of dry storage.

rate of water uptake, whereas the rate of water uptake of Sonia roses had much decreased with respect to controls not exposed to air. Frisco roses may be among the cultivars that are most resistant to dry storage.

The difference between Sonia and Frisco roses was probably partially related to the rate of stomatal closure after cutting. Stomatal behaviour, however, does not fully explain the difference between these two cultivars. The stomates of Frisco and Sonia roses in stems from one greenhouse showed the same rate of closure, but rehydration after prolonged dry storage was still better in Frisco than in Sonia (Fig. 4). Leaf water loss during the period of 24-36 h of dry storage was much lower in Frisco than in Sonia (Fig. 4). This indicates that cuticular transpiration is lower in the former cultivar. The rate of cuticular transpiration, therefore, is an important determinant in the difference between these two cultivars.

Table 4. Percentage bent stems 24 h after placement in water, following exposure to air of Sonia and Frisco roses, for various periods of time, in stems showing a similar rate of stomatal closure during the first 2 h of dry storage

Exposure to air (h)	Percentage bent stems	
	Sonia	Frisco
0	0	0
24	0	0
36	100	0
48	100	40

However, the rate of stomatal closure during dry storage, or the rate of cuticular transpiration, did not explain the differences between cultivars such as Sonia, Madelon, and Cara mia. Following a 3 hperiod of exposure to air at 20°C Sonia stems showed rapid water uptake and regaining of turgidity. The high rate of water uptake was apparently due to the low water potential (Fig. 3) because it initially largely surpassed the rate of transpiration and then decreased until it reached the transpiration rate (Fig. 1). In cut wheat leaves that were exposed to air Barrs and Weatherley (1962) also found a high rate of water uptake associated with the elimination of the water deficit.

Cut flowering stems of cultivars such as Madelon and Cara mia did not show this rapid water uptake after 3 h of exposure to air, and those of Cara mia even remained wilted. Upon recutting the stems under water the rate of water uptake was restored to rates similar to those of cut flowering Sonia stems (Fig. 1), indicating that a resistance to flow of water had developed in the basal part of the stem. This resistance apparently develops in all cultivars investigated, but with a different time course.

No apparent differences were found in the number of the xylem elements, nor in the thickness of the walls of these elements. Since no deposition of material in the lumen of the xylem conduits was found, this too could not account for the differences between the cultivars.

Previous experiments showed that during exposure to air (of stems that had been held in water) the number of bacteria in the basal 5 cm of Sonia stems increased, even with the same rate as in stems placed in water (Van Doorn and De Witte, 1991); this was correlated with a decrease in water uptake (unpublished results). In the present experiments the development of micro-organisms during exposure to air was avoided as the stems were not placed in water after cutting. Routine checks, using the methods described in Chapter 2, showed no bacteria in the stems after exposure to air. The results

show, therefore, an occlusion which is independent of the presence of bacteria on the cut surface or in the stem interior.

Our results confirm the findings of Zieslin et al. (1978) who compared the vase life of Cara mia with some other rose cultivars, including Samantha, after a period of dry storage. The latter cultivar tended to show a lower incidence of stem bending indicating its lower sensitivity to air exposure.

The Chapters 7-9 describe experiments in which three hypotheses about the cause of the occlusion developing during dry storage were tested. The differences in sensitivity between the cultivars proved an important tool for discrimination between the possible causes of this occlusion.

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CHAPTER 7

ROLE OF THE XYLEM WALL PATHWAY FOR WATER IN THE VASCULAR OCCLUSION DEVELOPING IN CUT FLOWERING ROSE STEMS UPON EXPOSURE TO AIR

Summary

Upon exposure to air at 20°C and 60% RH an occlusion to water uptake developed in the basal stem segment of cut Sonia roses. The development of the occlusion was delayed by removing the leaves before storage, whereas placing the stem ends at 100% RH during dry storage had no effect. Scanning electron microscopy did not show plant gums or tyloses in the xylem vessels of the lowermost 10 cm stem segment.

The hypothesis by Scholander et al. (1955, 1957) that water uptake into stems held in air and then placed in water occurs through conduit walls rather than the lumen was tested using stems in which the lumen was experimentally plugged and the walls left open to water flow, or using stems of which the cut surface was covered with laboratory grease and part of the bark was removed (girdled stems). Experimentally plugged conduits and open walls resulted in low water uptake, which is inconsistent with the hypothesis. Girdled stems placed in water remained fully turgid when the girdled area was about 60 mm^2 or more. Water uptake was strongly inhibited in girdled stems exposed to air for a prolonged period. Inclusion of a surfactant (nonylphenoxypolyethoxy ethanol) in the vase water facilitated water uptake after dry storage of normal cut flowering stems but did not improve water uptake into the girdled stem system.

It is concluded that the vascular occlusion developing upon exposure to air cannot be explained by decreased flow in the wall pathway for water.

INTRODUCTION

Scholander et al. (1957) measured uptake of water into stems of a liana (*Tetracera* sp.) after leaving the cut stem in air for 15 min. Although 80 ml of air was aspired by the stems during exposure of the cut end to air, the subsequent rate of water uptake was the same as before the stems were cut. They concluded that the xylem lumen had become embolized during exposure to air and that water entered the stem through tracheids and vessel walls. In experiments with a grapevine species (*Vitis labrusca* L.), however, Scholander et al. (1955) found that water uptake was not inhibited by exposing the cut end of the stem to gas for 5 min, whereas exposure for 10 or 30 min did inhibit water absorption. In this species the occlusion was possibly due to exudation of a gummy substance at the cut surface (Scholander et al., 1955; Scholander, 1958).

In cut Sonia rose stems we found no blockage of water uptake after 3 h exposure to air at 20°C and 60% RH but noted a progressive occlusion after 24 or 36 h of exposure. The occlusion was apparently not solely due to the presence of air in the lumen of the xylem conduits as air uptake ceased within 20 min and the occlusion was not present within 3 h of exposure to air (Chapter 5). In Sonia roses we noted no exudation of substances at the cut surface. According to the literature, however, the lumen of the xylem conduits in plant stems may become occluded with gums or tyloses after the vessel lumen has become devoid of water (Klein, 1923; Zimmerman and McDonough, 1978; Zimmermann, 1983).

The present paper examines the location of the occlusion, the ultrastructure of the occluded xylem tissue, and the possibility that the blockage relates to the xylem-wall pathway for water.

MATERIALS AND METHODS

Plant material

Flowering Sonia rose stems of 65 cm length were obtained from the Department of Horticulture of the Wageningen Agricultural University or from commercial growers.

Exposure to air

The flowering stems were placed in water immediately after cutting and brought to the laboratory within 4 h. In the laboratory 30 cm was recut under water and the stems were placed in appropriate

solutions or suspensions or held dry by placing them horizontally on a bench in a climatized room at 20°C, 60% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}, from fluorescent tubes, from 7 a.m. to 7 p.m.

In one series of experiments the stem ends were placed in translucent pvc tubes, through a hole in the rubber lid of the tubes. The lids closely adhered to the bark. A wet cotton plug was placed inside the tube. Condensation drops indicated the atmosphere to be saturated with water vapour. Controls were placed in air and in tubes in which the cotton plug was not wetted.

During exposure to air, all leaves were either left attached to the flowering stem or removed at the onset of treatment. After exposure to air the stems were placed individually in vases containing deionized water, with or without recutting the stem base in air or under water, and were placed in the climate-controlled room under the ambient conditions mentioned above.

Stem girdling

In some experiments the cut surface was covered with a thick layer of laboratory grease (Dow Corning, Midland, MI). In these stems the lowermost leaf was removed and the resulting leaf scar also covered with grease. These flowering stems served as controls for treatments in which the lowermost parts of the bark were carefully girdled. Girdling was done by hand in order to avoid cutting of the underlying cell walls. In the girdled flowering stems the cut surface, the margin of the bark, and the leaf scars in the girdled segment were covered with a layer of laboratory grease (Fig. 1).

Filling the lumina of xylem elements with paints

Freshly cut flowering stems were placed in aqueous suspensions of 1 mg l^{-1} acrylic latex paint (Ace Hardware Corp., Oak Brook, IL), 4 mg l^{-1} india ink (Rotring Werke, Hamburg, Germany) or 4.5 mg l^{-1} water colour no. 331 (Talens, Apeldoorn, NL) for 1 or 5 h. Any residue attached to the cut surface of these stems placed in the suspensions was removed by gently scraping the cut end with a razor blade after which the stems were placed in water.

Transport of dye solutions and paint suspensions

Flowering rose stems were held dry for 24 h or longer and then placed, for various periods of time, in solutions containing one of the following dyes: 10 mg l^{-1} fast green FCF (Allied Chemical and Dye Corporation, New York, NY), 10 mg l^{-1} Evans' blue (Eastman Organic Chemicals, Rochester, NY), or 1 mg l^{-1} acid fuchsin (Coleman & Bell Co, Norwood, Ox. UK). Alternatively, stems were placed

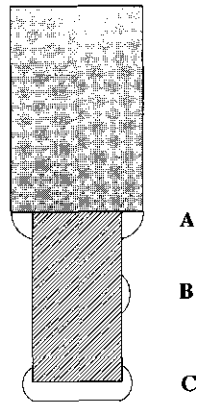

Fig. 1. The girdled stem system. Laboratory grease was placed on the margin of the cut bark (A), the leaf scars in the girdled area (B) and on the cut surface (C).

in the abovementioned aqueous paint suspensions. Freehand horizontal cross sections of the stems were made at intervals from the basal end and examined microscopically.

Water uptake

The rate of water uptake was determined by attaching the stems to a pipette containing distilled water. Pipettes and stems were placed horizontally on the laboratory bench. Other stems were placed in vials containing water or an aqueous solution of 0.25 mg l^{-1} of the surfactant nonylphenoxy-polyethoxy ethanol, with an average number of ethoxy units of 8.5 (Agral, from ICI, Rotterdam). Experiments occurred at 20°C, 60% RH and a photon flux density (PAR) of 15 µmol m^{-2} s^{-1}. Ten replications were used per treatment.

Scanning electron microscopy

After 0, 3 or 36 h of exposure to air the stem adjacent to the cut end was sectioned at 1 cm intervals. The 0-1, 2-3 or 4-5 cm portions were retained for electron microscopical examination. The techniques used in the electron microscope study are described in Chapter 3.

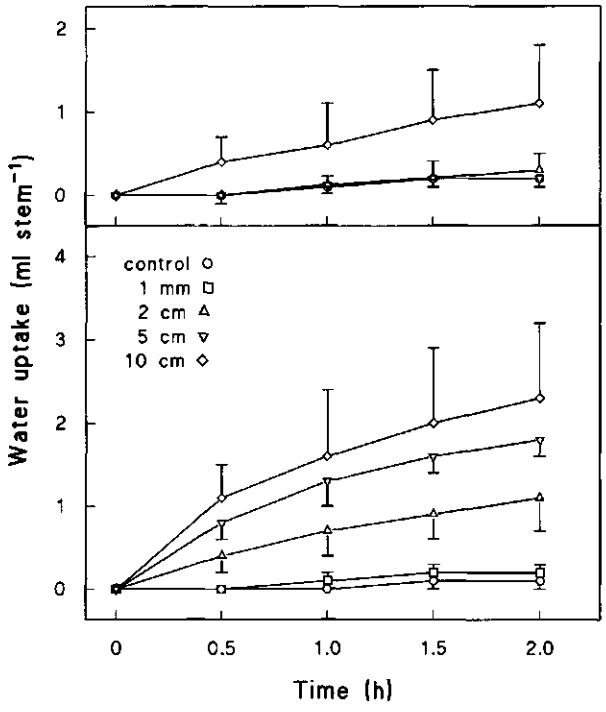

Fig. 2. Accumulated water uptake into Sonia rose stems after 36 h of exposure to air, following recutting segments of various lengths from the stem base in air (upper figure) or under water (lower figure). Data are means of 10 replications, ± SD.

Antimicrobial compounds and assessment of bacterial numbers in stems

The number of bacteria in stems was held low by including antimicrobial compounds such as 400 mg l^{-1} hydroxyquinoline citrate (La Quinoléine, Oissel, France) or 200 mg l^{-1} dichloroisocyanuric acid (Merck) in the water. The number of bacteria in the stems was determined as described in Chapter 2.

Statistical treatment

All experiments involved 8-10 flowering stems per treatment and were repeated at least once. Statistical analysis (analysis of variance, t-test) was carried out with the GENSTAT V Statistical Package.

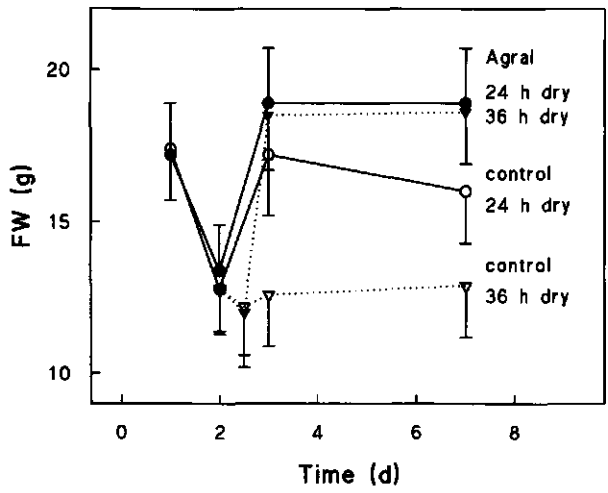

Fig. 3. The fresh weight of Sonia rose stems exposed to air for 24 h (solid lines) or 36 h (dotted lines), and placed in water (o, v) or in a solution of nonyl phenoxypolyethoxy ethanol (Agral, ●, ▾). Data are means of ten replications, with SD.

RESULTS

Bacterial counts

Stems that were not held dry (water controls) contained less than 1000 cfu per gram fresh weight in the basal 5 cm of the stems when an antimicrobial compound was included in the vase water. The number of bacteria in stems exposed to air was below the detection limit.

Water relations of ungirdled stems

After 36 h of exposure to air the rate of water uptake into cut flowering rose stems was very low. Recutting the basal 2 or 5 cm in air did not affect the blockage to water uptake, but recutting the basal 10 cm in air partially alleviated the blockage (Fig. 2).

Recutting 0.1 cm from the basal end of the stems under water had no effect on the rate of water uptake. Recutting 2 cm restored uptake rate to half the control value, and recutting 5 cm or more under water restored water uptake to control levels (Fig. 2).

Table 1. Rate of water uptake after 36 h of exposure to air of cut flowering Sonia roses. Results are means of ten flowering stems, ± S.D., and reflect uptake during the first 3 h.

	Rate of uptake after exposure to air ($\mu l\ stem^{-1}\ min^{-1}$)
Control (exposed to air at 20 °C and 60% r.h.)	8.5 ± 6.3
High humidity (exposed to air with 100% humidity at the stem cut surface)	7.9 ± 5.8
Leaves removed (exposed to air after removal of the leaves)	36.4 ± 11.7
0.25 mg l^{-1} nonylphenoxypolyethoxy-ethanol (exposed to air as controls, then placed in water containing the surfactant)	86.3 ± 14.1

Removal of all leaves prior to 36 h exposure of the flowering rose stems to air and subsequent placing the stems in water resulted in a higher rate of water uptake than in foliated controls (Table 1) and in restoration of full turgidity of the flower, wheras the control flowering stems remained bent. Holding flowering stems in air for 36 h with the cut surface in an atmosphere saturated with water vapour had no effect on the occlusion or on wilting (Table 1).

When stems were held dry for 36 h and placed in an aqueous solution of nonylphenoxypolyethoxy ethanol (0.25 mg l^{-1}) the rate of water uptake was much higher than in controls (Table 1). The fresh weight of surfactant-treated stems readily recovered when placed in water after 24 or 36 h of dry storage, whereas the control stems only partially recovered after 24 h and failed to do so after 36 h of dry storage (Fig. 3).

Water relations of girdled stems

Freshly-cut flowering rose stems of which the cut surface was covered with laboratory grease, the basal leaves stripped and the leaf scars also covered with laboratory grease, lost turgidity within 3 h

Table 2. Effects of girdling on water relations of cut Sonia roses of which the cut surface and the leaf scars were covered with laboratory grease. Ten stems per treatment. The number of turgid flowering stems was determined 48 h after placing the stems in the solutions. Solute uptake refers to the first 3 h after placing the stems in the solutions. Solute uptake data: means ± SD.

	Solute uptake ($\mu l\ stem^{-1}\ min^{-1}$)	Turgid flowering stems (%)
No girdle	1.6 ± 2.3	0
Girdle 0.5 cm long	29.4 ± 8.0	40
Girdle 1.0 cm long	53.7± 6.9	100
Girdle 2.0 cm long	65.9 ± 7.3	100
Girdle 4.0 cm long		
Girdle 4.0 cm long, in air for 36 h, then in water	7.8 ± 5.6	0
Girdle 4.0 cm long, in air for 36 h, in water containing 0.25 mg mg l^{-1} nonyl-phenoxypolyethoxyethanol	6.3 ± 4.8	0

after placing them in water. When similar stems were girdled at the stem base the stems stayed fully turgid for more than 7 days. The minimum length of the girdle to prevent visible symptoms of water stress was about 1 cm, i.e. an area of 60 mm^2 (Table 2).

When the girdled stems were held dry for 36 h, and again placed in water, none of the stems regained turgor. Turgor was not restored either when the girdled stems were placed in water containing 0.25 mg l^{-1} nonylphenoxypolyethoxy ethanol (Table 2).

Histological and anatomical observations

Scanning electron micrographs of the basal 0-1 cm, the 2-3 cm segment and the 4-5 cm segment of the stem after 0, 3, 24 and 36 h of exposing the cut flowering stems to air, showed no gum deposits

Table 3. Effect of experimentally plugging the lumen of the water-conducting elements of Sonia roses, on the percentage of bent stems and the percentage turgid flowers two days after the stems were placed in water. Stems were not held dry between plugging and placement in water.

Plugging material	Bent pedicels (% of total)	Turgid stems (% of total)
India ink (4 mg l^{-1})	90	0
Acrylic latex paint (1 mg l^{-1})	100	0
Water colour (4.5 mg l^{-1})	30	0

and no tyloses in the xylem vessels. Stems held in air had the same ultrastructure as control stems not exposed to air.

When the flowering stems were not exposed to air and placed in a dye solution the dye readily penetrated all walls of the xylem elements, up to the flower head. Dyes were not found to penetrate the walls of cells outside the xylem. When placed in an aqueous suspension of paint (an emulsion unable to penetrate the cell walls or the vessel-to-vessel pit membranes) the paint was only found in the lumen of all xylem elements opened by cutting.

When the flowering rose stems were held dry for 24 h and placed in vase water either containing a dye or a paint both classes of substances were most abundantly present in the innermost xylem elements, close to the pith, and were virtually absent from the elements close to the cambium. Upon longer exposure to air the distribution gradually became more confined to the innermost elements.

Water relations of stems in which the xylem lumen was experimentally plugged

The lumen of the xylem conduits can be experimentally occluded by suspensions with particles larger than the size of the pores in the vessel-to-vessel pit membranes. Flowering rose stems were held in three such suspensions (latex paint, india ink, and water colour) until they showed bending of the stem (1-5 h). The material at the cut surface was then carefully scraped off with a razor blade. After treatment with latex paint or india ink the flowering stems, placed in water, did not regain turgor. After the treatment with water colour most stems slowly regained turgor, but none of the stems became fully turgid (Table 3).

DISCUSSION

Scanning electron microscopy and light microscopy showed no evidence of tyloses, gums or any other deposited material in the xylem conduits, even after 36 h exposure to air, indicating that the occlusion that developed during this period was not due to deposition of material in the lumina. Bacterial counts showed that bacteria in the xylem conduits can also be excluded.

Removal of the basal 1 mm under water did not remove the occlusion, indicating that it was not due to a process at the cut surface of the stem. Removal of the basal 5 cm under water, however, completely overcame the blockage. The location of the occlusion is correlated with the presence of water-conducting elements filled with air. In Chapter 5 it was concluded that air was taken up only by the xylem conduits opened by cutting, not by adjacent conduits.

Recutting the stems in air was also effective in overcoming the occlusion, but a larger part had to be removed in air to obtain the same effect as recutting under water. The reason for this is not known. One possibility is that effective recutting in air requires the removal of more cavitated xylem elements than recutting under water.

Our dye experiments indicate that water is able to flow in the walls of the water-conducting elements, before exposure of the stem to air. Läuchli (1976) discussed water flow through cell walls in relation to the arrangement of the cellulose molecules, which form a chain lattice (micelles). The diameter of the capillaries between micelles is about 1 nm, greater than the diameter of water molecules (0.4 nm). Micelles are arranged in microfibrils, normally with about six micelles per microfibril. The space between microfibrils is about 10 nm. Water, therefore, may flow in intermicrofibrillar spaces. Whether it readily flows in intermicellar spaces is less clear. On the one hand, capillary forces may enhance water flow. On the other hand, intermicellar spaces are lined by pectins and glycoproteins and water flow may be impeded by these molecules as they contain numerous negative charges.

Whether, and how much, water is able to flow through the xylem walls would depend on the connections of the micro-capillaries. Electron microscopical methods have indicated water flow in cell walls, using materials such as Ag^{+} and La^{3+}, which are able to penetrate voids as small as 2 nm (Rudman, 1966; Bannister, 1971; Peterson et al., 1986; Thomson et al., 1973). The distribution of lanthanum showed access of water in the primary and secondary walls of xylem conduits (Rudman, 1966; Wisniewski et al., 1987).

Exposure of more than about 60 mm^2 of xylem wall surface by girdling was adequate to bypass the vascular blockage at the basal part of the stem. Using this system we found that water was able to enter the stem through the walls. This experiment, therefore, apparently supports the hypothesis of Scholander et al. (1955) that after exposure to air, water is able to enter the xylem through the cell walls.

The occlusion found in stems after exposure to air could possibly be related to dehydration of the conduit walls. Upon placing the stems in water, the water molecules would, in this hypothesis, bypass the emboli by flowing through the walls unless this flow would be stopped by dehydration of the walls. The withdrawal of free water by transpiration reduces the water potential of the stem cells which may result in withdrawal of water from the walls. Removal of the leaves delayed the development of the occlusion, consistent with the idea that the reduction of the water potential by transpiration is, indeed, a main cause. The effects observed in girdled stems that were held dry are also consistent with this hypothesis. As water uptake was not affected by keeping the stem end in air saturated with water vapour, evaporation of free water from the walls into the xylem lumen and then to the atmosphere is apparently not the primary cause of the occlusion.

The experiments, however, do not all support the hypothesis that the occlusion is principally due to a restriction in the xylem wall pathway for water. Firstly, the girdled area necessary for maintenance of full turgidity was relatively large compared to the xylem wall area exposed at the cut surface of non-girdled stems, calculated to be about 4 mm^2. This calculation did not include, however, the inner wall area possibly in contact with water. Secondly, filling the lumen of all cut xylem conduits with paint and placing the walls at the cut surface in water did not result in restoration of full turgidity, which indicates that the mere exposure of the xylem walls at the cut surface may not be adequate for maintenance of turgidity in the flowering rose stems. Finally, in cut flowering stems held dry for 36 h, water uptake was greatly facilitated by placing the stems in an aqueous solution containing a wetting agent (0.25 mg l^{-1} nonyl- phenoxypolyethoxy ethanol), and similar treatment with Tween-20 improved water uptake in cut chrysanthemum flowers exposed to air (Durkin, 1980). We found, however, that the surfactant did not facilitate water uptake into the girdled stem system (Table 2). Especially this finding falsifies our hypothesis that the vascular occlusion in cut flowering rose stems is mainly based on occlusion of the xylem wall pathway for water.

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CHAPTER 8

RELATIONSHIP BETWEEN VASCULAR OCCLUSION OF CUT FLOWERING ROSE STEMS EXPOSED TO AIR AND PENETRATION OF WATER INTO XYLEM CONDUITS OPENED BY CUTTING

Summary

Cut Sonia rose stems held in air in the light at 20°C and 60% RH for 24 h or more showed a resistance to water uptake in the stem base. Placing stems (that had not previously been exposed to air) in an aqueous suspension of India ink indicated the length of the water-conducting elements opened by cutting. Penetration of india ink at 2 cm from the cut surface was found in about 100 elements and maximum penetration depth was about 25 cm. Following short exposure to air (5-180 min) penetration occurred into 20-40 elements at 2 cm from the cut surface, and maximum penetration depth was about 10 cm. When exposed to air for 24 h or more penetration depth of india ink was about 5 cm. Placing the stems in a detergent solution (0.25 mg l^{-1} nonylphenoxypolyethoxy ethanol) after 24 h of exposure to air fully reversed the effect of exposure to air on the number of filled vessels at 2 cm and partially reversed the effect of exposure to air on the maximum penetration depth. It is concluded that in this rose cultivar the vascular occlusion due to exposure to air may, at least partially, be explained by the absence of penetration of water into the lumina of the conducting elements opened by cutting. In Cara mia roses, however, the occlusion developed more rapidly, but the inability of water to enter the vessels opened by cutting occurred at about the same time as in Sonia. Occlusion in this cultivar, therefore, cannot be due to the inability of water to enter conduits opened by cutting.

INTRODUCTION

When cut flowering rose stems are held dry an occlusion develops in the basal 5 cm of the stems. The blockage is not solely due to the presence of air in the conduits opened by cutting, as it developed after more than 3 h of exposure to air whereas aspiration of air ceased within 10-20 min of exposure to air (Chapter 5).

Using girdled stems of which the cut surface and the leaf scars were covered with laboratory grease, it was found that lateral water transport through more than about 0.6 cm^2 of exposed xylem wall was adequate to maintain turgor in cut flowering rose stems. When such girdled stems were held in air the occlusion occurred after about the same time as in ungirdled stems (of which the cut surface was not covered with laboratory grease). Placing stems in a solution containing a detergent is known to facilitate water uptake after prolonged exposure to air. When applied to the girdled stem system, however, this treatment did not increase water uptake, which was inconsistent with the hypothesis that water uptake after exposure to air occurs through the xylem walls (Chapter 7).

Drying of cell walls might result in decreased wettability of the walls, thereby preventing the entry of water into the conducting elements. As Siau (1983) pointed out, the rise of a water column in a capillary depends on the wetting angle, a measure of wettability of the capillary inner surface. When the stems are placed in water after exposure to air the water might, therefore, not be able to penetrate the lumen of the conducting elements.

The present investigation addresses the question to which extent water is able to penetrate the lumen of the conducting elements opened by cutting, after exposure of the stems to air, and whether vascular occlusion can be explained by the penetration of water into the lumina.

MATERIALS AND METHODS

Plant material

Cut flowering Sonia rose stems were obtained from a commercial grower or from the greenhouse of the Department of Horticulture, Wageningen Agricultural University. Stems of Cara mia roses were also obtained from the latter. When obtained from commercial growers stems were 70 cm long and had been placed in water immediately after cutting. Stems were kept at 5°C for some hours and following transport to the laboratory they were stored in water at 5°C until required. Before use 30 cm was recut from the stem base, under water. The water contained an adequate antibacterial compound (generally 8-hydroxyquinoline citrate, La Quinoléine, Oissel, France). The stems obtained from the Dept. of Horticulture were cut under water, held in water and transferred to the laboratory within 20 min after cutting.

Treatments with suspensions

Stems of 40 cm length cut under water from intact plants or stems of 70 cm length from a commercial grower from which 30 cm were cut off under water were placed in aqueous suspensions of 4 mg l^{-1} India ink (Rotring, Hamburg, Germany), 4.5 mg l^{-1} water colour 331 (Talens, Apeldoorn, NL) or 1 mg l^{-1} latex paint (Ace Hardware Corp, Oak Brook, IL.) for 1 or 5 h in a climatized room at 20°C, 60% RH and a photon flux (PAR) of 15 µmol m^{-2} s^{-1}.

Other stems were held dry by placing them horizontally on the table in the climatized room (20°C, 60% RH in the light) for various periods of time, following which they were placed in one of the above suspensions.

The methods of using India ink, water colour and latex paint have been previously described by Salleo et al. (1984), De Stigter and Broekhuysen (1986) and Zimmermann and Jeje (1981), respectively.

Observation of conducting elements filled with macromolecular matter

Thin freehand serial transections were made at 1 cm intervals along the stems up to 25 cm from the cut surface. The total number of conducting elements filled with macromolecular matter was counted using light microscopy.

Treatments with surfactant

After exposure to air stems were placed in deionized water or in an aqueous solution of 0.25 mg l^{-1} nonylphenoxypolyethoxy ethanol, with an average number of ethoxy groups of 8.5, using a commercial preparation (Agral, from ICI, Rotterdam, NL), or in a suspension of 4 mg l^{-1} India ink and the above concentration of the surfactant.

All treatments included ten replications, and all experiments were repeated at least twice. Results were compared by analysis of variance, using the GENSTAT V program (Rothamsted Experimental Station, UK) and t-test at $P>0.05$.

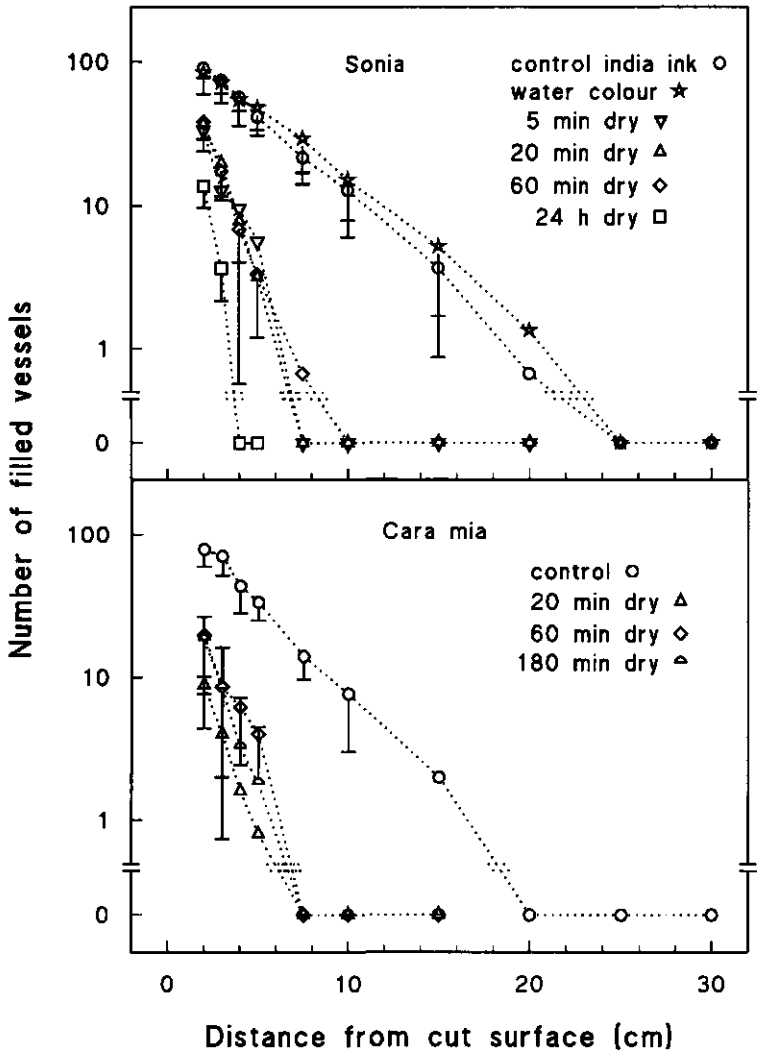

Fig. 1. Effect of dry storage on the number of water-conducting elements filled with macromolecular matter, after standing of cut Sonia and Cara mia rose stems in their aqueous suspensions for 1 h. Sonia stems were freshly cut, recut under water and placed in 4.5 mg l^{-1} of water colour 331 (five pointed star) or freshly cut, recut under water and placed in 4 mg l^{-1} India ink (o). Other stems were exposed to air at 20°C for various periods as indicated, then placed in India ink. Cara mia stems were freshly cut, recut under water and placed in India ink (o), or held dry for various periods as indicated, and placed in the India ink suspension. Data are the means of 10 replications, ± SD.

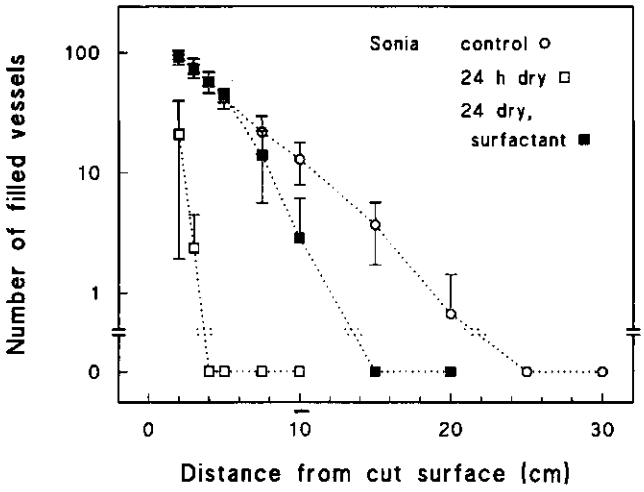

Fig. 2. Effect of a surfactant on the number of water-conducting elements filled with India ink after 24 h exposure to air of cut Sonia rose stems. Controls were freshly cut, not held dry, recut under water and placed in an aqueous suspension of 4 mg l^{-1} India ink for 1 h (○). Other stems were held dry, were not recut and placed in the India ink suspension for 1 h (□) or in a suspension of India ink also containing 0.25 mg l^{-1} nonylphenyxypolyethoxy ethanol for 1 h (■). Data are the means of 10 replications, ± SD.

RESULTS

Distribution of macromolecular matter in the xylem

When Sonia or Cara mia roses were cut under water and placed in a suspension of India ink for 1 h the xylem conduits at the cut surface (about 1500) were filled with ink. At 2 cm from the cut surface only about 100 water-conducting elements were filled with ink (Fig. 1). The number of filled elements declined exponentially until at 25-30 cm from the cut surface no vessels were filled with ink. The same distribution was found when using water colour (Fig. 1) or latex paint (results not shown).

Effect of exposure to air

When the cut flowering stems were held in air and then in a suspension of India ink for 1 h, the number of filled vessels at any transverse section was lower than in control stems. When Sonia roses were held in air no difference was found between stems held in air for 5 min, 20 min or 1 h (Fig. 1).

After 5-60 min of exposure to air the maximum penetration depth was about 10 cm, and the number of filled elements at 2 cm from the cut surface was about 40, less than half the number of controls cut under water (Fig. 1). The same was found after 3 h of exposure to air (results not shown). After 24 h exposure to air the number of filled elements at 2 cm and 3 cm from the cut surface had further decreased (Figs. 1 and 2), although the effect at 2 cm was not significant in all repeat experiments. The maximum penetration depth after 24 h of exposure to air was reduced to about 5 cm (Fig. 2). In the figures 1 and 2 the results of standing in the suspension for 1 h are shown. Standing in the suspension for 5 h gave the same results as 1 h (not shown).

In cut Cara mia roses, which show vascular blockage at a faster rate than Sonia, the distribution of vessels filled with India ink was similar to that in Sonia, before exposure to air (Fig. 1). When exposed to air the reduction of penetration depth and of the number of vessels filled at 2 cm was also similar to stems of Sonia (Fig. 1).

Effect of a surfactant

When stems were held dry for 24 h and placed in a suspension of India ink containing a surfactant (0.25 mg l^{-1} nonylphenoxypolyethoxy ethanol) the number of elements filled with ink at 2 cm from the cut surface increased with respect to controls held in air (and then placed in the suspension of India ink), and was not significantly different from controls cut under water. Use of the surfactant resulted in penetration of ink to about 15 cm depth (Fig. 2).

Bacterial numbers

The number of bacteria in the lowermost 5 cm of the stem, after a period of dry storage was below 10^4 cfu per gram FW.

DISCUSSION

Flowering Cara mia and Sonia rose stems cut under water and placed in an aqueous suspension containing macromolecular material showed maximum penetration to 25-30 cm distance from the cut surface. This distance corresponds with the maximum vessel length in flowering stems of Sonia roses (Chapter 2). Similar techniques have been previously used to study the length of conducting xylem elements (Greenidge, 1952; Skene and Balodis, 1968; Zimmermann and Jeje, 1981; Middleton and Butterfield, 1990). In these studies the suspension particles were found not to pass through the pores

in the pit membranes. These studies also showed that the conducting elements of different lengths have a random spatial distribution. An exponential relationship was established between the length of vessels and the number of vessels in a length category, most vessels occurring in the shortest length category (Zimmermann, 1983). This was now also found in stems of cut rose flowers.

Zimmermann and Jeje (1981) and Zimmermann (1983) objected to the method of using India ink for determination of vessel length. They suggested that because of their diameter and their ragged surface, particles of India ink might not penetrate up to the tapered vessel ending, and therefore recommended the use of latex paint rather than India ink. We found no difference between results using India ink, water colour or latex paint. Moreover, it was not our primary aim to determine exactly the length of the vessels opened by cutting and possibly in none of the methods used the vessel end was effectively penetrated by the tracer. If this problem occurred we would have underestimated the difference between controls cut under water and those stems in which the cut surface was exposed to air.

A clear difference between the controls and stems exposed to air was already evident within 5 min. This correlates with the aspiration of air at the cut surface which was already high after about 5 min (Chapter 5). The difference in penetration depth of the controls and the stems that were held dry, therefore, apparently shows the presence of air emboli in the latter.

Even after prolonged (24 h) exposure to air and subsequent standing in the India ink suspension, ink was found in some conducting elements close to the cut surface, indicating that the emboli became compressed by the suspension. This compression of the emboli was apparently inadequate for uptake of water, which was low after 24 h of exposure to air (Chapter 5).

When the cut flowering Sonia stems were exposed to air for 3 h, and then placed in water for one or more hours, penetration of ink at 2 cm from the cut surface occurred in about 40 conducting elements, as compared to 80-90 in controls cut under water. After this treatment, the maximum penetration depth was about 10 cm. This agreed with a high rate of water uptake and a rapid restoration of turgor in these flowering stems (Chapter 5). The number of elements filled with ink at 2 cm from the cut surface was about the same, but the maximum penetration depth was reduced when the flower-

ing stems had been exposed to air for 24 h or more. This was correlated with a low rate of water uptake (Chapter 5).

Stems of cut Cara mia roses showed a vascular occlusion after 3 h of exposure to air, similar to the occlusion in Sonia roses found after 36 h exposure to air (Chapter 6). The changes of penetration of ink after various periods of exposure to air, however, were the same in both cultivars. This indicates that factors other than the penetration of water into the lumina of the water-conducting elements opened by cutting are involved in the occlusion in this cultivar.

Surfactants have been found to facilitate water uptake into cut flowering rose stems exposed to air for an extended period (Chapter 7). The effect of one of these surfactants, used after 24 h of exposure to air, was reflected in the penetration of India ink. The treatment resulted in an increase in the number of filled elements at 2 cm from the cut surface and in the maximum penetration depth. The surfactant acts, therefore, apparently by facilitating the entry of water into the lumen of the xylem conduits opened by cutting. The effect of the surfactant indicates the importance of the contact between water and the walls of the conducting elements. Dehydration of the cell walls during prolonged exposure to air may prevent water from entering the lumina of the conducting elements. When the walls are not dry, or when the surface tension is overcome by a wetting agent, the emboli may be compressed, removed, or even dissolved.

The results described in this Chapter indicate, therefore, that vascular blockage in Sonia rose stems exposed to air is related to the inability of water to penetrate into the capillary lumen of the xylem elements opened by cutting. In Cara mia roses the inability of water to enter the elements opened by cutting occurs later than the vascular blockage. The hypothesis that in this cultivar cavitation of the (intact) xylem vessels is the main cause of the occlusion is investigated in the next Chapter.

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CHAPTER 9

RELATIONSHIP BETWEEN CAVITATIONS AND VASCULAR OCCLUSION IN CUT FLOWERING ROSE STEMS EXPOSED TO AIR

Summary

Cavitations in the stems of Cara mia, Madelon, and Sonia roses were determined using two methods: a) attaching one end of 2.5 cm stem segments to air at low pressure (0.1 MPa) and measurement of the air-conductivity of the segments, and b) assessing the number of ultrasonic acoustic emissions (UAEs) at the stem surface.

On sunny days the stems of intact rose plants showed conductivity to air. The stems became cavitated during the morning. In Cara mia and Sonia the cavitations were repaired during the late afternoon; in Madelon plants the repair occurred overnight. At similar temperature and RH the stems of Cara mia roses were more cavitated than those of Madelon and the latter more than Sonia. When the stems were cut early in the morning and held dry (in the light at 20°C and 60% RH) the air conductivity increased in a similar manner in the three cultivars investigated.

UAEs were determined during dry storage. The onset of a high frequency of UAEs preceded the onset of vascular occlusion in the stem, which occurred after 3-4, 9-12 and 24-36 h, in Cara mia, Madelon and Sonia roses, respectively. In these cultivars, the burst of UAEs occurred after 2.4 ± 0.7 h, 6.8 ± 4.3 h and 19.8 ± 9.0 h of exposure to air, respectively, which corresponded with a water potential of -1.7, -2.9 and -3.8 MPa, respectively.

The possible role of cavitation in the vascular occlusion developing during exposure to air is discussed.

INTRODUCTION

When a microphone is connected to plants or plant parts, sudden short sounds (clicks) can be observed. Such acoustic emissions have been measured at audible frequencies (AEs, Milburn and Johnson, 1966; Crombie et al., 1985; Ritman and Milburn, 1991) and by the use of appropriate filters

also at ultrasonic frequencies (UAEs, West and Gaff, 1976; Tyree and Dixon, 1983; Tyree et al., 1986; Sperry et al., 1987). In small blocks of xylem the total number of acoustic emissions corresponded with the number of water-conducting elements (Tyree and Sperry, 1989). The acoustic emissions have, therefore, been interpreted to represent cavitations, i.e. the filling of water-conducting elements with gas. The mechanisms by which water-conduits can become cavitated have been reviewed by Zimmermann (1983) and Tyree and Sperry (1989). Cavitation may be initiated by a gas bubble entering the water-conducting element from the wall. Because of the negative pressure in the element the bubble expands when entering, leading to the acoustic emission. The element will initially be filled with water vapour, but dissolved air in the wall will soon diffuse into the lumen. After the initial cavitation several others may follow as the air may enter the adjacent water-conducting elements through the pores in the pit membranes. In cut plant parts the latter mechanism of cavitation may also occur, as air will enter the xylem elements from the conduits opened by cutting (Crombie et al., 1985). In some woody species an inverse relationship between the number of UAEs and hydraulic conductivity was established (Sperry and Tyree, 1988; Tyree and Sperry, 1988).

In Samantha roses Dixon et al. (1988) found that keeping the stems in air resulted in reduction of hydraulic conductivity. Until a water potential of about - 2.0 MPa no clear evidence for UAEs was found, although hydraulic conductivity was reduced to 20% of the controls. At lower water potential UAEs were recorded and it was concluded that cavitations might contribute to the vascular occlusion in cut roses that are held in air. This hypothesis was investigated in rose cultivars that represent three groups of tolerance to air exposure, as determined in Chapter 5.

De Stigter and Broekhuysen (1989) described another method of determining cavitations: stem segments of 2.5 cm were connected for 1 min to air at a pressure of 0.1 MPa, and the amount of gas passing through the segments was measured. We also used this method to determine cavitation during dry storage, in the above three cultivars.

METHODS AND MATERIALS

Plants

Cut flowering Cara mia, Madelon, and Sonia roses were obtained from a greenhouse of the Department of Horticulture, Wageningen Agricultural University, or (Madelon and Sonia) from commercial growers. Stems of about 70 cm were cut, immediately placed in water and transported in

water to the laboratory. Time between cutting and the experiment was about 15 min when using stems from the Horticulture Department and 3 h when using stems from commercial growers.

In experiments on the effect of exposure to air, thirty cm of the basal stem end was cut under water and the stems were subsequently held dry at 20°C, 60% RH and a photon flux of 15 μmol m^{-2} s^{-1}.

Acoustic emissions

The method for monitoring the UAEs was essentially that of Tyree and Dixon (1983). Stems were placed horizontally and the ultrasonic microphones (Model 8312 broad band transducer from Bruel and Kjaer, Naerum, Denmark) were clamped onto the stems at about 5 cm from the basal end using a force of about 20 kN, without removing the bark. The microphones contain a pre-amplifier. The signal was amplified using Bruel and Kjaer 2638 wideband conditioning amplifiers in which it passed a high pass filter with a frequency cut off at 100 kHz. The lowest frequency detected was 2 MHz.

The signal was recorded on a chart recorder and/or a personal computer using the Labtech Notebook program (version 5.03 from Laboratories Technologies Corporation, Wilmington, MA). The program recorded the number of acoustic emissions and the time of their occurrence, and displayed these figures on the screen, as well as on a detailed time chart of the last 20 min of the experiment, also displayed on the screen. Experiments were performed using two stems in two complete set-ups. All background spikes were eliminated by using a mains radiofrequency interference filter (150 KHz-30 Mhz, 35 dB attenuation, from RS Components Ltd, Corby, Northants., U.K.).

UAE experiments were conducted in winter, and stems were collected early in the morning. Of each cultivar ten stems were tested. The experiments were once repeated.

Air conductivity of stem segments

The method used was described by De Stigter and Broekhuysen (1989). In short: stems of 50-70 cm length were harvested and immediately placed in water. Stems were either measured immediately after cutting, or were exposed to air as described above. When the stems were measured immediately after cutting 25-30 cm was recut under water and the leaves and the flower head were removed. The stem was then taken out of the water and segments of 2.5 cm length were cut and mounted in stoppers which were placed on glass jars. The air in the jar was connected to air at 0.1 MPa pressure for one min. The other side of the segment was connected with tubing to the lower end of a vertical glass capillary which was closed at the upper end. At the lower end the capillary had an overflow for water that is replaced by air. The air entering the capillary accumulated at the upper end and the volume was

determined by reading the scale on the capillary wall, and expressed per unit surface of the stem segment. When the stems were exposed to air about 30 cm was recut under water before air exposure. After the period of exposure to air the leaves and the flower head were removed and the stem was cut into segments of 2.5 cm length, after which air conductivity was measured as described.

The apparatus used was a gift from dr. De Stigter. The experiments included 7-10 replicate stems, and were conducted at least twice.

Water potential

The water potential was determined in 6 replications in the 3rd leaf from the flower head, or in the flower head cut at 5 cm from the sepals, using a Scholander pressure chamber constructed by TFDL at Wageningen.

Number of bent stems

From the sample of flowering stems of which UAEs were measured other stems were exposed to air at 20°C, 60% RH and a photon flux (PAR) of 15 µmol m^{-2} s^{-1} for varying periods of time. After this dry storage period ten stems were placed in water, without recutting. The number of bent pedicels was recorded daily.

RESULTS

Measurement of air-conductivity in 2.5 cm segments showed that the xylem of flowering stems attached to rose plants in the greenhouse contained more gas on warm and sunny days than on cool, overcast days. In Cara mia and Sonia roses air conductivity was zero at 7 a.m. but on warm days it increased during the morning and early afternoon, and was always zero again at 7 p.m. (Fig. 1). In Madelon roses the conductivity to air was zero at 4 a.m. and had increased already at 7 a.m. In the example shown in Fig. 1 the conductivity remained about the same at 11 a.m. (not shown), 3 p.m. and 7 p.m.

When measured at similar days with regard to temperature and RH the conductivity to air at 11 a.m. and 3 p.m. was always progressively higher in Sonia, Madelon and Cara mia (Fig. 1).

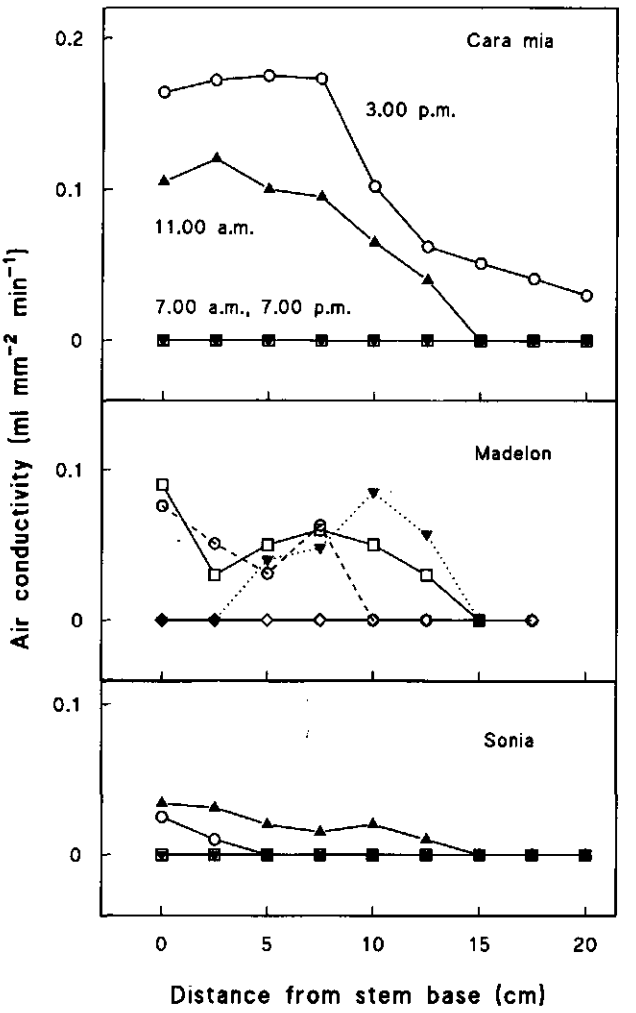

Fig. 1. Air conductivity of 2.5 cm segments from attached stems of Cara mia, Madelon and Sonia roses in the greenhouse. Measurements from a sunny day. The average temperature from 7 a.m. to 7 p.m. was 23°C and average RH was about 60%. ◊: 4 a.m. (Madelon only); □: 7 a.m.; ▲: 11 a.m.; ○: 3 p.m.; ▼: 7 p.m. a.m. and 3 p.m. was always progressively higher in Sonia, Madelon, and Cara mia (Fig. 1).

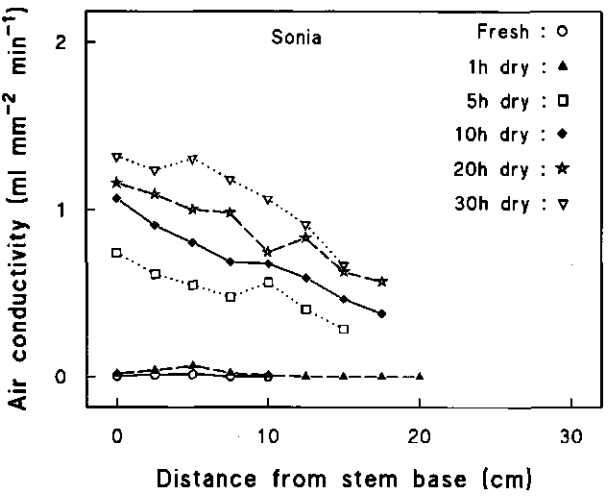

Fig. 2. Air conductivity of 2.5 cm segments along the stem of Sonia roses, cut at 7 a.m. and held dry for various periods at 20°C, 60% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}.

When the stems were cut at 4 a.m. (Madelon) or 7 a.m. and held dry in the light at 20°C, the conductivity to air had not increased after 1 h, but was higher after 5 h, and tended to further increase with the period of dry storage. The conductivity at various periods of dry storage is shown for Sonia in Fig. 2. No difference was found between the three cultivars tested (Fig. 3).

Ultrasonic acoustic emissions were recorded by attaching a microphone to the stems of dry-stored flowers. Each ultrasonic emission results in a voltage spike. Flowering rose stems exposed to air showed UAEs first at a low rate, later at a high rate (Fig. 4). This was generally followed by a gradual decrease in frequency, but could be followed by one or more other peaks and in some experiments it stayed at a high level. The time to the onset of a high rate, determined as the time to reach 50% of the maximum frequency of the first peak (shown in Fig. 4 for Cara mia roses), depended on the investigated cultivar. The high frequency started first in Cara mia roses, then in Madelon, and only after 20 h in Sonia (Fig. 4), at progressively lower water potentials (Table 1). As shown in Chapter 8, the rate of change in water potential was similar in the three cultivars. The onset of high-frequency UAEs was related to the percentage of pedicels that remained bent after exposure to air (Table 1).

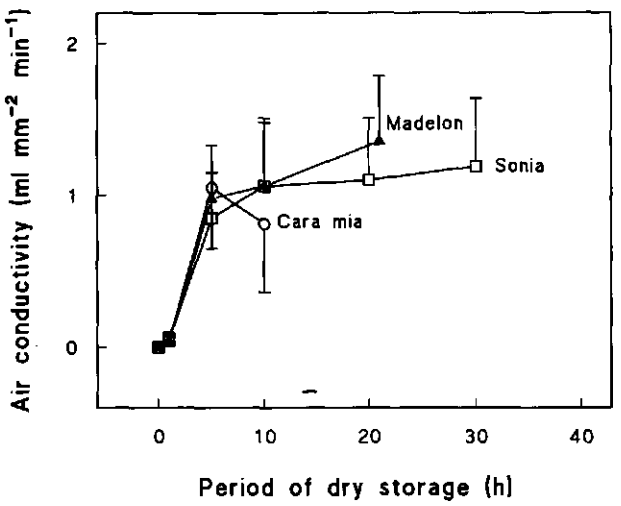

Fig. 3. Air conductivity of the basal 2.5 cm segment from stems cut early in the morning (4 a.m. for Madelon and 7 a.m. for Cara mia and Sonia roses) and held dry for various periods at 20°C, 60% RH and a photon flux (PAR) of 15 µmol m^{-2} s^{-1}.

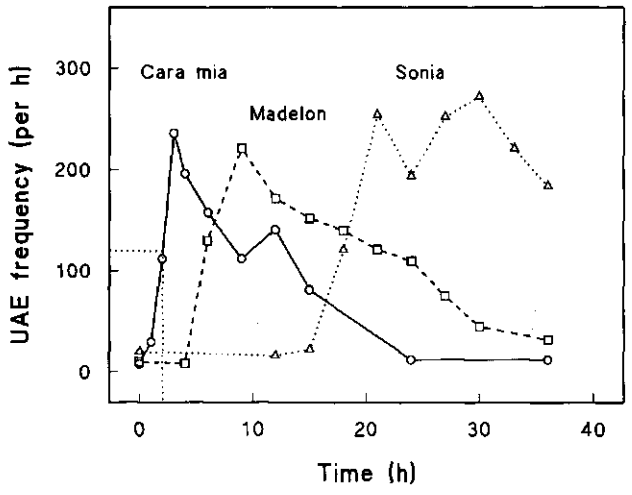

Fig. 4. The frequency of UAEs from the stems of cut Cara mia, Madelon and Sonia roses, during dry storage at 20°C, 60% RH and a photon flux (PAR) of 15 µmol m^{-2} s^{-1}. The graph shows representative individual stems, and the method to determine the time to 50% of the maximum in the first frequency peak.

Table 1. Time (I) to the onset of a high frequency of ultrasonic acoustic emissions (UAEs) from the stems of cut Cara mia, Madelon, and Sonia roses held in air at 20°C, 60% RH and a photon flux (PAR) of 15 μmol m^{-2} s^{-1}, as related to time (II) to high hydraulic resistance, the water potential at time I of the third seven-leaflet leaf beneath the flower head, and the percentage of bent pedicels one day after placement in water, following 3, 9 and 24 h of exposure to air. The stems were not recut before placement in water.

Cultivar	Time to high frequency of UAE (h) (I)	Time to high hydraulic resistance (h) (II)
Cara mia	3-4	2.4 ± 0.7 (n=10)
Madelon	9-14	6.8 ± 4.3 (n=10)
Sonia	24-36	19.8 ± 9.0 (n=10)

	Leaf water potential (MPa)	Bent pedicels (%) after 24 h in water, and previous dry storage for		
		3h	9h	24h
Cara mia	-1.7 ± 0.4 (n=7)	100	100	100
Madelon	-2.9 ± 0.7 (n=7)	0	40	100
Sonia	-3.8 ± 0.8 (n=7)	0	0	60

DISCUSSION

UAEs have been reported to occur upon lowering the water potential of plants or plant parts (Tyree and Sperry, 1989). Both in conifers and in dicotyledonous species differences were found in the water potential at which the first cavitations occurred (Tyree et al., 1984; Sperry, 1986; Tyree and Sperry, 1988; Sperry and Tyree, 1990). In conifers such as *Abies balsamea* and *Tsuga canadensis* a number of UAEs occurred at -3 MPa, in *Juniperus virginiana*, however, not before the xylem water potential had reached -7 MPa. In hardwood species a comparable number of UAEs was reached at -4 MPa in *Acer saccharum* and *Cassipourea elliptica*, and at -5 MPa in *Rhizophora mangle*. The differences

between species have been related to their ecology. The mangrove species *Rhizophora mangle*, for example, normally has a xylem water potential which is much lower than the water potential of its inland relative *Cassipourea elliptica* (Sperry et al., 1988).

We now also found considerable differences within one species; the onset of a high frequency of UAEs in different rose cultivars started at a water potential ranging from -1.7 to -3.8 MPa.

Zimmermann (1983) showed, on theoretical grounds, that the pressure difference which results in cavitation is higher when the diameter of the largest pore is smaller. This may also be the explanation for the differences found in the three rose cultivars investigated, and this hypothesis will be tested in future experiments.

In cut Samantha roses Dixon et al. (1988) observed the first UAEs when the water potential dropped to about -2.0 MPa, a value similar to that of other temperate species such as *Vitis* grapevines (Sperry et al., 1987), and of Cara mia roses. In Samantha roses UAEs started when the flowering stems had lost about 30% of their fresh weight. By then hydraulic conductance had already decreased by about 80%, indicating processes other than cavitations in the development of vascular occlusion. In these experiments, however, microbiological control was absent. The stems may have been placed in water prior to dry storage and bacteria may therefore account for the blockage.

The present data on Cara mia, Madelon and Sonia roses show that a high number of cavitations occurred at a considerable difference in time after dry storage (from 2.4 to 19.8 h), but in all three investigated cultivars it occurred prior to the development of the blockage. This indicates a causal relationship between cavitation and vascular blockage.

Measurement of the conductivity to air (at 0.1 MPa) in 2.5 cm stem segments showed cavitations in attached flowering stems on plants in the greenhouse, on sunny days. During the same day highest conductivity was observed in Cara mia and the lowest in Sonia roses. The water potential of plants in the greenhouse was similar when measured at the same time (Chapter 6, and unpublished results). The air-conductivity data, therefore, apparently confirm the UAE measurements showing that the onset of a high frequency of UAEs (cavitated conduits) occurs at progressively higher water potentials in Cara mia, Madelon and Sonia.

Using AE- or UAE-measurements on intact plants growing in the field, cavitation of the xylem elements has been found on warm days. It has been inferred that these cavitations become repaired by root pressure, during the night (Tyree and Sperry, 1989). We now found in intact Cara mia and Sonia rose plants that the cavitations become already repaired at the end of the afternoon, between 3 and 7 p.m. Such repair is only possible at a low rate of transpiration (Yang and Tyree, 1992). The earlier repair in Cara mia and Sonia plants as compared with Madelon may relate to earlier stomatal closure.

Measurement of air conductivity of stems that were stored dry confirmed that the xylem conduits became devoid of water. This was, however, already detectable after 5 h in all three investigated cultivars, whereas a clear increase in UAEs occurred within 5 h in Cara mia and after more than 5 h in Madelon and Sonia. Zimmermann (1983) hypothesized that large-diameter conduits cavitate earlier than conduits with a small diameter. In rose stems, a number of UAEs always occurred prior to the high frequency of the emisssions, and these may be due to cavitations in the widest conduits. The presence of air in uncut vessels after 5 h of exposure to air may, therefore, be due to the a few cavitations in the largest of these vessels. Moreover, even when the initial cavitations occur irrespective of conduit size, the air-conductivity method may show a relatively high increase after only relatively few cavitations.

Inhibition of water uptake has been found only when more than two thirds of all conduits had been blocked (Chapter 3). Cavitation in the first elements, even when these are the widest ones, does, therefore, not lead to vascular blockage. Only when a large number of elements is cavitated the occlusion becomes apparent. Although the differences between the cultivars were not detectable using the air-conductivity method, they were shown using ultrasonic emission.

Chapter 7 described that including a surfactant in the vase solution overcame the blockage. The surfactant, apart from facilitating the penetration of the water column in the xylem elements opened by cutting (Chapter 8), may also help to re-establish the water column in cavitated xylem conduits.

It is concluded that the occlusion in dry-stored rose stems may be due to the presence of a high percentage of cavitated xylem elements, not opened by cutting. This is especially true for sensitive cultivars such as Cara mia, as in this cultivar a correlation was found between the occlusion and a high frequency of UAEs, whereas no correlation was found between the occlusion and the inability of water

to penetrate the xylem conduits opened by cutting (Chapter 8). In Sonia, on the contrary, vascular occlusion was also correlated with the inability of water to penetrate the conduits opened by cutting.

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CHAPTER 10

GENERAL DISCUSSION

The occlusion in stems of cut rose flowers, leading to premature wilting of the leaves and petals, to bending of the stem underneath the flower, and to inhibition of flower opening, was found to be mainly located in the basal stem segment. According to the literature this occlusion may be due to a stem-induced process, to microbial effects, or to air in the stems (Chapter 1). We tested the hypothesis that the blockage is stem-induced and separated the effects of microbial growth and those of air absorption into the xylem conduits opened by cutting, and into the intact conduits. The roses were placed in the vase solution directly after cutting, thereby avoiding effects of aspired air, or were held dry without prior placement in water, thereby avoiding effects of microbial growth.

Occlusion as a stem-induced process

Several studies suggested that the blockage in stems of cut rose flowers standing in water is due to a reaction of the stem. After cutting substantial amounts of ethylene are produced at the cut surface, but the occlusion was not affected by inhibition or stimulation of ethylene production. Silver thiosulfate, an inhibitor of ethylene action, also had no effect on the occlusion, whereas the effect of free Ag^+ was correlated with its inhibitory effect on bacterial growth. These results indicate that ethylene production as a response to wounding is not the cause of the occlusion (Chapter 3).

Durkin (1967) suggested that some active process might lead to accumulation of phenolic compounds, such as lignins and tannins, in the xylem. Others, however, found little staining with ferric chloride or with phloroglucinol-HCl, thus excluding the presence of lignins or tannins (Burdett, 1970; Gilman and Steponkus, 1972; Parups and Molnar, 1972). We investigated the hypothesis of phenol polymerization by using vase solutions of pH 4 to pH 8, the activity of the key enzyme in phenol polymerization being zero at pH 4 (Vámos Vigiázó, 1981). After seven days vascular occlusion was independent of pH, which is evidence against the above hypothesis (Chapter 4).

Amorphous plugs have been found in the xylem conduits and these plugs were considered to be due to a response of the rose stem to cutting (Parups and Molnar, 1972). The amorphous vascular plugs were mainly found at about 15-30 cm from the cut surface (Burdett, 1970; Lineberger and Steponkus, 1976). With the exception of Burdett (1970) no author has shown that hydraulic conductance was low in this region of the stem. Instead they found the blockage to be present in the basal stem segment (De Stigter and Broekhuysen, 1986, Durkin and Kuc, 1966) and we found the same for Sonia, Ilona, Motrea, Madelon and Jack Frost roses (Chapter 3).

Water uptake of cut Sonia roses was found to be unaffected when a razor blade was introduced into one third or two thirds of the stem cross-sectional area. All conduits above the blade did not conduct water, as was shown by placing the stems in 1% aqueous fuchsin solution (Chapter 3). This indicates that a large proportion of the vessels has to become blocked before water uptake is reduced. From SEM studies Rasmussen and Carpenter (1974) reported that up to 4% of the vessels contained amorphous plugs. In transverse stem sections, Burdett (1970) detected plugs in 3-11% of the vessels, Lineberger and Steponkus (1976) showed a maximum of 20%, and Dixon and Peterson (1989) a maximum of 8-23%. In cut Sonia roses we found no gum-like occlusions when using light microscopy, while only a few amorphous plugs were observed with the scanning electron microscope (Chapter 3). The number of conduits with amorphous plugs, therefore, is far too low to account for a detectable blockage.

The vascular plugs in cut rose stems were found to stain with ruthenium red (Burdett, 1970; Parups and Molnar, 1972). We isolated several bacterial strains from the stems of cut rose flowers and these strains all produced slime, some strains even in copious amounts (De Witte and Van Doorn, 1988). Bacterial slime mainly consists of polysaccharides (Sutherland, 1977; Wilkinson, 1977). Accordingly, we found that the slime of isolated bacterial colonies stained red with ruthenium red. In SEM micrographs of xylem conduits the structure of the amorphous plug material was like that of the slime of isolated bacterial colonies growing on agar (Chapter 2). The amorphous plugs in cut rose stems were found to contain not only carbohydrates, but also lipids and protein-like material (Parups and Molnar, 1972) which was also detected in the slime excreted by bacteria (Pier et al., 1978). The plugs in stems of cut Samantha roses contained polysaccharides as indicated by the periodic acid-Schiff's test, as well as proteins, which also indicates the microbial origin of the plugs (Dixon and Peterson, 1989).

Marousky (1969, 1971) held the stems of cut Better Times roses in a hypochlorite solution for some minutes and then transferred them to sterilized bottles with sterile water, either containing a buffer at pH 6 or pH 3 or 8-hydroxyquinoline citrate (HQC). After two days of vase life most of the bottles contained no bacteria in the vase water. Yet, the hydraulic conductance was lower in controls than in the HQC treatment and lower at pH 6 than at pH 3. From this it was inferred that pH 3 and HQC, apart from being known as antibacterial agents, reduced some physiological blockage. We found, however, that in sterile vase water no bacteria may be found for several days, but that bacteria are growing in the stem. The experiments of Marousky (1969, 1971) were repeated with Sonia, Ilona, Polka and Frisco roses. Although no bacteria were present in the water, in all cases a reduction of hydraulic conductance was correlated with the presence of a high number of bacteria in the stems (Chapter 3).

When the growth of micro-organisms was prevented in stems standing in aqueous solution, no decrease in stem hydraulic conductance was ever observed, also indicating that there was no contribution by the stem cells to vascular occlusion. This was found both when chemicals were used to suppress bacterial growth and after a treatment in which the stems were held sterile (Chapter 3).

These results indicate, therefore, that in the investigated rose cultivars a stem reaction does not detectably contribute to vascular occlusion.

Occlusion by micro-organisms

A few yeasts have been observed at normal pH of the vase solution but no yeasts were observed at the cut surface and in the xylem interior (Chapter 2). The role of yeasts in vascular occlusion is, therefore, apparently minimal. Within 3-4 days, a few fungal hyphae are found at the cut surface. Because the development of fungi occurs after the development of the occlusion (Chapter 2), a role of fungi is also excluded.

The presence of a population of bacteria at the cut surface and inside the water-conducting elements preceded the onset of the occlusion (Chapter 2), and in the course of standing in the vase water a correlation was found between the number of bacteria associated with the stems and vascular occlusion (Chapter 3).

Burdett (1970) suggested that bacteria degrade cell walls or pit membranes by pectolytic or cellulytic activity. The fragments would then accumulate to form the occlusion. The pit membrane is a remnant of the primary wall in which the matrix material is apparently hydrolyzed during differentiation, and a cellulose microfibrillar web remains (O'Brien and Thimann, 1967; Butterfield and Meylan, 1982). The distribution of bacteria in stems, which indicates that bacteria do not pass the vessel-to-vessel pits (Chapter 3), is not consistent with the hypothesis that bacteria would degrade the pit membranes. Moreover, the bacteria isolated from vase water of cut rose flowers do not show pectolytic activity (De Witte and Van Doorn, 1988; Put and Klop, 1990) and no apparent degradation of the conduit walls was found (Chapter 2).

Burdett (1970) also hypothesized that bacteria exert an effect on the paratracheal xylem cells, which then produce plugs in the xylem conduits. This hypothesis was ruled out by our finding that blockage was related to the amount of material taken up by the stems, be it from a bacterial suspension or from a solution containing only extracellular slime from bacteria. The same relationship was found when the rose stems were kept at 1°C, in which a response to the presence of bacteria would have been inhibited (Chapter 4).

Cellulase was found to occlude the stem of cut rose flowers, although only at fairly high concentrations (more than 1 gram per liter, Mayak et al., 1974). We compared the effect of cellulase (about 50 kD) with ovalbumin, an inert protein of the same molecular weight, both at 1 gram per liter. Low hydraulic conductance was found shortly after exposure, and no differences between cellulase and ovalbumin occurred, indicating that any (globular) molecule with a molecular weight of 50 kD is able to block the pores in the pit membranes (Chapter 4). Blockage by suspensions containing a high number of living or dead bacteria occurred within 30 min, when given at 20°C, and resulted in a similarly rapid occlusion at 1°C, indicating that the occlusion by bacteria does not depend on physiological activity from the part of the bacteria (Chapter 4).

In a scanning electron microscopical investigation of bacterial colonies (*Pseudomonas aeruginosa*, isolated from rose stems) it was observed that the conventional preparation method completely dissolved the layer of extracellular polysaccharides (EPS) on the colonies. The EPS was well preserved and could be studied when using cryo-scanning techniques (Chapter 2). Accordingly, the cryo-techniques applied to the stems of cut rose flowers held in water showed much more EPS than

conventional SEM (Chapter 2). The role of EPS may be important and may have been underestimated in studies using conventional SEM (Rasmussen and Carpenter, 1974). The role of bacterial EPS in vascular occlusion related to micro-organisms has been discussed in Chapter 2: the EPS molecules block the pores in the pit membranes.

The above results indicate that the blockage by micro-organisms is a purely physical process. Living and dead bacteria, adhering together with their EPS, EPS itself, and the degradation products from bacteria, all block the pores in pit-membranes and possibly also the entry of water at the cut surface.

Occlusion upon exposure to air

Experiments with Sonia roses showed that air was taken up after cutting. In leafy stems the rate of air uptake correlated with the rate of transpiration, indicating that air was aspired into the conducting elements. When the lowermost leaf was present the amount of air taken up corresponded with the size of that leaf and air uptake only ceased when the leaf had completely desiccated. When the lowermost leaf was removed only about 0.04 ml of air was taken up, which corresponds with the lumen of the conducting elements opened by cutting (Chapter 5).

In Sonia roses, the absorption of air is completed within half an hour. The presence of air in the xylem lumen is initially no obstacle to the flow of water, but water uptake is inhibited after 24-36 h of exposure to air (Chapter 5). The mere uptake of air into the elements opened by cutting, therefore, is not responsible for the occlusion upon exposure to air.

A remarkable difference was found between rose cultivars in their sensitivity to air exposure. Frisco roses were more resistant than Sonia, wheras Cara mia roses were far more sensitive than Sonia. In Frisco roses the stomates generally closed more rapidly than in Sonia, but the main difference was the low cuticular transpiration in the former cultivar (Chapter 6). The differences between Cara mia, Madelon and Sonia roses were not related to their water loss, water potential, or xylem anatomy (Chapter 6).

The uptake of water into stems that were exposed to air could involve two pathways. Normally, in stems unexposed to air, the bulk of water transport will be through the lumina of the conducting elements, as the resistance for water transport in the walls is much higher (Läuchli, 1978). However, when gas is present in these elements water transport may also occur in the xylem conduit walls. Experiments with a girdled-stem system, in which a ring of bark was removed at the basal end of the stems and the cut surface was sealed with laboratory grease, showed that water uptake through the walls was adequate to maintain turgidity when stems were not held dry or held dry for a relatively short period. When the stems were held dry for a prolonged period the passage of water in the girdled-stem system was no longer adequate. These results could be interpreted to show that the blockage occurring during prolonged periods of air dehydration of cut Sonia stems would be related to the water pathway in the xylem conduit walls. The effect of surfactants, however, was not consistent with this hypothesis. Surfactants included in the vase solution promoted water uptake into stems exposed to air, but had no effect on the girdled stems (Chapter 7).

The results from experiments in which Sonia roses were placed in a dilute suspension of india ink demonstrated that the onset of the occlusion was correlated with inhibited penetration of water into the lumen of the xylem elements opened by cutting. When stems were exposed to air for a short period water was still able to penetrate into a relatively large number of elements, although not fully into the longest elements. Prolonged exposure to air resulted in a decrease in the maximum penetration depth. This was correlated with the uptake rate of water (Chapter 8). Inclusion of surfactants in the vase solution greatly increased water uptake after prolonged dry storage and promoted penetration of water into the xylem conduits that were opened by cutting. These results show that a decrease in the lumen pathway for water in the lowermost xylem elements may be a cause of the occlusion developing upon exposure to air, at least in Sonia roses (Chapter 8). Although in Cara mia roses the occlusion developed more rapidly than in Sonia roses, these cultivars showed the same time course with respect to water entry into the xylem conduits opened by cutting, during dry storage. The occlusion in Cara mia, therefore, cannot be explained by inhibited entry into the lowermost xylem elements only.

When the rose stems were exposed to air, ultrasonic acoustic emissions (UAEs) were detected at the stem surface. The literature shows that these emissions reflect cavitations in the intact xylem conduits (Tyree and Sperry, 1989). As the absorption of air at the cut surface ceased after about half an hour the cavitations probably did not result from transfer of air from the conduits opened by cutting.

Upon exposure to air of cut rose stems the onset of a high frequency of UAE was correlated with the development of the vascular occlusion in the stems: in Cara mia, Madelon and Sonia the onset of a high frequency of UAE preceded the occlusion (Chapter 9). Cavitations in the water conduits not opened by cutting may, therefore, is apparently the main cause of vascular blockage in Cara mia roses. In Sonia roses the inability of water to enter the conduits opened by cutting is also correlated with the blockage and may also be one of the causes of the occlusion.

Conclusions

The following picture emerges from these experiments:

1. When cut rose stems are held in water no apparent role was established for occlusion as a result of a reaction by the stem. Hydraulic conductance of cut stems remained as in freshly harvested stems when microbial growth was precluded. Treatments which interfered with ethylene production (which is increased as a result of cutting) had no effect on the occlusion. No visible occlusions such as tyloses were found, and only a few xylem elements were filled with gummy material of an undetermined origin.

2. Of the micro-organisms leading to vascular blockage bacteria are apparently much more important than fungi or yeasts. Living and dead bacteria, their extracellular polysaccharides, and the degradation products from bacteria block the water pathway by occluding the pores in the pit-membranes and by covering the cut surface. The mode of action of the bacteria is purely physical since both living and dead bacteria, and molecules originating from them, resulted in the same increased resistance to water flow, both at 20°C and at 1°C.

3. Water uptake is also inhibited upon exposure of the stems to air. Considerable differences exist between rose cultivars. The presence of air into the lumen of the xylem elements is not in itself an obstacle to water uptake. The development of the occlusion during dry storage is neither due to a reduced passage of water through the xylem walls, which could be used as a pathway for water when the lumina are blocked. In Sonia roses, but not in Cara mia roses, the blockage was correlated in time with a reduced penetration of water into the conduits which are opened by cutting. In Cara mia, Madelon and Sonia roses the occlusion was correlated in time with the onset

of a high number of ultrasonic acoustic emissions (cavitations) in the non-opened xylem elements in the stem. The results indicate that the early onset of a high number of cavitations is a main cause of blockage in sensitive cultivars such as Cara mia. The inability to penetrate the conduits opened by cutting may also be a cause of occlusion in intermediary sensitive and insensitive cultivars as Madelon, Sonia and Frisco.

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SUMMARY

The quality of cut rose flowers, a major horticultural crop in the Netherlands, is often unsatisfactory. During vase-life premature signs of water stress occur, such as slow growth of the bud which often results in poor flower opening, wilting of both the flowers and the leaves, and bending of the stem just underneath the flower. These symptoms are due to an inability to take up adequate amounts of water from the vase solution, which in turn is due to an occlusion in the lower part of the stem. Experiments in which a razor blade was introduced into the stem showed that more than 66% of all xylem conduits must become non-functional before a reduction in the rate of water uptake becomes apparent. This study distinguishes between the occlusion which occurs when the stems are placed in water immediately after harvest, and the occlusion which occurs as a result of dry storage.

When the stems are placed in water directly after harvest the blockage could be due to processes inherent in the stem, e.g. as a result of a wound-reaction, in which ethylene is generally involved. Alternatively, it could be due to microbial growth. Inhibition of the production or action of ethylene had no effect on the blockage. Light- and electron microscopy revealed no tyloses in the lumen of the xylem conduits (vessels and tracheids) and in only a few conduits a deposit of gummy material occurred in the absence of bacteria. This material stained with ruthenium red, a dye on polysaccharides. Slime on the colonies of bacteria isolated from the stems also stained with ruthenium red, indicating that the material found in some conduits might be bacterial polysaccharide that was able to pass the pit membranes between the conduits. These membranes contain small pores, through which the water flows from one conduit to the next. During vase-life a population of bacteria developed on the cut surface and inside the opened xylem conduits. The bacteria were always accompanied by extracellular polysaccharides. Fungi were also observed at the cut surface, but only after the occlusion had already occurred, and yeasts were not observed. When bacterial growth was excluded the blockage was absent. It was concluded, therefore, that the blockage occurring in the stems that are placed in water directly after harvest is caused by bacteria.

Isolated living or dead bacteria both resulted in vascular blockage, when given either at room temperature or at 1°C. This indicates that physiological activity, either from the part of the bacteria or from the part of the stem, is not a prerequisite in the bacterial blockage. Isolated bacterial polysaccharides and proteins such as cellulase and the inert ovalbumin, both with a molecular mass of

about 50 kilodaltons, also resulted in rapid blockage. It is concluded, therefore, that the occlusion is due to a purely physical effect of living bacteria with their extracellular polysaccharides, as well as dead bacteria and their degradation products. The blockage is probably mainly due to the obstruction of the passage of water at the pit membranes between the xylem conduits.

The effects of dry storage can also be partially microbial in origin, as the number of bacteria associated with the cut surface and the xylem interior increase during dry storage. When the stems are not temporarily placed in water after harvest, however, they do not become contaminated with bacteria. When such stems are held dry an occlusion also develops. Among rose cultivars great differences were found in the time-course of this blockage. In Cara mia roses, for example, it occurred within 3-4 h (at 20°C), in Madelon within 9-14 h, in Sonia after 24-36 h, and in Frisco after about 48 h of dry storage. The last cultivar apparently conserves water as its stomates often closed more rapidly, and its rate of cuticular transpiration was lower. However, in Cara mia, Madelon, and Sonia roses stomatal response, water loss, and water potential during dry storage, showed the same time course.

The blockage that is not microbial in origin could be due to processes in the stem, for instance as a response to the low water potential. A microscopical investigation, however, did not show evidence for any material, be it tyloses, amorphous plugs or hydrophobic substances, in the xylem lumen.

After cutting air absorption occurs at the cut surface, due to the receding water columns in the xylem conduits opened by cutting. This absorption ceased already within half an hour. In the absence of a leaf nearby the cut surface the amount of absorbed air corresponded with the volume of the lumen of the opened conduits. The occlusion related to dry storage, however, occurs only after three hours in the most sensitive cultivar tested. It was concluded, therefore, that the mere presence of air in the lumen of the conduits opened by cutting is no obstacle to the subsequent flow of water.

Three hypotheses were tested as to the origin of the blockage due to dry storage:

Firstly, when the lumen of the conduits that are opened by cutting is blocked by the presence of air, the water might follow the xylem walls until reaching non-opened conduits filled with water. When the walls would dehydrate this second pathway could become inoperative. This hypothesis was tested using rose stems of which the cut surface was covered with laboratory grease, and a part of the bark

higher on the stem was cut away (girdling), thus exposing cell walls adjacent to the xylem. Girdled Sonia rose stems which were placed in water without dry storage remained turgid, provided that the wall area in contact with water was more than 0.6 cm^2. When the girdled stems were held dry and then placed in water the uptake of water was strongly inhibited, with a time course reminiscent of the blockage in normal stems. These results support the above hypothesis. When the dry-stored girdled stems were placed in a surfactant solution, however, the rate of water uptake was not improved, whereas the rate of uptake was greatly improved in dry-stored non-girdled stems. It was concluded, therefore, that a reduced access via the cell wall pathway is not the cause of the occlusion.

Secondly, water might initially be able to partially compress the embolus in the conduits opened by cutting, thereby providing contact with adjacent conduits filled with water. An occlusion would occur only when the walls dehydrate to the extent that water, because of its surface tension, is no longer able to contact these walls. This hypothesis was tested by placing stems in a suspension of india ink for 1-5 h, after dry storage. After 5-180 min of dry storage water was still able to partially penetrate the conduits of Sonia roses, and even after 24 h this was true, although the penetration depth was smaller after 24 h as compared to 3 h. When the stems were placed in a surfactant solution after 24 h of dry storage the penetration depth of the water was much higher than after 5-180 min of dry storage, but not as high as in controls that were not stored dry. These results are in agreement with the hypothesis. In Cara mia roses, however, the results were the same as in Sonia. As the occlusion occurs already within 180 min in the former cultivar, it is not correlated in time with a reduction of the penetration depth of water in the conduits opened by cutting.

Thirdly, the occlusion may be related to cavitation of the conduits that are not opened by cutting. Cavitation is the sudden filling with gas of a liquid-filled conduit; it may occur spontaneously when the water potential becomes low, or as a result of pulling gas from an adjacent conduit that is already gas-filled. Cavitation results in ultrasonic acoustic emissions (UAEs) which can be detected by placing a microphone at the surface of the stem. The frequency of UAEs always increased prior to the development of the occlusion, in Cara mia, Madelon, and Sonia roses. As these cultivars show a different time until the occlusion occurs, the results suggest that cavitation is an important cause of the occlusion. In Cara mia roses cavitation appears to be the only cause of the occlusion, in the other cultivars tested the incapacity of water to penetrate the conduits opened by cutting may also be part of the blockage.

SAMENVATTING

De kwaliteit van snijrozen is dikwijls matig omdat tijdens het vaasleven verschijnselen van watergebrek optreden: geringe groei van de bloemknop, hetgeen ertoe leidt dat de bloemen niet openkomen, slappe bloemen en bladeren, en knikken van het stengeldeel net onder de bloem. Deze verschijnselen zijn het gevolg van een geringe wateropname, veroorzaakt door een verstopping in het onderste deel van de stengel. Uit experimenten waarbij een scheermes horizontaal in de stengel werd gestoken werd geconcludeerd dat er pas sprake is van verstopping als meer dan tweederde van alle houtvaten zijn uitgeschakeld. In dit onderzoek werd een scheiding aangebracht tussen de verstopping die ontstaat als de rozen direct na de oogst in water worden gezet, en de verstopping die optreedt als gevolg van droge bewaring.

Eerstgenoemd type verstopping zou het gevolg kunnen zijn van processen in de stengel, bijvoorbeeld als gevolg van een wondreactie; waarbij ethyleen veelal een rol speelt. Deze verstopping zou bovendien het gevolg kunnen zijn van micro-organismen. Het snijvlak bleek een geruime hoeveelheid ethyleen te produceren, maar remming van de productie of de werking van ethyleen had geen effect op de verstopping. Uit onderzoek met de licht- en electronenmicroscoop bleek dat zich geen tylosen bevonden in het lumen van de vaten en dat er in slechts enkele vaten materiaal aanwezig was dat niet samenging met de aanwezigheid van bacteriën in hetzelfde vat. Dit materiaal werd rood na behandeling met ruthenium-rood, een kleurstof op polysacchariden. Ook het slijm van bacteriën die op agar werden opgekweekt kleurde met ruthenium-rood, hetgeen er op duidt dat het materiaal dat in enkele vaten werd aangetroffen bestaat uit een fractie bacteriële polysacchariden die de stippelmembranen tussen de vaten kan passeren. In deze (fysische) membraan bevinden zich kleine poriën waardoor het water moet passeren als het van het ene naar het andere vat stroomt. Tijdens het vaasleven ontwikkelde zich op het snijvlak en in de opengesneden houtvaten een populatie bacteriën. Ook werden schimmels waargenomen aan het snijvlak, maar deze waren pas zichtbaar nadat er verstopping was. Gisten werden niet waargenomen. Als de ontwikkeling van bacteriën op het snijvlak en in de stengel werd onderdrukt was er geen verstopping. Uit deze experimenten werd daarom geconcludeerd dat de verstopping in stengels die direct na de oogst in water worden geplaatst het gevolg is van de ontwikkeling van bacteriën.

Geïsoleerde levende en dode bacteriën, toegediend aan het vaaswater, gaven zowel bij 20° als bij 1°C vatverstopping. Dit duidt erop dat fysiologische activiteit van de bacteriën noch van de stengels noodzakelijk is voor de verstopping door bacteriën. Toevoeging van eiwitten (cellulase en het inerte ovalbumine, beide met een moleculaire massa van ongeveer 50 kD) of geïsoleerd bacteriëel polysaccharide aan het vaaswater gaf eveneens verstopping. Hieruit werd geconcludeerd dat de verstopping een fysisch verschijnsel is. Waarschijnlijk wordt de wateropname vooral belemmerd bij de stippelmembraan tussen de houtvaten, waar het water moet passeren om van het ene naar het andere vat te kunnen stromen. De poriën in deze membraan kunnen al door moleculen ter grootte van eiwitten of polysacchariden worden geblokkeerd.

Bij droge bewaring treedt er een toename van het aantal bacteriën op in de stengel hetgeen mede kan verklaren waarom na droge bewaring een verstopping wordt geconstateerd. De toename van de aantallen bacteriën, tijdens droge bewaring, treedt echter alleen op als de stengels tevoren in water zijn geplaatst, dwz. als er een initiële bacteriepopulatie is. Als de stengels direct na de oogst droog worden gehouden, cq. geen bacteriën bevatten en droog worden gehouden, treedt ook een verstopping op. Tussen de cultivars werden sterke verschillen geconstateerd in de snelheid waarmee deze verstopping optrad. In Cara mia rozen, bijvoorbeeld, was deze (bij 20°C) al na 3-4 uur aanwezig, bij Madelon na 9-14 uur, Sonia na 24-36 uur en Frisco na ongeveer 48 uur droge bewaring. De laatste cultivar verliest tijdens droge bewaring minder water omdat de huidmondjes soms sneller sluiten en de cuticulaire verdamping geringer is. Tussen Cara mia, Madelon en Sonia werden daarentegen geen verschillen gevonden in de snelheid waarmee de huidmondjes sluiten, in de verdampingssnelheid, noch in de waterpotentiaal tijdens droogliggen.

De verstopping als gevolg van droge bewaring zou het gevolg kunnen zijn van een reactie van de stengel, bv. op de daling van de waterpotentiaal, waardoor een fysische blokkering in het lumen van de vaten zou kunnen ontstaan. Er werden echter met de scanning-electronenmicroscoop geen tylosen waargenomen en evenmin was er amorf of hydrophoob materiaal in de lumina van de vaten.

Na aansnijden wordt aan het snijvlak lucht aangezogen, hetgeen binnen 30 minuten tot stilstand komt. Als er geen blad nabij het snijvlak aanwezig is komt het volume van de aangezogen lucht overeen met het volume van de lumina van de opengesneden houtvaten. De verstopping treedt echter

pas na drie uur op in de meest gevoelige cultivar, en is daarom niet het gevolg van de aanwezigheid van lucht in de opengesneden houtvaten.

Drie hypothesen omtrent de oorzaak van de verstopping werden getoetst:

Als eerste zou het water door de wanden van de opengesneden houtvaten kunnen stromen wanneer zich lucht in het lumen van de vaten bevindt. Pas wanneer door uitdroging de wanden geen water meer zouden doorlaten zou er sprake zijn van een verstopping. Deze hypothese werd getoetst door het snijvlak van rozen af te dichten met vaseline en een stukje van de bast te verwijderen. Om de houtvaten te kunnen bereiken moet het water in dit systeem door de wanden stromen. Deze geringde rozen werden in water gezet en bleven volledig turgescent als het weggesneden oppervlak meer dan 0.6 cm^2 bedroeg. Als geringde Sonia rozen droog werden bewaard was er onvoldoende wateropname na eenzelfde periode als bij niet-geringde rozen. Als de droog bewaarde 'geringde' rozen in een oplossing van een uitvloeier werden geplaatst was er ook geen toename van de wateropname, terwijl er bij normale stengels wel een sterke toename van de wateropname is als gevolg van de uitvloeier. Uit deze proeven werd daarom geconcludeerd dat een verminderde toegang via de celwanden niet de oorzaak is van de verstopping.

Ten tweede werd onderzocht of het water de lucht in de opengesneden vaten gedeeltelijk kan samenpersen, zodat er opnieuw kontakt ontstaat met de niet-opengesneden vaten, die nog water bevatten. Wanneer de wanden uitdrogen kan het water, vanwege de oppervlaktespanning, de wanden niet langer bevochtigen en kan het de capillairen niet meer binnendringen. Deze hypothese werd onderzocht door de stengels gedurende 1-5 uur in een suspensie van oost-indische inkt te plaatsen. De verdeling van de inkt geeft aan tot hoever en in hoeveel vaten het water kan indringen. Na 5-180 minuten droge bewaring van Sonia rozen was het water in staat de vaten gedeeltelijk binnen te dringen, hoewel er een vermindering was van de penetratiediepte van het water en van het aantal vaten waarin zich water bevond. Na 24 uur droge bewaring was de penetratiediepte verder gedaald. Als de stengels na droge bewaring in een oplossing met een uitvloeier werden geplaatst nam de penetratiediepte sterk toe. Deze resultaten zijn in overeenstemming met de hypothese. Bij Cara mia rozen werden echter dezelfde resultaten gevonden als bij Sonia rozen. Omdat er bij eerstgenoemde cultivar al een verstopping is na 180 minuten is deze niet gecorreleerd met een afname van de penetratiediepte van het water in de opengesneden vaten.

Als derde mogelijkheid werd nagegaan in hoeverre de verstopping kan worden toegeschreven aan cavitatie van de niet-opengesneden vaten. Cavitatie is het plotselinge breken van de waterkolom in een vat, waarbij het water wordt vervangen door gas. Dit kan spontaan optreden door de onderdruk in vaten die worden omgeven door vaten waarin zich nog water bevindt, en kan ook optreden als in een naastgelegen vat al gas aanwezig is. Dit gas wordt bij lage waterpotentiaal door de poriën in de stippelmembraan getrokken. Bij cavitatie ontstaat een ultrasoon geluid (ultrasonic acoustic emission, UAE). Door een microfoon op het stengeloppervlak te drukken kunnen de UAEs worden gemeten. De UAE-frekwentie nam tijdens droge bewaring sterk toe, steeds op een tijdstip voorafgaand aan de verstopping, bij Cara mia, Madelon en Sonia rozen. Omdat deze cultivars zich onderscheiden in de snelheid waarmee de verstopping optreedt geeft de correlatie in de tijd met de UAEs een sterke aanwijzing dat de verstopping in belangrijke mate het gevolg is van cavitatie. Bij Cara mia rozen is cavitatie waarschijnlijk de enige oorzaak van de verstopping; bij de andere onderzochte cultivars kan de penetratie-dipete van water in de opengesneden houtvaten ook een rol spelen.

CURRICULUM VITAE

De auteur van dit proefschrift werd in Ede geboren op 10 december 1951. Na het doorlopen van de middelbare school studeerde hij biologie aan de Rijksuniversiteit te Utrecht, van 1970 tot 1976, met als hoofdvak plantenfysiologie en nevenvak chemische dierfysiologie. Het doctoraal-diploma werd cum laude behaald.

Op uitnodiging van prof. dr. R. Brouwer kwam hij in 1976 in dienst van de Rijksuniversiteit Utrecht. Zijn taak was het verrichten van (promotie)onderzoek en het geven van onderwijs, in de vakgroep Algemene Plantkunde. Het onderzoek had betrekking op de effecten van hoge grondwaterstand op de fysiologie van de intacte plant. Door het plotselinge overlijden van prof. Brouwer is het beoogde proefschrift echter niet voltooid.

Na een periode van onderzoek aan de relatie tussen de vegetatie-samenstelling en de waterhuishouding van de bodem, aan het Rijksinstituut voor Natuurbeheer te Leersum, kwam hij in 1984 in dienst van het toenmalige Sprenger Instituut. Het onderzoek betrof de houdbaarheid van snijbloemen, in het bijzonder de vatverstopping bij rozen en andere gewassen. Toen het Sprenger Instituut in 1989 opging in het Instituut voor Agrotechnologisch Onderzoek (ATO-DLO) kon hij het werk aan dit onderwerp voortzetten. Een deel van dit onderzoek is beschreven in dit proefschrift.

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Printed at Ponsen and Looijen, Wageningen, The Netherlands.

Van Doorn, W.G., 1993.

Vascular occlusion in stems of cut rose flowers.

Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands.

152 pp. ISBN 90-5485-139-2