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The influence of vase water pretreatment on the accumulation of microparticles, microcompounds and bacterial cells on the cut surface xylem of *Rosa* cv. 'Kardinal' observed by SEM

Der Einfluss einer Vasenwasservorbehandlung auf die Akkumulation von Mikroteilchen, Mikrokompounds und Bakterien auf der Schnittfläche der Xylemgefässe von *Rosa* cv. 'Kardinal'

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Summary

Scanning electron microscope (SEM) observations of stem end samples of *Rosa* cv. 'Kardinal' after 48 and 72 h vase life showed that citrate-phosphate buffering of the vase fluid to pH 3 decreased and minimised the accumulation and adherence onto the cut surface xylem and lower parts of the xylem vessel wall of: (i) microparticles and particulate matter in local tap water after passing through a 0.47 /µm micropore filter; (ii) bacterial cells (pure cultures of a *Bacillus* and a *Pseudomonas* species) which were added to the vase water (5×10^5 ml^{-1}). The decreased microparticle and bacterial adherence onto the *Rosa* xylem resulted in an increased water uptake, water potential and bud development. Rosa stems placed in non-inoculated micropore filtered DI water of pH 3 obtained the best bud development. SEM images of the cut surface compared with similar binocular microscopy observations at 45 X after application of the Latex Red test showed that the Latex Red test can be applied as a rapid and reliable method to indicate, at an early stage, the accumulation and adhesion of microparticles on the xylem surface of cut *Rosa* stems, but SEM techniques, at high magnifications (>100–5000 X) are essential to prove and study the anatomy of the cut Rosa xylem, and conditions leading to accumulation and adhesion of microparticles, particulate matter and bacteria onto the xylem vessels. Further investigations are needed to unveil the underlaying mechanism of the pH 3 phenomenon of cut *rosa* stems

Zusammenfassung

Elektronenmikroskopische (SEM) Untersuchungen von *Rosa* cv. 'Kardinal' am unteren Ende des Stengels nach 48 und 72 Stunden im Vasenwasser zeigten folgendes Verhalten: Eine Phosphat-Zitratpufferlösung mit einem pH 3 minimalisierten die Akkumulation und die Adhäsion auf der Schnittfläche der unteren Teile der wasserführenden Xylemgefässe von (i) Kleinstteilen im lokalen Leitungswasser; (ii) Kleinstteilen im lokalen Leitungswasser, welche ein 0.47 /µm Mikrofilter passieren; (iii) Zellen von *Bacillus subtilis* und *Pseudomonas aeruginosa* inokuliert mit 5×10^5 ml^{-1} deionisiertem Wasser. Die Störung des Wassersflusses im Xylem durch Kleinstteile und Bakterien wird damit (bei pH 3) minimalisiert und die Wasseraufnahme, das Wasserpotential und die Knospenentwicklung der *Rosa* werden optimiert. Verwendung von deinosiertem, nicht inokuliertem Wasser und Filterung in 0.47 /µm Mikrofilter maximalisiert die Balance zwischen Wassertransportleistung, Knospenentwicklung und Haltbarkeit der Rosenstiele. Das „Latex Red"-Bild, beobachtet mit einem 45 X Binokular, ermöglicht einen frühzeitigen Nachweis der Akkumulation von Kleinstteilen und Bakterien auf der Schnittfläche der Xylemgefässe. SEM Techniek bei einer Vergrößerung von >100–5000 X ist aber essentiell zur Beobachtung der Xylem-Anatomie, Kleinstteile- und Bakterienzellmorphologie und zur Prüfung der Kondition, wodurch Akkumulation und Adhäsion von Kleinstteilen und Bakterien am Xylem stattfinden. Der Einfluß des pH-Wertes auf die untersuchten Parameter ist weiter zu analysieren.

Introduction

Aarts (1957) found that vase life was above all dependent on the water balance, which is the relation between the capacity of the flower for water uptake, water transport, and transpiration. Since the work of Aarts (e.c.)many factors of the post harvest physiology of cut flowers have been studied in greater depth, but only a few new facts were revealed clearly. Halevy and Mayak (1979, 1981) concluded again that maintenance of an optimal water status was the most important factor in cut flower longevity but that the underlying mechanism(s) leading to disturbed water balance were still unresolved (Mayak and Tirosh, 1995).

The benefits of low pH of vase water have long been recognised by Aarts, and many of the cut flower preservative formulations commercially called 'flower food' contain an acid to reduce the pH. It is however not yet clear whether the beneficial effects of vase water acidification is the pH itself or the anionic part of the pH lowering compound e.g. $(PO_4)^{3-}$ or citrate $(C_6H_5O_7)^{3-}$ and the concentration thereof (Van Doorn and Perik 1990). The effects of low pH was attributed to a reduction of microbial populations in vase water, retarding stem blockage by micro-organisms (Aarts 1957). How-

ever, even in bacteria-free water, retardation of stem blockage of roses occurred (Durkin 1979a,b). Marousky (1971) measured an increase in water flow rates through *rose* stem segments with a decrease in pH from approx. pH 6 to pH 3. Previous work revealed: (1) a decrease of water flow through rose stems may occur even when very low numbers of (viable and non-viable) microbial cells were assessed in the vase fluid and physical blockage of the xylem vessels was not evident in SEM observations (Put and Jansen 1989); (2) physical blockage of the *rose* stem xylem may result from inclusions of high molecular microbial exopolysaccharides (EPS) or particulate macromolecular matter (dextran) in the vase fluid (De Stigter and Broekhuysen 1986); (3) a decreased water flow through xylem vessels was observed when very low concentrations of microbial EPS or purified microbial pectic enzymes, representing scant or no enzymatic activity were added to the vase fluid (Put and Rombouts 1989); (4) disruption of the water uptake and *rose* bud development may also occur when low concentrations of small (low molecular weight) microbial metabolites, passing through a molecular filter with a cut-off at 1000 daltons, were added to the *rose* vase water (Put and Klop 1990); (5) SEM observations of stem ends showed that microparticles found in local tap water may cause phenomena resembling microbial particles and their effects on the water balance of *roses* (Durkin *et al.* 1995). The latter paper not only focused on physical obstruction of xylem vessels by microparticles, but also on physiological obstruction related to particulate matter or chemical compounds passing a 0.47 /µm filter; (6) Durkin (1979b) and Durkin *et al.* (1995) revealed, in addition, the beneficial effects on the bud development and the water balance of 'Kardinal' *roses* by lowering the vase water pH to pH 3.

The aim of the present study was to get a better insight and discrimination of: factors in vase water influencing the adherence of microparticles, microcompounds and bacteria onto the xylem vessel cross wall of *rose* stems disturbing the water status of the flower.

Materials and methods

Plant material

Flowery stems of *Rosa* cultivar 'Kardinal' were harvested from the greenhouse of Rutgers Plant Science Department. The bud development was at stage 2 (Berkholst l980). Immediately the stems were graded by cutting with a sterile razor blade to 50 cm, followed by 30 minutes' hydration in micropore filtered (MP, 0.47/µm pore) deionized (DI) water and vase life evaluation (Durkin 1979b). The MP filtration was at 27 inches vacuum (84.78 Kpa) through membranes of 0.47 /µm pore diam. size (Millipore Co., Bedford MA 01730, USA).

Micro-organisms

Bacillus subtilis and *Pseudomonas aeruginosa* var. pyocyanin negativ, Put (1990), were inoculated on nutrient agar, incubated for 72 h at 30 °C. The surface bacterial growth was washed off into sterile deionized water, Vortex mixed and diluted in sterile deionized water. Cell concentrations were assessed microscopically, and by a colony forming unit (cfu) most probable number (MPN) method on plate count agar. The suspensions were used within 24 h and stored at 5 ^{0}C.

Vase water base

(i) Local tap water (tap); (ii) micropore filtered tap water, pore diam. 0.47 /µm (MP-tap); (iii) deionized water (DI); and (iv) micropore filtered deionized water (MP-DI).

The local tap water was supplied to Rutgers University by the New Brunswick Water Department. It is a surface-collected water, which is purified and chlorinated to make a sanitary drinking water. The DI water was micropore filtered to decontaminate and was comparable with MP-tap treatment.

Buffer solutions

Stock solutions: citric acid (0.1 M) and sodium phosphate (0.2 M, McIlvain buffer) in sterilized distilled water. Dilutions of the stock solutions added to the vase water: 2 mM citric acid and 16 mM sodium phosphate (pH 7), 5 mM citric acid and 10 mM sodium phosphate (pH 5), 8 mM citric acid and 4 mM sodium phosphate (pH 3). The pH of the vase water was measured at point zero and after 96 h of vase life.

Vase water inoculation

0, zero test non inoculated; **B**, inoculated with Bacillus subtilis or **Ps**, *Pseudomonas aeruginosa* up to $>10^5$–$<10^6$ ml^{-1}.

Vases

Calibrated conic vases, each vase was filled with 600 ml vase water, composition as given in the above paragraph, and six 'Kardinal' roses. The vases, covered with aluminium foil, were placed in a clean air flower room regulated at 28 °C +/–1 °C and illuminated at 65 /µmol M^{-2}S^{-1}PAR (Photon Lux).

Vase life observations

Vase life was measured from emplacement in the vase solution (point zero) to the loss of flower turgor. Daily observations consisted of: water uptake, ml^{-1} rose, bud development and ornamental value. After 4 d vase life under normal conditions, bud development was up to stage 7, a fully blooming rose (Berkholst 1980), whereupon the rose may remain as such for another 4 d. The end of vase life is marked by a visually decreased ornamental value to < 50 % of the 'normal' flower bud development in sterile deionized vase water (Put and Rombouts 1989). At point zero and 4 d of vase life the microbiological state of the vase fluids was assessed by determining bacterial cfu ml^{-1} vase fluid on plate count agar (PCA), fungal cfu's on potato dextrose agar (PDA) and incubation for 2–4 d at 25–30 °C. In addition, microbial cell numbers were assessed microscopically (Put and Clerkx l988).

Latex red test

After 48 h of vase life individual flower stems of each vase water combination were immersed up to 10 mm in a 0.3 % v/v red acrylic latex paint for 2 min, than held in air in a vertical position for 30 min to allow the paint on the cut end to dry. Transverse slices, 1–2 mm thick were cut from the stem base for examination under a 45 X binocular stereo objective (Durkin *et al.*, 1998).

Acid fuchsin test

After 96 h vase life stems of two flowers of each vase water combination were immersed up to 5 mm stem in an acid fuchsin red solution of 0.5 % w/v in 50 % ethanol, removed after 30 min and dripped dry. The epidermis and the cortex of the stems were peeled off and obstruction of the water transport was revealed by the extent and height (cm) of the red coloration of the vessels (Put and Klop l990).

Water potential

At day four (96 h), two rose stems from each factorial combination based on: MP-tap and MP-DI, were removed above the uppermost internode, trimmed to a flower with 5 cm peduncle, and inverted into a Scholander pressure bomb for water potential (-Mpa) measurements (Durkin 1979$_b$). Omitted from the pressure bomb test were *rose* stems from tap water, which senesced at $<$48 h, and from DI water, comparable with MP-DI.

SEM specimen preparations

After 48 h vase life, pieces of the stem end (10 mm long) were cut with a sterile razor blade initially fixed in 2 % w/v gluteraldehyde in cacodylate buffer 0.1 M, pH 7.2. Some specimens were taken after execution of the latex red test. The fixed specimens were transported and stored at 5 °C until the dehydration process, which was executed within four weeks of sampling preparation. The specimens were then washed twice in the buffer and dehydrated through a graded series of ethanol/water of 10 % v/v to 100 %, leaving the specimens in each solution for 10 min. They were then freeze-fractured in the 96 % solution, critical point dried (Fison-Polaron) and mounted with Leit-C conductive carbon paste on aluminium stubs. Finally, the specimens were coated with gold-palladium (80–20) and examined in a Philips 535 scanning electron microscope at 15 kv accelerating Voltage (Durkin et al. 1998).

Abbreviations

AOC	assimilable organic carbon
cfu	colony forming units
EPS	ExoPolySaccharides
MP	Micropore filtration (Millipore, 0.47 /µm pore diam.)
MPN	Most probable number
n	number of observations, statistical units
PCA	Plate count agar
PDA	Potato dextrose agar

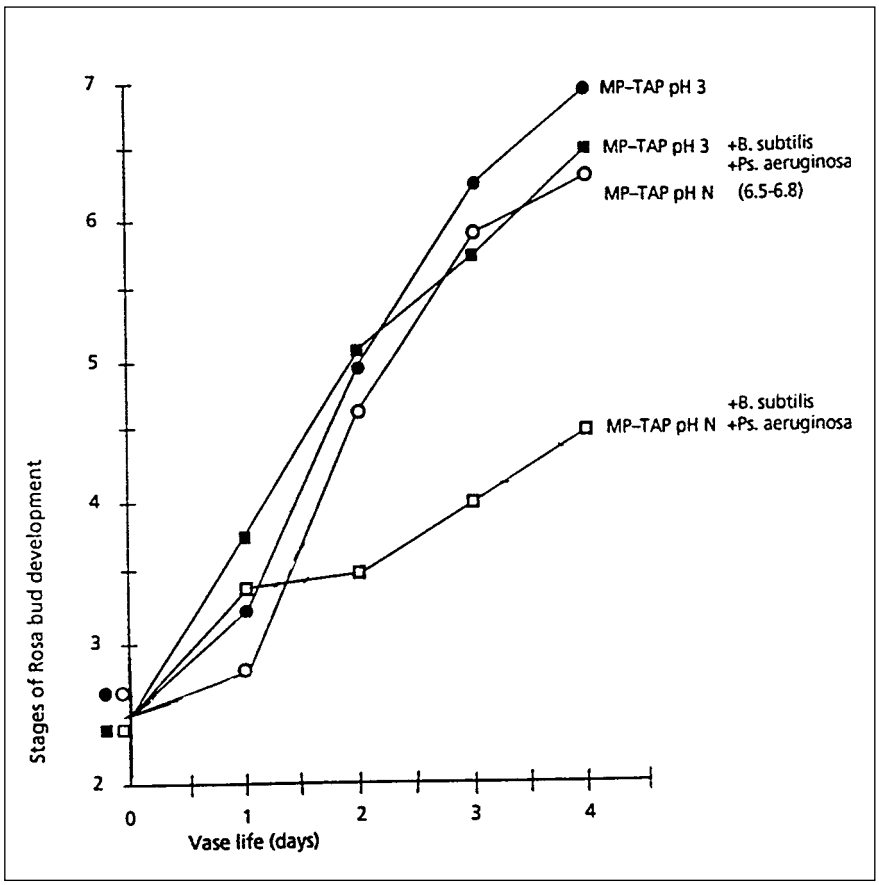


Fig. 1. Bud development of *Rosa* cv. 'Kardinal' during 4 d vase life in MP tap water: ○, no pH regulation=pH 6.5; ●, buffer pH 3; □, inoculated with bacterial cells 10^6 ml^{-1} no pH regulation; ■, inoculated with bacsterial cells 10^6 ml^{-1} buffer pH 3.

Results

Flower wilting was mainly observed 24 to 48 h preceding the appearance of 'bent neck' (bending of the *rose* stem at the peduncle). When *roses* were placed in tap water, 'bent neck' occurred suddenly between 24 h and 48 h, whether non-inoculated or inoculated vase water was used. MP minimized in part the negative effect of tap vase water on flower turgor, showing in addition the beneficial influence on the *rose* vase life of buffering at pH 3 (Figure 1). Together, these factors minimized the negative influence on *rosa* bud development of tap water and bacterial cells. Use of the phosphate-citrate buffer at pH 7 diminished bud development and flower life, which may have been the consequence of the relatively 'high' phosphate concentration at pH 7 (16 mM sodium phosphate, 368 mg L^{-1}). It did not refer to a physical vessel obstruction, as shown by SEM, and it occurred even in non-inoculated DI or MP vase water, so free entry of the phosphate into the xylem vessel system might have been a bit toxic and cumulative.

Table 1. Latex red test of *Rosa* cv. 'Kardinal' cut surface after 48 h vase life in different water
Latex–Rot-Test an Stengelquerschnitten der Sorte 'Kardinal' nach 48 Stunden in Vasenwasser unterschiedlicher Qualität

pH	Tap water	Micropore filtered tap water	Deionized water	Micropore filtered deionized water
	Zero samples: not inoculated			
N	4*	2*	2	1
3	0–1	0–1	0–1	0–1
5	3–4*	1–2	1–2	1
7	2*	2*	1–2	1–2
	Inoculated with ca. 10^6 ml^{-1} *B. subtilis*			
N	4*	3*	3	3
3	0–1	0–1	0–1	0–1
5	1–2*	1	2	2
	Inoculated with ca. 10^6 ml^{-1} *Ps. Aeruginosa*			
N	4*	4*	3–4	0–2
3	0–2	0–2	1–3	0–2
5	3–4	3	3	3

n, 4 or 6; N, no pH regulation = pH 6.5–7
Latex red test validations: **0**, no latex adherence; **1**, weak adherence on 25 % of the xylem vessels; **2**, on 50 %; **3,** on 75 %; **4**, nearly all xylem vessels covered with a thin layer of latex red particles; *****, thick bulging and glistening latex drops on the xylem cross section.

Table. 2. Acid fuchsin test *of Rosa* cv. 'Kardinal' stems after 96 h vase life in different water
Fuchsin-Test an Stengelquerschnitten der Sorten 'Kardinal' nach 96 Stunden in Vasenwasser unterschiedlicher Qualität

pH	Tap water			Micropore filtered tap water			Micropore filtered deionized water		
	Acid fuchsin uptake: cm up into the stem xylem *)								
	10	20	30	10	20	30	10	20	30
	Zero samples: vase water not inoculated								
N	0	0	0	2	1	0	3	2	1
3	1	1	0	2	2	1	3	3	1
5	1	0	0	2	1	1	2	1	0
7	1	1	0	1	0	0	1	0	0
	Vase water inoculated with ca l0^6 ml^{-1} *B. subtilis*								
N	0	0	0	1	1	0	2	1	1
3	2	2	1	2	2	1	2	2	1
5	2	1	1	2	2	1	2	1	1
	Vase water inoculated with ca 10^6 ml^{-1} *Ps. aeruginosa*								
N	1	0	0	1	0	0	2	1	1
3	1	1	0	2	2	1	2	2	1
5	1	1	0	1	1	0	2	1	1

Mean values of two or three consecutive vase life tests.
n, 4–6; N, no pH regulation = pH 6.5–7
*****) validation of the acid fuchsin uptake: **0**, none; **1**, 25 %; **2**, 50 %; **3**, 75 %; **4**, 100 % of xylem vessels shows fuchsin red uptake

Water uptake

The purer the vase water used (MP-DI), the more improved was the water uptake by the flowers. This phenomenon was masked by buffering vase water to pH 3, showing an optimization of water uptake even when lower purity vase water was used.

The pH 3 treatment, although to a lesser extent, did lead to optimization of the uptake of vase water inoculated with 10^6 ml^{-1} of bacterial cells.

Latex red test, at 48 h of vase life

The latex red test (Table 1) demonstrated a sensitive differentiation of the rate of vessel obstruction, consistent with the decrease in water uptake and flower life, comprising a certain predictability of the flower life.

Acid fuchsin test, at 96 h of vase life

The acid fuchsin test (Table 2) confirmed results of observations reported above. They were comprised of vessel obstruction caused by tap water and inoculation of bacterial cells. Roses placed in phosphate (16 mM) citrate (2 mM) buffer at pH 7 showed a strongly increased disruption of the fuchsin red uptake into non-physical obstructed vessels.

Pressure bomb test, at 96 h of vase life

Water potential results (Table 3) reflected observations made on vase life and water uptake. These data pointed again to the negative effects of water impurities and bacterial inoculations on the water uptake and bud development of the *roses*. The pressure bomb test revealed the benefits of acidification (pH 3) which extended water potential values to normal ranges varying between −0.2 and −0.4 Mpa (Durkin 1979a).

Table 3. Water potential measurements (Mpa)* of *Rosa* cv. 'Kardinal' peduncles after 96 h of vase life in different water
Wasserpotentialmessungen an Blütenstielen der Sorte 'Kardinal' nach 96 Stunden in Vasenwasser unterschiedlicher Qualität

Vase water pH	Micropore Filtered tap water	Micropore filtered deionized water
Zero samples: vase water not inoculated		
N	− 1.93	− 0.88
3	− 0.29	− 0.46
5	− 0.83	− 0.36
Vase water inoculated with ca 10^6 ml^{-1} *B. subtilis*		
N	− 2.24	− 1.47
3	− 0.59	− 0.29
5	− 1.40	− 1.07
Vase water inoculated with ca 10^6 ml^{-1} *Ps. aeruginosa*		
N	− 1.88	− 1.46
3	− 0.66	− 0.29
5	− 2.30	− 1.27

*) Mean values of duplicate measurements.
N, no pH regulation = pH 6.5–7

Microbiological analysis, at 96 h of vase life

Inoculated vase water of pH 5–7. Plate counts showed increases in the inoculated bacterial cells to approx. 10^7 cfu ml^{-1}. The microflora remained pure and no fungal growth was observed Non-inoculated vase water of pH 5–7. No fungi and some bacterial species were shown to have developed mainly up to $<10^4$ and max. up to 10^5 cfu ml^{-1}. Their origin may have been the rose stem or air borne microflora.

Scanning Electron Microscopy (plates 1–12)

SEM plates of the xylem cross section and the lower parts of the xylem vessels show: (1) the purer the vase water the lower the accumulation of microparticles on the xylem; (2) by buffering of vase water to pH 3 microparticle and bacterial accumulation on the xylem was strongly reduced; (3) reduction of microparticle accumulation was not observed when the vase water was buffered to pH 5 or 7; (4) the latex red test increased the observation of microparticles and bacteria on the xylem cut surface and xylem vessels.

Discussion

Influence on the bud development and vase life of Rosa cv. 'Kardinal' of the composition of vase water.

No bacteria inoculated

When *rose* stems were placed in local water, within two days of the vase life, a sudden 'bent neck' occurred, even when non-inoculated tap vase water was used. The results reported here, confirmed previously executed vase life tests (Durkin *et al.* 1995), showing that the tap water used did contain microscopic particles and particulate matter which collected and accumulated on the cut surface and the lower parts of the xylem vessels of *roses* (Plate 1). Therewith a strong obstruction of the water uptake occurred, disturbing the bud development and decreasing the vase life of the *roses*.

By use of MP filtration, particles >0.47 /µm were essentially removed from the tap water. Particles <0.47/µm however, remained in the filtrate fluid, as shown by SEM of a 0.22 /µm micropore filter through which the MP water was refiltered (Put and Clerkx unpublished). The size of particles on the 0.22 /µm filter was not only smaller, but also the number of particles per volume was much lower than observed on the 0.47/µm MP filter. Not withstanding the large decrease in microparticle numbers and size by 0.47 /µm MP filtration, it resulted in a slightly enhanced water uptake and bud development. The latex red test and the acid fuchsin tests revealed a slightly reduced vessel obstruction, compared to non-treated tap water (Tables 1 and 2). SEM observations however, showed scant adherence onto the cut surface by a small number of very small microparticles and granular material which may indeed have passed the MP 0.47 /µm filter. These could only be made visible by magnifications >2500 X of the SEM samples. The low amount of microparticles however, could not have resulted in the poor bud development and low water uptake reported. Thus, other phenomena may have played here an additional role, such

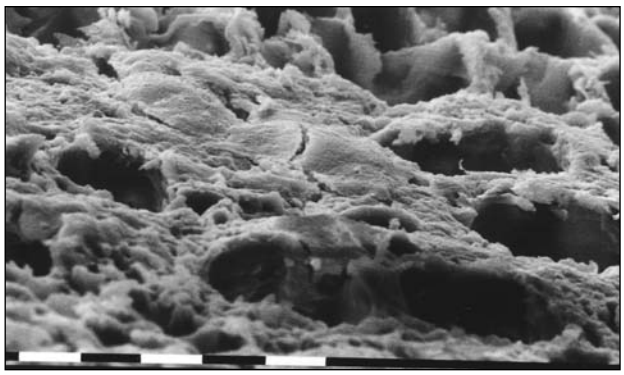


PLATE 1. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 48 h in tap water (pH 6.5). Bar=10/µm.
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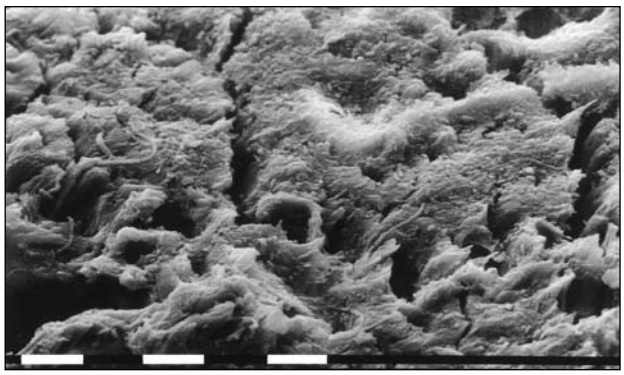


PLATE 2. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 48 h in tap water (pH 6.5) inoculated with 10^6 ml^{-1} *B. subtilis* cells. Bar=10/µm.
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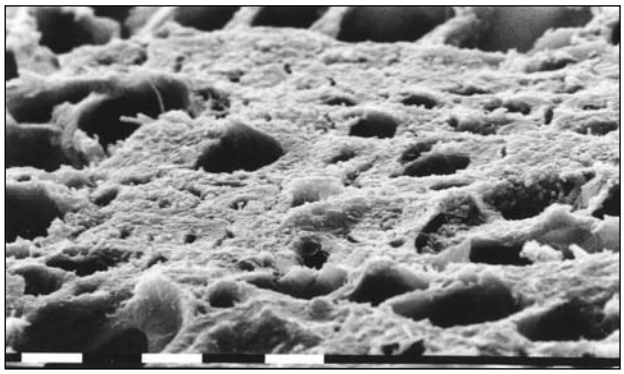


PLATE 3. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 48 h in MP-DI water (pH 6.5) inoculated with 10^6 ml^{-1} *B. subtilis* cells and latex tested. Bar=10/µm.
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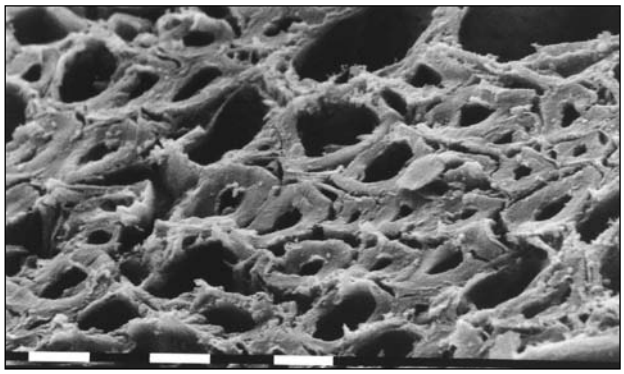


PLATE 4. Cut surface cross section of xylem of a *Rosa* cv 'Kardinal' held at 48 h in MP-tap water at pH 3, inoculated with 10^6 ml^{-1} *B. subtilis* cells and latex tested. Bar=10/µm.
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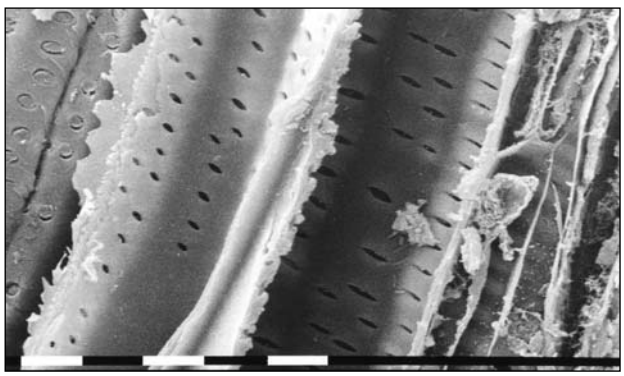


PLATE 5. Length section of the xylem of a *Rosa* cv. 'Kardinal' handled as in **Plate** 4. Bar=10/µm.
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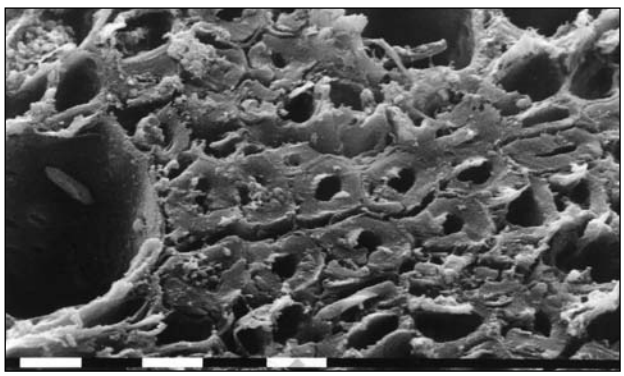


PLATE 6. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 72 h in MP-tap water at pH 3, inoculated with 10^6 ml^{-1} *B. subtilis* cells. Bar=10/µm.
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as adsorption by the flower of injurious materials from the tap water used. Indications for this phenomenon were: chemical analysis of solutes of the tap water and X-ray microanalytical spectra of microparticles on the 0.47/µm micropore filter. These showed the presence in tap water of: e.g. Al, Si, Mg, Fe, C, O and diatom skeletons, pointing to trace metals, organic compounds s.a. *AOC (*easily Assimilable Organic Carbon), colloidal and particulate matter. These microelemental materials, as well as microparticles and particulate matter <0.47/µm, may not have been eliminated by micropore filtration. This then most likely contributed to the observed disturbance pattern of the water balance and *rose* bud development (Halverson and Stacey 1986; Dixon and Peterson 1989; Van der Kooij 1999).

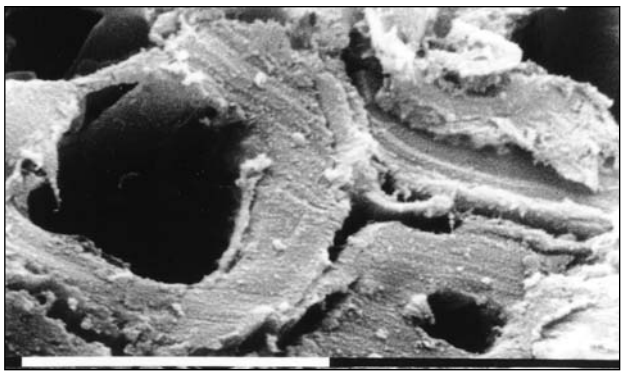


PLATE 7. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 72 h in MP-DI water at pH 3, inoculated with 10^6 ml^{-1} *B. subtilis* cells. Bar=10/µm.
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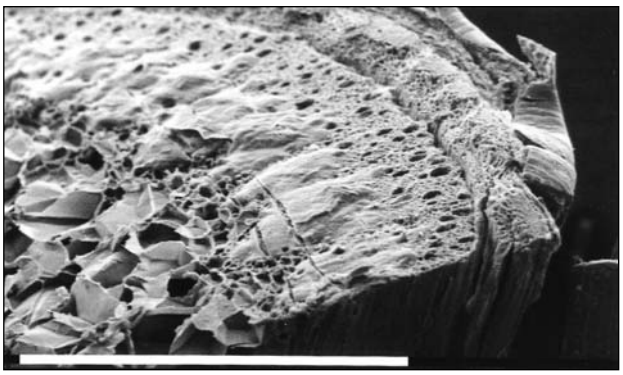


PLATE 8. Cut surface cross of xylem of a *Rosa* cv. 'Kardinal' held at 48 h in MP-tap water (pH 6.5) inoculated with 10^6 ml^{-1} *Ps. aeruginosa* cells, and latex tested. Bar=1 mm.
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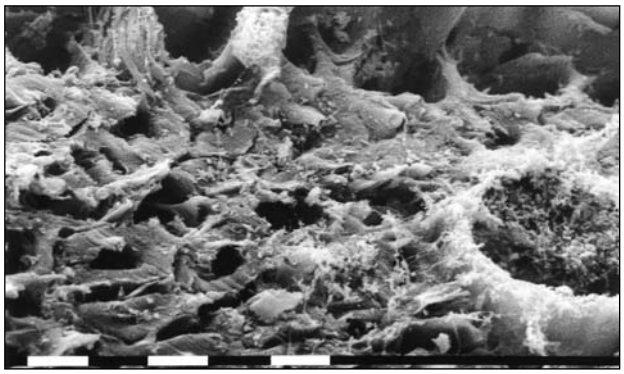


PLATE 9. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 72 h in MP-DI water (pH 6.5) inoculated with 10^6 ml^{-1} *Ps. aeruginosa* cells. Bar=10/µm.
deutsche Abbildungsbeschriftung (bitte auf Disc)??

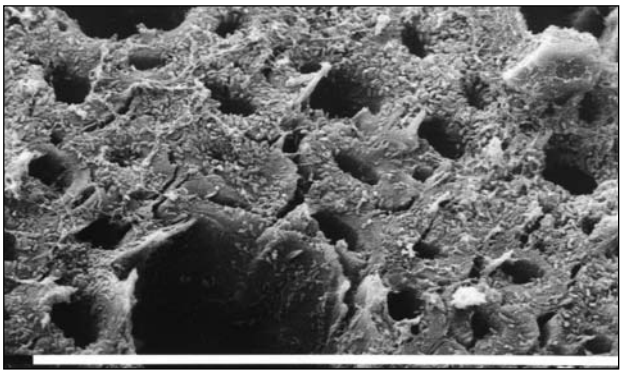


PLATE 10. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 48 h in MP-DI water at pH 3, inoculated with 10^6 ml^{-1} *Ps. aeruginosa* cells, latex tested. Bar=0.1 mm
deutsche Abbildungsbeschriftung (bitte auf Disc)??

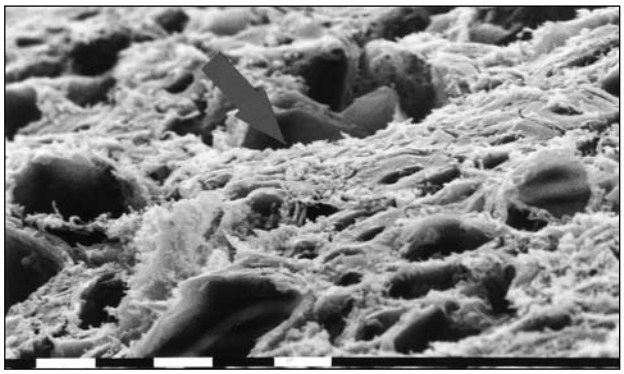


PLATE 10. Cut surface cross section of xylem of a *Rosa* cv. 'Kardinal' held at 72 h in MP-DI water at pH 3, inoculated with 10^6 ml^{-1} *Ps. aeruginosa* cell. Bar=10/µm.
deutsche Abbildungsbeschriftung (bitte auf Disc)??

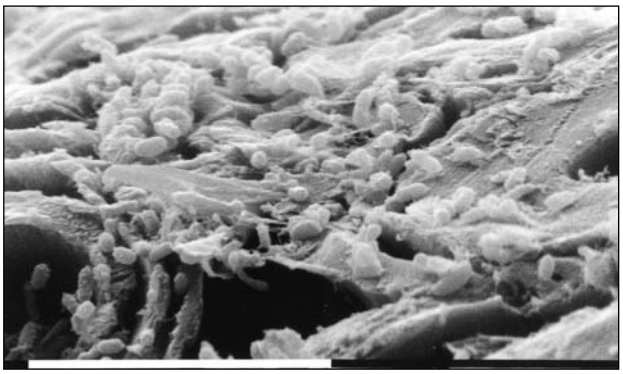


PLATE 12. Enlarged detail of **Plate 11** (arrow). Bar=10/µm.
deutsche Abbildungsbeschriftung (bitte auf Disc)??

The DI process resulted in complete particle removal, even those passing a 0.47/µm micropore filter, removing also an 'unknown' amount of dissolved (ionogenic) salts, colloids and particulate matter thus increasing the water uptake of the *rosa* stems. Physical and physiological obstruction was minimized, and flower bud development was optimized as shown in the results of the latex red test, the acid fuchsin test, the pressure bomb test and SEM images. The lower the pH (pH 3), the purer the vase water (MP-DI), the less the obstruction for water uptake, the more harmonious the bud development of the 'Kardinal' *rose*. The $(PO_4)^{-3}$ concentration for buffering to pH of >5 however was shown to disturb the normal water uptake regulation of the flower. This phenomenon may point to the relatively 'high' phosphate concentration in the buffers at

pH 5 and 7 (10 and 16 mM sodium phosphate resp.) rather than to the pH itself. This showed that the *rose* physiology was influenced by the external (buffer) medium composition, for it occurred almost independent of the purity of the vase water applied. Also by SEM at 2500 X of stem specimen of *roses* from the purest vase water, no particle accumulation on the cross section and xylem vessel walls was shown. Supported by the data already obtained, the beneficial influence upon the *rose* vase life by acidification of vase water to pH 3, was again clearly demonstrated.

Inoculation of bacteria

Buffering at pH 3 of DI and MP vase water, diminished and minimized the obstructive influence on water uptake of *roses* caused by bacterial cells, *B. subtilis* and *Ps. aeruginosa*, inoculated up to $>10^5-<10^6$ ml^{-1} vase fluid (**Plates 2–7**, *B. subtilis*; **Plates 8–12**, *Ps. aeruginosa*).

The latex red test, at 48 h of vase life, reinforced considerably the visibility of microparticles adhering on the xylem vessels of *roses*, infiltrating and mainly blocking the smaller xylem vessels. Differentiation between the adherence of *Rosa* stems placed in: tap, MP-tap and MP-DI, non buffered vase water, versus buffered vase water at pH 3, became therewith more clearly pronounced and observable and thereby simplified differentiation of bacterial cells and microparticles (**Table 1**; SEM **Plates 3, 4 and 5**, *B. subtilis;* **Plates 8 and 10**, *Ps. aeruginosa*).

The pH 3 vase life maximization of *roses* was visually shown to be related to deprivation or diminishing of microparticle adherence, including bacterial cells, onto the *Rosa* xylem vessel cross wall (**Plates 4–7**, *B. subtilis;* **Plates 10–12**, *Ps. aeruginosa)* as well as into the lower parts of the xylem vessels (**Plate 5**, *B. subtilis*). Infiltration of *Ps. aeruginosa* cells at pH 3 was reduced from obstruction of vessels by a mass of bacterial cells into a scarce infiltration of free cells and a few micro cell clusters (**Plate 5**, *B. subtilis).* Images made at 72 h vase life were comparable with those obtained after 48 h vase life (**Plates 6 and 7**, *B. subtilis;* **Plates 11 and 12**, *Ps. aeruginosa).*

Multiplication, accumulation and adherence of bacterial cells on stem xylem cross sections was somewhat higher when *Pseudomonas*, than when *Bacillus* was inoculated in vase water. Factors needed for growth and multiplication of both species differ essentially. *Ps. aeruginosa* achieves growth and biofilm formation using a single organic substrate at a low concentration as the sole carbon and energy source (AOC), Van Der Kooy (1999). *B. subtilis*, however, requires a more complicated substrate for multiplication. The generation time (doubling time) of *Ps. aeruginosa* cells may therefore have been shorter than that of *B. subtilis* cells under the same growth conditions. On the contrary, *Ps. aeruginosa* grows poorly, does not form oxidized products or slime when oxygen is not readily available, while growth of *B. subtilis*, is not restricted by submerged conditions. Besides, the structure and composition of the cell envelope of Gram-positive *Bacillus* and the Gram-negative *Pseudomonas* differ essentially as well as the conditions for their formation (Poxton, 1993).

Ps. aeruginosa and vegetative cells of *B. subtilis* are non- acidophilic. These bacteria lose their viability at pH 3, their motility, their cell wall membrane protection, probably also their cell wall electrical (-) charge may change and consequently also their adhesivity onto the *rose* xylem cross wall and vessel wall (Van Loosdrecht *et al.* 1989). The plant's xylem, submerged in a pH 3 vase fluid, on the other hand, may have lost its attractiveness towards bacterial cells, bacterial spores, microparticles and colloidal compounds, causing the stem base to repel microparticles or to prevent their association to sizes large enough to interfere with solution uptake (Fletcher 1996).

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