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# Hydroxyquinoline Citrate and Low pH Prevent Vascular Blockage in Stems of Cut Rose Flowers by Reducing the Number of Bacteria

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***Abstract.*** **Stems of cut rose flowers (Rosa *hybrids* L., cvs. Sonia, Ilona, Polka, and Frisco) were held in a sodium hypochlorite solution and then placed in distilled water or in a buffer at pH 6.0. After 2 days, many bacteria were found in the basal end of the stems, even when the number of bacteria in the water was below the detection limit. The hydraulic conductance of 5-cm stem segments was reduced whenever the number of bacteria exceeded =$10^6$ cfu/g fresh weight. Adding HQC or a buffer at pH 3.0 limited the number of bacteria in stems. Hydraulic conductance of the stems held in these solutions for 2 days was as in stems of freshly harvested flowers. Thus, HQC and low pH prevent vascular blockage by reducing the number of bacteria in the stems. No evidence was found for the hypothesis that HQC and low pH inhibit a stem-induced vascular blockage.**

During the vase life of cut rose flowers, the basal parts of the stems become blocked, reducing the uptake of water (Mayak et al., 1974; de Stigter, 1980, 1981). The nature of the occlusion in the stems, however, is not clear. It has been supposed that the occlusion is due partly to air embolism (Durkin, 1979), the presence of microorganisms (Aarts, 1957), or to processes in the stem induced by cutting.

Although the literature often refers to a role for physiological processes in vascular blockage of cut roses, the evidence supporting this concept is scarce. Some authors showed that the xylem of cut rose flowers contained amorphous plugs (Burdett, 1970; Lineberger and Steponkus, 1976), but others have seriously questioned the relevance of these plugs for vascular blockage, as they were found in only a few xylem elements (Rasmussen and Carpenter, 1974).

The most direct evidence for a role of physiological processes in the xylem blockage of cut rose flowers was given by Marousky (1969, 1971), 'Better Times' rose stems were placed in 3% to 5% chlorine bleach (sodium hypochlorite) and then in sterile water. After 2 days, no bacteria were found in the vase water. Nevertheless, when 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. The author, therefore, inferred that the differences between the treatments indicated a physiological blockage in the stems.

The cut surface of rose stems was found to produce considerable amounts of ethylene (van Doom et al., 1989), and ethylene induced the presence of vascular plugs in *Ricinus communis* (VanderMolen et al., 1983). Olien and Bukovac (1982) reported that ethephon treatment resulted in a blockage in xylem vessels of *Prunus cerasus* (Rosaceae). HQC is known to inhibit the production of ethylene in carnations (Parups and Peterson, 1972) and to inhibit ethylene production by the cut surface of rose stems (van Doom et al., 1989). After the stems are cut, an impermeable layer of suberin-like material maybe deposited on the cut surface (Cline and Neely, 1983). Enzymes involved in polymerization processes leading to deposition of lignin and suberin are inhibited at low pH (Vamos Vigyazó, 1981). Both HQC and a low pH may therefore inhibit vascular blockage that results from a wound reaction.

We repeated the experiments described by Marousky (1969, 1971) using four rose cultivars. The number of bacteria in the vase solution was determined, as well as the number of bacteria associated with the stems.

## Materials and Methods

*Plant material.* 'Sonia', 'Polka', and 'Frisco' rose flowers were obtained from a commercial grower, and 'Ilona' from a greenhouse of the Agricultural Univ. at Wageningen. Roses obtained from the commercial grower were kept dry after cutting, stored at 4C, and brought to the laboratory within 3 to 4 hr. Roses obtained from the greenhouse were brought to the laboratory within 30 min after cutting.

Upon arrival in the laboratory, all but the top seven leaves were removed, In some preliminary experiments, stems were recut with a nonsterile knife and placed in sterilized bottles containing sterilized water. Experiments on the effect of HQC were according to the methods described by Marousky (1971). Briefly, glass bottles (containing 150 ml of water) were autoclave for 20 min at 120C. Sucrose (30 g·liter$^{-1}$) was added before autoclaving, and HQC (200 mg·liter$^{1}$, 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. Flowers were placed with the lower 15 cm immersed in the hypochlorite solution. Stems were then placed in autoclave 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.

The pH experiments were according to Marousky (1971). The protocol in these experiments was slightly different from the HQC tests (Marousky, 1969). Bottles with 150 ml of a citrate-phosphate buffer solution at pH 3.0 or pH 6.0 were autoclave for 20 min at 120C. The basal 5 cm was cut from the stems and stems were placed in 5% sodium hypochlorite solution for 5 rein, after which another 5 cm of the stem was cut and stems were placed once more in the 5% sodium hypochlorite solution

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Abbreviations: cfu, colony-forming units; HQC, hydroxyquinoline citrate.

level 15 cm) for 5 min. The stems were then placed in the autoclave solutions at a level of 7 cm. A cotton plug around the stem closed the bottles. At the end of the experiments, the pH of the solutions was checked with a Philips PW 9410 meter (pH measurements were made after, taking samples for measurement of the number of bacteria in the water). All experiments included ten 40-cm-long replicate flowers, placed in individual bottles. The ambient temperature was 20C, RH was 60%, and photosynthetically active quantum flux at leaf level was 15 $\mu mol \cdot s^{-1} \cdot m^{-2}$ for 12 hr/day. Experiments were conducted four times.

*Bacteria.* The number of bacteria in the water was determined after 2 days by plating a water sample on Plate Count Agar (Oxoid, Basingstoke, Hants., U.K.). After incubation at 30C for 48 hr, the number of colonies was counted.

The number of. bacteria associated with 5-cm stem segments was measured by peeling off the bark with sterile equipment and cutting the stem into small segments. The stem segments were placed in a sterile plastic bag and weighed. Ten times this weight of sterile 0.870 NaCl solution was added. The bags were placed in a Stomacher apparatus for 1 min (Sharpe and Jackson, 1972). The solution was then plated on Plate Count Agar with a spiral plate machine (model C, Spiral Plate Systems, Cincinnati). After incubation at 30C for 48 hr, the number of colonies was counted. Measurements were made on each of three stem segments at the start of experiment and after 2 days.

*Hydraulic conductance.* Conductance of the basal 5 cm of the stems was determined as described by van Doom et al. (1989). Conductance measurements were made on six stems not used for measurement of the number of bacteria. The data were compared by analysis of variance and LSD test.

## Results

*Relationship between the number of bacteria in vase water and in stems.* In the preliminary experiments with 'Sonia' roses, no bacteria were found in the water for several days, 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 $3 \times 10^6$ cfu/g fresh weight before bacteria were found in the water (Table 1).

*Effect of hydroxyquinoline citrate.* After 2 days of vase life, the number of bacteria in the vase water generally remained below the level of detection. When bacteria were present in the water, the flowers were not used for determination of hydraulic conductance, Even when no bacteria were present in the water, many bacteria (up to $10^6$ cfu/g fresh weight) were 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 2). 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 2).

Table 1. 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 (vases 1 and 2).

| | No. of bacteria in vase water (cfu/ml) | | No. of bacteria in the basal 5 cm of stems (cfu/g fresh wt) | |
|---|---|---|---|---|
| Day | 1 | 2 | 1 | 2 |
| 0 | 0 | 0 | $<1.2 \times 10^2$ | $<1.2 \times 10^2$ |
| 0 | 0 | 0 | $1.2 \times 10^3$ | $2.2 \times 10^3$ |
| 4 | 0 | 0 | ND[z] | ND |
| 7 | 0 | $7 \times 10^3$ | $4.8 \times 10^4$ | $3.0 \times 10^6$ |

[z]ND = Not determined.

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

*Effect of low pH.* The number of bacteria in vase water of cut roses that were treated twice with sodium hypochlorite, and then treated with buffer at pH 3.0 and 6.0, was invariably below the detection limit. The number of bacteria in stems of flowers treated at pH 3.0 was also below the detection limit, whereas the number of bacteria in stems of flowers held at pH 6.0 was variable. The highest number was $2.2 \times 10^4$ cfu/g fresh weight (Table 3).

Hydraulic conductance of stem segments from the pH 6.0 treatment was the same as those from the pH 3.0-treated flowers and from freshly harvested stems (Table 3).

## Discussion

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 are present in the xylem vessels and the cut surface. Hydraulic conductance of the stem was found to be decreased when the number of bacteria was $=10^6$ cfu/g fresh weight (Table 2) or higher (results not shown).

In stems of cut 'Red American Beauty' rose flowers, Lineberger and Steponkus (1976) found bacteria close to the cut surface and amorphous plugs higher up the stem. Since staining reactions indicated that no bacteria were present in the amorphous material, the latter was thought to be a physiological occlusion. The amorphous material was mainly found at =10 cm above the water level in the vase. In our experiments (water level at 7 cm), the hydraulic conductance in the stem segments above the water level was the same or only slightly lower than in stem segments from freshly harvested flowers [data on 'Sonia' and 'Ilona' roses in van Doom et al. (1989); unpublished data on 'Polka' and 'Frisco' roses]. This result indicates that the plugs in the segments above the water level, if present, did not appreciably affect the passage of water. Our data indicated that only a few xylem vessels contained these plugs (van Doom et al., 1989). Instead, we found that the basal 5 cm of the stems limited the flow of water and that this blockage was correlated with a high number of bacteria in this stem segment (van Doom et al., 1989).

The experiments of Marousky (1969, 1971) on cut 'Better Times' roses were based on the observations of Kuc (1964), who worked with the same cultivar. She found a decline in the uptake of water, even under apparently sterile conditions. Flasks and water were autoclave and rose stems were placed in a 3% solution of commercial bleach for 5 min before placing them in the vase water. Recutting the stems under water largely prevented the decline in water uptake rates. She streaked swabs from the upper, middle, and lower portions of the stems onto potato dextrose agar (pH 5.0) and proteose agar (pH 7.0). Because no microorganisms were detected, it was concluded that microbial contamination could not be the explanation for the decrease in water uptake, and a physiological blockage was inferred. The method used to detect bacterial colonization of the stem was not described in detail. The method might have failed to show the presence of bacteria.

As we did not investigate 'Better Times' roses, the present

Table 2. Number of bacteria and hydraulic conductance in 5-cm segments of stems from cut 'Sonia' roses, held in various solutions for 2 days. No bacteria were present in the vase water.

| Stem segment (cm) | Treatment | | | |
|---|---|---|---|---|
| | Control | HQC | Sucrose | HQC + sucrose |
| | *No. of bacteria (cfu/g fresh wt)* | | | |
| 0–5 | $8.4 \times 10^5$ | $<1.2 \times 10^2$ | $9.2 \times 10^5$ | $<1.2 \times 10^2$ |
| 5–10 | $5.4 \times 10^4$ | $<1.2 \times 10^2$ | $4.1 \times 10^4$ | $<1.2 \times 10^2$ |
| | *Hydraulic conductance (ml/30 min)*[z] | | | |
| 0–5 | 2.6 ± 0.5 a | 3.5 ± 0.4 b | 2.0 ± 0.2 a | 3.4 ± 0.6 b |
| 5–10 | 3.6 ± 0.4 b | 3.2 ± 0.6 b | 3.4 ± 1.0 b | 3.8 ± 0.4 b |

[z]Mean separation for six replications (± SD) at $P < 0.05$. The mean (± SD) hydraulic conductance of freshly harvested stems was 3.4 ± 0.5 and 3.6 ± 0.4 ml/30 min at 0-5 cm and 5-10 cm from the cut surface, respectively.

Table 3. Number of bacteria and hydraulic conductance in the basal 5-cm segment of stems of cut rose flowers, briefly held in a chlorine bleach solution, and then at pH 6.0 or pH 3.0 for 2 days.

| | No. of bacteria (cfu/g fresh wt) | | Hydraulic conductance (ml/30 min)[z,y] | | |
|---|---|---|---|---|---|
| Cultivar | pH 6.0 | pH 3.0 | Day 0 | pH 6.0 | pH 3.0 |
| Sonia | $1.4 \times 10^3$ | $<1.2 \times 10^2$ | 3.1 ± 0.4 b | 2.8 ± 1.4 b | 3.4 ± 1.0 b |
| Ilona | $<1.2 \times 10^2$ | $<1.2 \times 10^2$ | 2.0 ± 0.4 a | 2.1 ± 0.7 a | 1.9 ± 0.2 a |
| Polka | $<1.2 \times 10^2$ | $<1.2 \times 10^2$ | 1.7 ± 0.5 a | 1.6 ± 0.3 a | 2.0 ± 1.0 a |
| Frisco | $2.2 \times 10^4$ | $<1.2 \times 10^2$ | 3.1 ± 0.9 b | 3.3 ± 1.0 b | 3.2 ± 1.2 b |

[z]Mean separation for six replications (± SD) at $P < 0.05$.
[y]Analyses for pH 6.0 and 3.0 after 2 days of holding at 20C.

results do not exclude the possibility of a detectable physiological blockage in this cultivar. In the cultivars we investigated, however, a blockage due to physiological processes, if present, did not appreciably decrease the hydraulic conductance of the stems.

In our pH experiments, a number of bacteria high enough to reduce hydraulic conductance was not reached. In these experiments, the stems were placed twice in sodium hypochlorite, whereas they were placed once in sodium hypochlorite in the HQC experiments. In various repeat experiments, our controls (pH 6.0 or no HQC) showed a variable number of bacteria. We suspect that this variability relates to the age of the bleach and/or to the composition of the bacterial flora. Marousky (1971) had to discard =1070 of the flowers at pH 3.0 and a higher percentage of flowers at pH 6.0 because the solutions were contaminated. In our pH experiments, contamination of the water was not found in any of the bottles, including the controls at pH 6.0. Thus, bacterial growth was more adequately suppressed in the present pH experiments, and our methods may explain why we did not find a decrease of hydraulic conductance at pH 6.0, while Marousky (1971) found a clear reduction in hydraulic conductance.

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