Sources of the Bacteria Involved in Vascular Occlusion of Cut Rose Flowers

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ABSTRACT. Cut rose (*Rosa hybrida* L.) flowers placed in water often wilt prematurely, which is partially due to bacterial accumulation in the stems. Bacterial strains in the stems are mainly pseudomonads and enterobacteria. The possible sources of these organisms were investigated in 'Sweet Promise' (trade name Sonia) roses. No bacteria were found in the xylem of intact plants. Cutting the stems with sterile secateurs introduced no bacteria at the cut surface or the stem interior, but cutting with nonsterile secateurs used by rose growers did. The secateurs sampled at rose growers contained *Enterobacter agglomerans* along with several other bacteria not found inside the xylem of cut flowers but did not contain pseudomonads. Although the plant surface may contain bacteria, freshly cut stems placed in water introduced no bacteria. Bacteria rapidly developed on the cut surface and inside the water-conducting elements when rose stems were placed in tap water, even when the stems had been surface-sterilized. However, there were no bacteria in vase water when the water and the stem surface had been sterilized. Since the stem and the secateurs are not a main source of bacteria inside stems and tap water contains pseudomonads and *Enterobacter* spp., we conclude that tap water is the main source of the bacteria inside cut rose stems.

Cut rose flowers placed in water often wilt prematurely because of an occlusion in the xylem conduits at the basal end of the stem (Mayak et al., 1974). The occlusion is mainly due to rapid accumulation of bacteria at the cut surface and in the xylem combined with the effect of cavitated xylem conduits (van Doorn, 1997). Bacterial strains found in stems that had been in water for some days included pseudomonads (≈80% of the total number of colonies); enterobacteria (≈5% to 10%); and some infrequently occurring genera, such as *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Citrobacter*, and *Flavobacterium* (van Doorn et al., 1991).

Bacteria have been found in xylem conduits of intact plants (Gardner et al., 1982) and also may be present in the xylem of flowering rose stems before harvest. Bacteria also are found on plant surfaces (Billing, 1976; Blakeman and Brodie, 1976; Klinecare et al., 1971) and, hence, may be transferred to the cut end by the secateurs. Cell sap, usually found on the secateur blades, may be a good medium for bacterial growth, which may lead to selection of specific strains that subsequently can be introduced at the cut surface.

Shortly after harvest, the flowers generally are placed in tap water for rehydration, which will be repeated by retailers and consumers. Several bacterial strains have been identified in tap water (Clark et al., 1982), and these strains also may be a source of the strains associated with vascular occlusion.

Our investigation was aimed at establishing the sources of the bacterial population found in stems of cut rose flowers during their vase lives.

Materials and Methods

PLANTS. Commercially mature cut flowering rose stems ('Sweet Promise', trade name Sonia) were obtained from commercial growers in The Netherlands or from the Dept. of Horticulture, Agricultural Univ., Wageningen, The Netherlands.

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BACTERIAL POPULATIONS IN THE XYLEM OF INTACT PLANTS. Stems, ≈100 cm long, were cut in the greenhouse and brought dry to the laboratory within 30 min of cutting. A sterile razor blade was used to cut 30 cm off of both ends. The bark of the remaining segment was peeled off aseptically and, using standard sterile equipment, stem internodes were cut into 1-cm segments that were split longitudinally. The segment halves were incubated in tryptone glucose yeast extract broth (TGYEB) (Oxoid, Basingstoke, Hants, U.K.) or tryptone soya broth (TSB, Oxoid) for 7 d at 30 °C. The broth then was plated on plate count agar (PCA) (Oxoid), and the number of bacteria was determined as described by van Doorn et al. (1989). This experiment was performed six times at various times of the year.

BACTERIAL POPULATIONS ON THE STEM AND LEAF SURFACE. Individual leaflets and 20-cm-long stem segments of greenhouse plants were cut using a sterile razor blade, and the parts were placed individually in sterile plastic bags and transported to the laboratory. Using standard sterile equipment, sections 1 cm long were cut from the stem internodes. The bark of these sections was pressed on the surface of freshly prepared PCA by rolling the segment until the whole circumference of the stem had been in contact with the agar. Similarly, sections from the leaflets were pressed on PCA. The plates were incubated at 30 °C for 48 h.

Table 1. Effect of the secateurs, stem surface, vase water, and vases on the number of bacteria in the basal 5 cm of the stems of cut, flowering Sonia roses after placing stems in vase water for 3 d at 20 °C.

Treatment	Bacteria in the basal 5 cm of stems, fresh mass basis (cfu/g) ^a
Cut with sterile secateurs, placed in sterile water (and sterile vase)	<1.6 × 10 ²
Cut with nonsterile secateurs, placed in sterile water (sterile vase)	5.1 × 10 ³
Cut with sterile secateurs, placed in fresh tap water (nonsterile vase)	8.3 × 10 ⁶
Cut with sterile secateurs, placed in fresh tap water (sterile vase)	1.9 × 10 ⁷

^aThe detection limit was 1.6 × 10².

The previously described procedure also was followed after stem and leaf segments had been held in high relative humidity at 23 °C for 48 h by placing them in a closed vial lined with wet filter paper, according to the method of Watson and Koons (1973).

The number of bacteria transferring to the solution was measured after plant material incubation in TSB. Leaflets, stem internodes, and nodes with one attached petiole were selected randomly in a greenhouse, cut with sterile equipment, placed in TSB, and kept at 20 °C for 16 h. After thorough shaking, a sample of the broth was plated on PCA and incubated at 30 °C for 48 h.

Plant material also was placed in sterilized distilled water and was ground using a Waring blender. The suspensions were plated on either PCA, glutamic acid-starch-phenolred agar to which penicillin and pimaricin were added (Kielwein, 1969), or TSB with agar no. 3 (Oxoid) and crystal violet at 12 g and 2 mg·L⁻¹, respectively. These plates were used to select aerobic bacteria, *Aeromonas* and *Pseudomonas* species, and enterobacteria plus pseudomonad species, respectively (Kielwein, 1969; Oxoid, 1980).

There were five replications of the previously described experiments, and each experiment was repeated at least once.

DETERMINATION OF THE BACTERIAL POPULATIONS AT THE CUT SURFACE AND INSIDE THE STEMS. The number of bacteria in the xylem conduits and at the cut surface was determined in 5-cm stem segments as described by van Doorn et al. (1989). To separate the cut surface from the stem interior, in some experiments, the bark was peeled off and the basal 1 to 2 mm of the stems were separated from the next 4.8 to 4.9 mm using a sterile razor blade. The lowermost 1-mm segment was placed in 10 mL 0.8% NaCl and was ground in a mixer at 14,000 rpm for 1.5 min, after which the suspension was placed on PCA. Incubation was at 30 °C for 48 h.

EFFECT OF CUTTING ON BACTERIAL POPULATIONS AT THE CUT SURFACE. Stems were cut with sterile equipment and were pressed on the surface of PCA, which was incubated using standard procedure. Other stems were surface-sterilized using cotton and 98% ethanol or were left unsterile and then were cut with a pair of secateurs (Leyat, Lausanne, Switzerland) whose blades were surface-sterilized with 98% ethanol or left unsterile. The secateurs were used by a rose grower. Another series of stems were cut with a rotating device (Metabo, Nürtingen, Germany) used in horticultural practice for grading rose stems. The cut surface of these stems was pressed on PCA, and the number of bacteria in the basal 1 mm of the stems was determined after grinding the stem material as previously described. There were five replications, and experiments were repeated once.

In another series of experiments, the secateurs were used with

or without sterilization and flowers were placed in sterile tap water in vases that had been sterilized before use. The number of bacteria in the basal 5 cm of the stems was determined after 3 d at 20 °C. There were five replications, and the experiments were repeated once.

BACTERIAL POPULATIONS ON SECATEURS. Secateurs were sampled at two commercial greenhouses, one pair of secateurs per business, using cotton swabs that were rolled over the cutting blade. Swabs were stored in a vial with 5 mL peptone physiological salt solution and kept in ice during transport to the laboratory. The secateurs were sampled using three replications in time. Each time the secateurs were in full use for harvesting roses and had been in use for several weeks before sampling. The bacteria were grown on various media as previously described.

EFFECT OF BACTERIA IN WATER. Flowering rose stems were cut with sterile secateurs and placed in sterilized bottles containing either tap water or sterilized tap water. Another series of stems was placed in water at the growers' premises for 3 d. This water was held at 4 °C and had been routinely used for rehydration of cut flowering rose stems; it contained 3×10^6 colony forming units (cfu) of bacteria per milliliter at the onset of the experiment. There were five replications, and the experiment was repeated once.

IDENTIFICATION OF BACTERIA. Bacteria originating from the surface of the leaves and the stems of rose plants from the cut surface or xylem interior were selected using the methods previously described and were grown to pure culture. Twenty random isolates from secateurs at two rose growers (one per grower) were grown in tryptone yeast medium for 36 h, washed with physiological salt solution, centrifuged, and resuspended in physiological salt solution. All strains were characterized using the Biolog system as described by Klingler et al. (1992). Additional tests, if required, were made using the Enterotube (Oberhofer, 1979) and the Oxi/Ferm (Oberhofer, 1979) systems and the tests to validate a determination as described for the various species in Bergey's Manual (Buchanan and Gibbons, 1974).

Results

BACTERIA IN THE XYLEM OF INTACT ROSE STEMS AND ON THE SURFACE OF STEMS AND LEAVES. Isolated stem segments whose bark was removed and that were cut longitudinally showed no bacterial growth, even when they were incubated in favorable media (TGYEB or TSB) for 7 d at 30 °C. Pressing the surface of stem segments, without removal of the bark or the leaflet surface, on PCA did not result in bacterial growth, even when this was performed after the plant material had been held at saturating moisture and 23 °C for 48 h.

After segments of stems or leaves were placed in TSB at 10 °C for 16 h and the broth subsequently was incubated on PCA, some bacteria grew. Some strains were catalase and Gram-positive cocci and were identified as *Staphylococcus xylosus*; other strains were aerobic spore-forming rods identified as *Bacillus* sp.

EFFECT OF CUTTING WITH STERILE OR NONSTERILE BLADES. After a surface-sterilized stem was cut with a pair of sterilized secateurs, the number of bacteria per gram of fresh mass introduced on the cut surface and in the xylem interior remained below the detection limit (1.6×10^2 cfu) and pressing the cut surface on PCA did not cause bacterial growth. We found the same result when the stem surface was not sterilized.

When the stems had not been surface-sterilized before cutting with a nonsterile pair of secateurs in use for rose harvesting or with a nonsterile rotating cutting device that was used in practice, the number of bacteria introduced still was under the detection limit,

Table 2. The bacteria in water used for storage of cut, flowering Sonia roses at the premises of three commercial growers and the bacteria in the basal 5 cm of the rose stems held in water for 3 d at 1 or 4 °C.

Grower	Bacteria in water (cfu/mL) ^{z,y}	Bacteria in stems, fresh mass basis (cfu/g) ^{z,y}
A	2.0×10^6	5.8×10^5
B	8.5×10^2	1.4×10^4
C	3.5×10^5	3.8×10^5

^zThe detection limit was 1.6×10^2 .

^yNumbers are the average of two sampling dates. Grower A (water held at 4 °C) used aluminum sulfate as an antimicrobial compound in the water, which had not recently been renewed; Grower B (water held at 4 °C) treated water with a commercial ultra violet system for bacterial control; and Grower C (water held at 1 °C) did not use an antimicrobial treatment.

but, after the cut surface was pressed on TCA, a few colonies were noticeable at the imprint of the cut surface edge.

Nonsterile secateurs introduced a low but detectable number of bacteria in the basal 5 cm of the stems, when sampling occurred after the roses had been in water for 3 d (at 20 °C) after cutting (Table 1).

EFFECT OF PLACING THE STEMS IN WATER. There was a low bacterial count in stems that had been cut with sterile secateurs and placed for 3 d at 20 °C in a sterile vase with sterile water (Table 1). Bacterial counts were high when the stems were surface-sterilized, cut with sterile secateurs, and placed in a nonsterile vase with (nonsterile) tap water (Table 1). Similar bacterial numbers were associated with the cut surface and the stem interior after surface-sterilized stems were cut with sterile secateurs and placed in a sterilized vase but nonsterile tap water (Table 1).

In horticultural practice, cut flowering rose stems often are stored or transported in water at low temperatures. After 3 d of such low-temperature storage (1 or 4 °C) at two rose growers' premises, the number of bacteria in the stems was high and reflected the number in the water (Table 2).

BACTERIA ON SECATEURS. Secateur blades, sampled during rose harvesting, contained 10^4 to 10^5 cfu per square centimeter (Table 3). The flora on the secateur blades contained no pseudomonads, but *E. agglomerans*, along with several other species, were identified (Table 4).

Discussion

The experiments showed no bacteria in stems of intact Sonia roses. Although bacteria may reside in the xylem conduits of other plant species, they apparently can be excluded as a source of bacteria involved in vascular occlusion of cut Sonia roses. A second source could be the strains transferred from the stem surface to the cut surface by the secateurs. However, cutting with a sterile razor blade or with sterilized secateurs did not result in inoculum proliferation, indicating that the chance of transferring bacteria from the stem surface is low.

When stems, with or without leaves, are placed in water, bacteria from the plant surface may transfer to that water. Nevertheless, the results suggest that this transfer normally does not occur. Placing stems or leaves in sterile water did not result in bacterial growth, even when the bacteria were first allowed to multiply on the plant surface in a high-humidity environment, a method previously used for detecting bacteria on the surface of chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitamura] leaves (Watson and Koons, 1973). Only after placing stem parts or leaves of Sonia roses in TSB was there some bacterial growth, but this medium does not reflect the conditions in vase water, which is extremely nutrient-poor compared to the broth.

We identified the species originating from stems and leaves and growing in TSB as *Staphylococcus* and *Bacillus*. Cocci, identified as *Micrococcus* (Klincare et al., 1971) and *Bacillus* spp. also have been observed on aerial surfaces of other plants (Blakeman and Brodie, 1976). Using a surfactant (0.05 % v/v Tween), bacteria have been isolated from the surface of Sonia rose stems, and the isolated strains were identified as *Alcaligenes*, *Citrobacter*, *Enterobacter*, and *Pseudomonas*. The latter genus was found only occasionally, and the other strains even less frequently (Put, 1990). We were unable to isolate any bacteria from the stem using a nutrient-poor aqueous medium without a surfactant. Although all four genera that have been previously isolated from the plant surface are associated with vascular occlusions, the fact that a surfactant was required to isolate them indicates that they normally

Table 3. Bacterial counts on the secateur blades at two Sonia rose growers; three replications in time (1 to 3) were used.^z

Sampling date	Bacterial count (cfu/cm ²) ^y	
	Grower A	Grower B
1	1.2×10^4	2.8×10^5
2	2.4×10^4	2.2×10^5
3	1.4×10^4	4.7×10^5

^zWhen sampled, the secateurs were in use for harvesting roses. Bacteria were sampled using cotton swabs and were grown on plate count agar. cfu = colony forming units.

^yThe detection limit was 0.8×10^2 .

Table 4. Identification of bacteria found on the blade of secateurs used for cutting Sonia roses at the premises of two growers.^z

Bacterial species	
Grower A	Grower B
<i>Acinetobacter radioresistens</i> (2) ^y	<i>Corynebacterium variabilis</i>
<i>Enterobacter agglomerans</i>	<i>Enterobacter agglomerans</i>
<i>Klebsiella pneumonia</i>	<i>Photobacterium phosphoreum</i>
<i>Leuconostoc mesenteroides</i>	<i>Shewanella putrefaciens</i>
<i>Pediococcus pentosaceus</i> (4) ^y	<i>Streptococcus equinus</i>
<i>Yersinia enterocolica</i>	<i>Xanthomonas campestris</i> (2) ^y
<i>Xanthomonas campestris</i> (4) ^y	
<i>X. oryzae</i> (2) ^y	

^zOf the 20 isolates sampled at each greenhouse, 16 could be identified at grower A's greenhouse, only seven at grower B's greenhouse.

^yThe number in parentheses shows the number of isolates determined to belong to a species if more than one isolate of that species was found.

do not transfer from the plant surface into vase water. Therefore, we conclude that the plant surface is not a major source of the bacteria involved in vascular occlusion.

The secateurs are another possible source of bacterial inoculum. When rose stems were cut with nonsterile secateurs and then placed in a sterile aqueous solution, some bacteria, but not many, were associated with the lowermost 5 cm of the stems. Secateurs blades used for harvesting Sonia roses did contain many bacteria (10^4 to 10^5 cfu per square centimeter), but, among the various strains, we found no pseudomonads. Therefore, the secateurs seem not to be a main source of inoculum. The blades did contain *E. agglomerans*, which is the main *Enterobacter* species found on the cut surface and inside rose stems (van Doorn et al., 1991). Although this species also occurs in water (Labonde and Festy, 1979), the secateurs could be a source.

The fourth possible source of bacteria is water. We found that predominantly *Pseudomonas* species and $\approx 10\%$, at most, *Enterobacter* species, were associated with the stems. These genera frequently have been found in tap water (Labonde and Festy, 1979; van der Kooij, 1977, 1984). Several strains of *Pseudomonas* grow in tap water even when it contained only trace amounts of organic compounds (van der Kooij et al., 1982a, 1982b). Apart from tap water, we identified no other source of pseudomonads.

Since pseudomonads represent most bacteria involved in vascular occlusion and they are present in tap water, we conclude that tap water is the main source of the bacteria associated with vascular occlusion. Tap water and secateurs are possible sources of *Enterobacter* species. Chance contaminants associated with vascular occlusion, such as *Alcaligenes*, *Bacillus*, and *Citrobacter*, have been isolated from aerial surfaces of the stems and leaves of

Sonia roses. Although other sources of these strains cannot be excluded, the difficulties experienced in removing them from the plant surface may account for their rare occurrence in vase water and stems.

Measures to prevent excessive bacterial growth in water and rose stems should be directed first against the pseudomonads in the water in which the stems are placed, either directly after harvest or by wholesalers, retailers, or consumers. Antimicrobial compounds included in water, such as chlorine bleach or aluminium sulfate, when used at the appropriate concentrations, can reduce the total bacterial count in rose stems adequately (van Doorn et al., 1990) and, hence, are active against the pseudomonads.

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