# Effects of Electrolyzed Neutral Water on the Bacterial Populations in a Flower Vase and in Stems of Cut Roses

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#### Summary

Stems of the flowering rose, 'Asami Red' were placed in 20 °C tap water (control) or electrolyzed neutral water (ENW; pH 6.8, 20 ppm available chlorine) for 7 days to evaluate their flower quality and shelf life. ENW reduced the occurrence and development of bent-neck, fresh weight loss and the percentage of wilted leaves after day 3. The numbers of bacteria in the water and in the basal 3 cm segments of stems were  $10^6$  CFU · ml<sup>-1</sup> and  $10^7$  CFU · gFW<sup>-1</sup> on day 5, respectively, whereas no bacteria was detected in the ENW. The bacterial population in the basal 3 cm of the control stems was 10 to 100 times higher than those in ENW throughout the holding period. Hydraulic conductance in the basal 3 cm of stems decreased dramatically by day 3, the decrease being greater in the stems in tap water than those in ENW.

Key Words: bent-neck, cut rose, electrolyzed water, vase solution.

#### Introduction

Cut rose flowers, placed in water, often senesce with bending of the neck because of an occlusion in a large majority of xylem elements at the lower end of the stem (Burdett, 1970). The vascular occlusion during vase life involves bacteria and their degradation products (van Doorn, 1997). The number of bacteria in the stems of cut rose flowers held in water was positively correlated with the number of bacteria in the water (van Doorn and de Witte, 1991). Therefore, the vase solutions including antimicrobial compounds such as metal salts, quinoline compounds, chlorine compounds and quaternary ammonium compounds have extended the vase life of cut roses (van Doorn, 1997).

Electrolyzed water is effective as a disinfectant for several foods (Hotta, 2000). ENW (pH 6.8), containing only 15 to 50 ppm available chlorine, reduced microbial populations on fresh-cut vegetables without causing discoloration (Izumi, 1999). ENW contains hypochlorous acid, which is generated by electrolysis of NaCl solution < 10% without a septum. Because chlorine in the ENW may have a bactericidal effect on cut roses as with fresh-cut vegetables, this work was conducted to determine the effects of ENW on vase life of cut roses and on bacterial populations in the solution and the rose stems submerged in it.

## **Materials and Methods**

Flowering 'Asami Red' roses, trade name 'Rote Rose', were obtained from a commercial grower in Wakayama in June 1997. The stems were cut to a length of 50 cm; the basal leaves were removed, leaving the uppermost three leaves. They were placed in tap water at 4 °C for 22 hr in the dark and then packed in a cardboard box lined with newspaper and held at 20 °C for 20 hr. Ten stems were removed from the box and placed in a 10-liter plastic container with 2 liters of tap water (0.3 ppm available chlorine) or ENW and held at 20 °C, 75% RH and 10  $\mu$ mol·m<sup>-2</sup>·sec<sup>-1</sup> irradiance from coolwhite fluorescent lamps for 7 days. The ENW (pH 6.8), containing 20 ppm available chlorine, was generated by electrolysis of 2.5 % NaCl solution using an ENW generator (Ameni Clean, Matsushita Seiko, Osaka). The concentration of available chlorine decreased from 20 ppm to 7 ppm, as measured by the sodium thiosulfate titration method, during the 7-day period.

Bacterial counts in the water containing 10 stems and in the basal 3 cm segments of 5 stems were determined by the method of van Doorn and Perik (1990). Bacterial counts in the vase water and the stems are expressed as  $log_{10}$  colony-forming units (CFU)·ml<sup>-1</sup> and CFU· gFW<sup>-1</sup>, respectively. Experiments were replicated three times. Bent-necks of 10 stems of each treatment were individually rated on a scale of 0 to 3 with 0 : normal, 1:bending slightly, 2: bending diagonally and 3: bending perpendicularly. Fresh weight and number of wilted leaves of 10 stems were determined daily and expressed as the percentage of initial weight and percentage of all leaves. Hydraulic conductance in the basal 3 cm segments of 6 stems from each treatment was determined as previously described (Izumi et al.,2000).

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### **Results and Discussion**

Bent-necks of control roses occurred on day 3; the scores of bent-necks increased with time (Fig. 1A). ENW reduced the occurrence and degree of bent-necks as well as the fresh weight loss (Fig. 1B) and percentage of wilted leaves (data not shown) after day 3. Based on time for the bent-neck to reach the score of 1 and/or to > 10% decline in fresh weight, the vase lives of control and ENW-treated roses were estimated as 3 and 6 days, respectively. These results indicate that ENW reduced water deficit in stems and leaves, thus contributing to a longer vase life of roses.

Bacteria was not detected in the holding solution on day 0. However, the bacterial population increased to about  $10^6$  CFU  $\cdot$  ml<sup>-1</sup> by day 5 in the tap water, while it remained non-detectable in the ENW after 7 days (Fig. 2A). Ohta and Harada (2000) reported that electrolyzed acid water (EAW; pH 2-3) also extended the vase life of cut roses, which they attributed to the low pH or the presence of hypochlorous acid. With EAW, the undissociated hypochlorous acid has a better bactericidal effect than has pH (Hotta, 2000). Since hypochlorous acid dissociates readily to chlorine gas at low pH or hypochlorite ions at high pH, neutral water allows hypochlorous acid to be stable and effective. The bacterial count in the basal 3 cm segments of the stems in tap water increased from  $10^4$  CFU  $\cdot$  gFW<sup>-1</sup> on day 0 to  $10^7$  $CFU \cdot gFW^{-1}$  on day 5. Hence, it was 10 to 100 times higher than those in ENW throughout the holding period (Fig. 2B). Van Doorn and Perik (1990) reported that hydroxyquinoline citrate (HQC) or a buffer at pH 3.0, as a vase solution, prevents vascular blockage by reducing the number of bacteria in the solutions and stems. ENW



Fig. 1. Changes in bent-neck score (A) and fresh weight (B) of cut roses held in tap water (control) or electrolyzed neutral water (ENW) at 20 °C for 7 days. NS, \*, \*\*: Nonsignificant, significant at P<0.05 or 0.01, respectively.</p>

in this study also effectively controlled bacterial proliferation in the stem by inhibiting bacterial growth in the vase solution.

Hydraulic conductance in the basal 3 cm of all rose stems decreased dramatically by day 3; the decrease was greater in the stems placed in tap water than those in ENW (Fig. 2C). The hydraulic conductance of rose stems decreased when the number of bacteria in the basal 5 cm stem segments exceeded  $10^6$  CFU · gFW<sup>-1</sup> (van Doorn and Perik, 1990). The hydraulic conductance of the basal 3 cm segments of stems decreased as the number of bacteria approached  $10^5 \text{ CFU} \cdot \text{ml}^{-1}$  in water and  $10^6$  CFU  $\cdot$  gFW<sup>-1</sup> in stems on day 3, which coincided with the occurrence of bent-necks. However, the hydraulic conductance of stem segments in ENW also decreased on day 3 even though no bacteria was detected; the bacterial count was less than  $10^6$  CFU  $\cdot$  gFW<sup>-1</sup> in the stems which indicates that the vascular occlusion probably resulted from nonbacterial blockage as proposed by Marousky (1971).

ENW did not develop visible chlorosis or leaf abscission (data not shown), so that ENW may be a beneficial,



Fig. 2. Number of bacteria in tap water (control) or electrolyzed neutral water (ENW) in the flower vase (A), and number of bacteria (B) and hydraulic conductance (C) in the basal 3 cm segment of stems of cut roses held in tap water (control) or ENW at 20 °C for 7 days. Vertical lines represent SE. SE bars were not shown when masked by the symbols. NS, \*, \*\*: Nonsignificant, significant at P<0.05 or 0.01, respectively. ND: Not detectable.

nontoxic means of extending vase life of cut roses by inhibiting the proliferation of bacteria.

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# 電気分解中性水がバラ切り花の生け水および 茎中の菌数に及ぼす影響

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#### 摘 要

バラ'アサミレッド'の切り花を水道水(対照)あるいは電 気分解中性水(電解水; pH 6.8, 有効塩素濃度 20 ppm)に, 20℃下で7日間生け,品質と保持期間を調べた.電解水は3 日目以降に,ベントネックの発生と進行,新鮮重の低下およ び葉の萎凋を抑制した.生け水中および水に生けた切り花の 茎基部(3 cm)中の菌数は,5日目にはそれぞれ10<sup>6</sup>CFU・ ml<sup>-1</sup>および10<sup>7</sup>CFU・g<sup>-1</sup>となった.一方,電解水中では菌は 検出されず,電解水に生けた茎基部の菌数よりも,水に生け た切り花の茎基部の菌数の方が,保持期間中を通して10から 100倍程度高かった.切り花の茎基部(3 cm)における水通導 性は3日目までに急速に減少したが,その減少量は電解水よ りも水に生けた切り花で大きかった.