

Extension of the Vase Life in Cut Roses by Treatment with Glucose, Isothiazolinonic Germicide, Citric Acid and Aluminum Sulphate Solution

Kazuo ICHIMURA^{1*}, Makoto TAGUCHI² and Ryo NORIKOSHI^{1,3}

¹ Research Team for Flower Quality, National Institute of Floricultural Science (Tsukuba, Ibaraki 305–8519, Japan)

² Tono Toki Agricultural Extension Center (Tajimi, Gifu 507–8708, Japan)

³ Tokyo University of Agriculture (Atsugi, Kanagawa 243–0034, Japan)

Abstract

Cut rose (*Rosa hybrida* L.) cv. Rote Rose was treated with glucose, fructose or sucrose at 10 g L⁻¹ in combination with a commercial preparation of isothiazolinonic germicide (a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; CMI/MI; Legend MK) at 0.25, 0.5 or 1 mL L⁻¹. To stabilize germicidal activity, the solution was acidified by the addition of citric acid to a final concentration at 30 mg L⁻¹. Of the sugars, glucose was the most effective in extending the vase life, followed by fructose. CMI/MI was most optimal at 0.5 mL L⁻¹. The addition of aluminum sulphate at 50 mg L⁻¹ to glucose plus CMI/MI considerably extended the vase life of cut roses more than glucose plus CMI/MI. Based on these results, a formulation comprising 10 g L⁻¹ glucose, 0.5 mL L⁻¹ CMI/MI, 30 mg L⁻¹ citric acid and 50 mg L⁻¹ aluminum sulphate was designated as GLCA and the effect of GLCA on the vase life of 8 cultivars was compared against 10 g L⁻¹ glucose plus 200 mg L⁻¹ 8-hydroxyquinoline sulphate (HQS). Treatment with GLCA extended the vase life of all the tested cultivars more than glucose plus HQS. Hydraulic conductance of stem segments in the control 'Rote Rose' roses decreased rapidly after harvest, but those for GLCA and glucose plus HQS were maintained at near their initial levels. The extension of vase life in cut roses by the addition of GLCA is attributed to the supply of sugars and the suppression of vascular occlusion without toxicity to cut flowers.

Discipline: Postharvest technology

Additional key words: cut flower, 8-hydroxyquinoline sulphate (HQS), *Rosa hybrida*

Introduction

The vase life of cut rose flowers is often short. The short vase life is attributed to vascular occlusion, which constricts the water supply to the flowers^{3,19}. Occlusions are thought to develop due to various factors, such as bacteria^{1,24,28}, air emboli^{6,26} and physiological responses of stems to cutting¹⁸. van Doorn et al.²⁴ reported a positive correlation between the abundance of bacteria and the decrease in hydraulic conductance of the stem. Furthermore, treatment with germicide, such as silver nitrate²⁰ or 8-hydroxyquinoline sulphate (HQS)^{7,14}, inhibited bacterial proliferation and maintained the hydraulic conductance of the stem. These findings suggest that bacterial proliferation is largely responsible for vascular occlusion, which shortens the vase life of cut rose flowers.

Commercial harvest of roses is usually done at the bud stage. For flower opening, large amounts of soluble carbohydrates are required for respiration and cell wall synthesis and as an osmolyte⁸. As sugar reserves in cut rose flowers are gradually consumed, the vase life of cut roses may be thus shortened¹⁴. Treatment with sugars, such as sucrose and glucose, in combination with some germicides was shown to extend the vase life of many cut flowers⁸. Sucrose plus 8-hydroxyquinoline compounds, such as HQS or 8-hydroxyquinoline citrate, is known to extend the vase life of cut rose flowers^{12,15,18}. However, Ichimura et al.¹³ previously reported that sucrose plus HQS did not extend the vase life of 5 out of 10 cultivars, including 'Rote Rose' and 'Noblesse', which are currently the leading cultivars in Japan. Similarly, in a preliminary experiment, some commercial preservatives did not extend the vase life of these cultivars. Therefore,

*Corresponding author: fax +81-29-838-6841; e-mail ichimu@affrc.go.jp

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there is a need for alternative effective formulations to extend the vase life of cut roses. Recently, Knee¹⁶ reported that Isocil (a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; CMI/MI), a commercially prepared germicide, was suitable for extending the vase life of cut roses, carnations and *Alstroemeria* flowers. Isocil also has other commercial names, including Legend MK and Kathon WT. Okabayashi and Yamamoto²¹ also reported that CMI/MI could be applicable to cut *Gloriosa* flowers.

Aluminum sulphate has been used as a microbial inhibitor in commercial preservatives⁹. Indeed, continuous treatment with aluminum sulphate has been shown to extend the vase life of cut 'Sonia' rose flowers¹¹ and this effect is attributed to its antimicrobial action. In addition, aluminum sulphate may improve the water relation of cut flowers because aluminum ions inhibit transpiration from leaves²³.

In the present study, therefore, we developed and tested a formulation composed of sugar, germicide and aluminum sulphate that is effective in extending the vase life of cut rose flowers. Furthermore, we also investigated the effectiveness of this newly developed formulation by comparing it with the effect of glucose plus HQS.

Materials and methods

1. Plant material and treatment

Roses (*Rosa hybrida* L.) cvs. Bridal Pink, Gold Strike, Noblesse, Pareo⁹⁰, Pretty Woman, Rote Rose, Saphir, and Thineke were obtained from a commercial grower at Ise, Mie Prefecture in 2001. Flowers were harvested at normal harvest maturity (stage 1 as described by Ichimura and Ueyama¹¹), and cut ends of the flower stems were immersed in tap water and kept at 5°C overnight. The next day, the cut flowers were transported to the laboratory and used in experiments within 1 h.

At the start of experiments, flower stems were trimmed to 45 cm, and all leaves except for the upper three leaflets were removed. Cut flowers were placed two per 500-mL beaker with 500 mL of distilled water (control), or 500 mL of 0.25, 0.5 or 1 mL L⁻¹ CMI/MI (Legend MK; Rohm and Haas Japan K.K., Tokyo, Japan) solution containing 11.3 g L⁻¹ 5-chloro-2-methyl-4-isothiazolin-3-one and 3.9 g L⁻¹ 2-methyl-4-isothiazolin-3-one as active ingredients with or without 10 g L⁻¹ glucose, fructose or sucrose. Solutions containing CMI/MI were acidified with 30 mg L⁻¹ citric acid to stabilize the active ingredients. In some experiments, flowers were treated with sugar plus CMI/MI in combination with aluminum sulphate at 50 mg L⁻¹ or 10 g L⁻¹ glucose plus 200 mg L⁻¹ HQS. Eight flowers were used in each treat-

ment. The cut flowers were then transferred to temperature-controlled chambers at 23°C under 70% relative humidity, with a 12-h photoperiod with 10 μmol m⁻² sec⁻¹ irradiance from cool-white fluorescent lamps. The fresh weight of cut flowers and the amount of solution uptake were measured daily. Solution uptake volume was corrected by subtracting evaporation of water from a beaker without cut flowers. Water loss was calculated by subtracting the increase in fresh weight from the amount of solution uptake. Vase life was defined as the period from the start of the continuous treatment to the time when either the petals lost turgor or the petals abscised.

2. Determination of sugar absorption by cut flowers

Vase solutions were diluted with distilled water for sugar concentration determinations by a Jasco HPLC system (Tokyo, Japan) equipped with a Shodex SUGAR KS-801 column (Showa Denko, Tokyo) and a refractive index detector. The column was kept at 80°C and analyte was eluted with water at a rate of 1.0 mL min⁻¹. The amount of sugar remaining in the vase solution was calculated using the volume of solution and the determined sugar concentration.

3. Measurement of stem hydraulic conductance

Hydraulic conductance of the stem segment was measured as previously described¹⁴. Briefly, the basal 5 cm portion of the stem was inserted into a silicon tube (internal diameter 4 mm) and 10 mM KCl solution at a head pressure of 130 cm was applied. After a 1-h equilibration, the KCl solution that had passed through the segments was collected in attached tubes for 1 h and the flow rate was determined. In one experiment, the rate was measured on two stem segments taken from one vessel and measurements were made in triplicate.

Results

1. Effect of glucose, fructose or sucrose in combination with CMI/MI on the vase life of cut 'Rote Rose' rose flowers

In preliminary experiments, glucose or fructose with concentrations higher than 10 g L⁻¹ plus CMI/MI caused damage to the leaves of cut 'Rote Rose'. Thus, we used sugar concentration at 10 g L⁻¹ for subsequent experiments.

Fig. 1 shows the effect of different concentrations of CMI/MI with or without glucose, fructose and sucrose on the fresh weight of cut 'Rote Rose' flowers. In the control treatment, flowers wilted before flower opening was complete. At 0.25 mL L⁻¹ CMI/MI, maximum fresh weights were obtained in glucose and fructose.

Decreases in fresh weight were latest in glucose treatments, followed by fructose. At 0.5 and 1 mL L⁻¹ CMI/MI, the maximum fresh weight and the start of the fresh weight decrease in glucose were almost similar to those in fructose. However, the stems in the 1 mL L⁻¹ CMI/MI treatment browned a few centimeters from the cut ends, making this concentration unsuitable. Table 1 shows the effect of CMI/MI with or without glucose, fructose and sucrose on the vase life of cut ‘Rote Rose’ flowers.

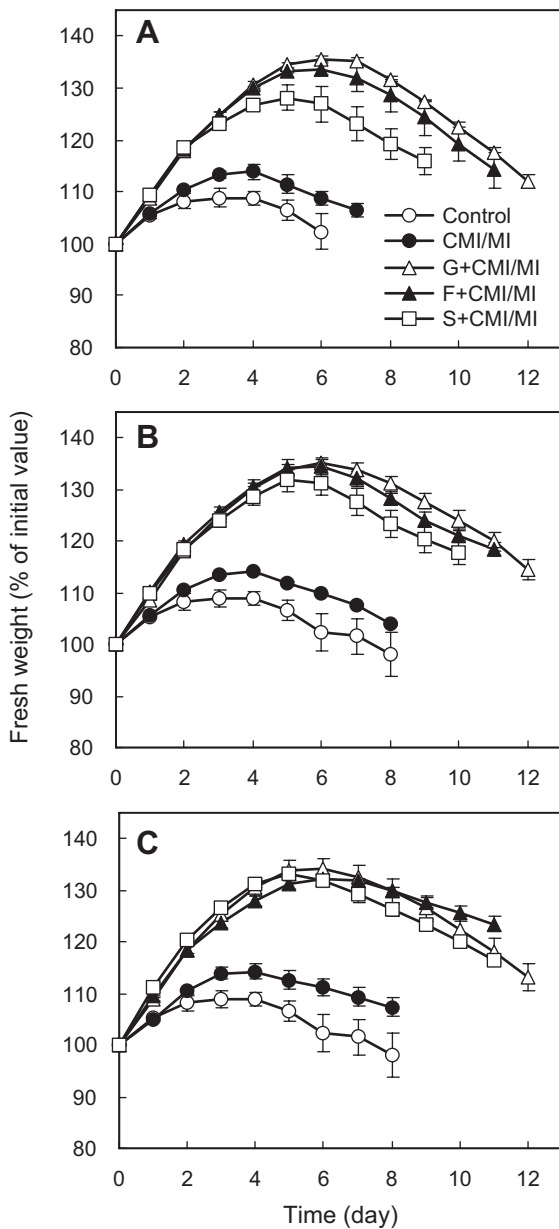


Fig. 1. Changes in fresh weight of cut ‘Rote Rose’ flowers treated with distilled water (Control), 10 g L⁻¹ glucose (G), fructose (F), or sucrose (S) plus CMI/MI at 0.25 mL L⁻¹ (A), 0.5 mL L⁻¹ (B), and 1 mL L⁻¹ (C)
 Values are the means of 3 replications ± SE.

Treatment of CMI/MI alone at all concentrations significantly extended vase life. Treatment with CMI/MI combined with sugars further extended the vase life and of the three sugars, glucose, followed by fructose, was the most effective.

Fig. 2 shows the changes in solution uptake by flowers for CMI/MI concentration of 0.5 mL L⁻¹. While uptake of all solutions tended to decrease with time, glucose solution was absorbed more than the other sugar

Table 1. Effects of glucose, fructose or sucrose in combination with different concentrations of CMI/MI on the vase life of cut ‘Rote Rose’ roses

Solution	Vase life (day)
Control (DW)	5.0 ± 0.3 a
0.25 mL L ⁻¹ CMI/MI	6.7 ± 0.2 bc
0.5 mL L ⁻¹ CMI/MI	6.2 ± 0.2 ab
1 mL L ⁻¹ CMI/MI	7.3 ± 0.4 bcd
Glucose + 0.25 mL L ⁻¹ CMI/MI	11.3 ± 0.3 gh
Glucose + 0.5 mL L ⁻¹ CMI/MI	11.7 ± 0.4 h
Glucose + 1 mL L ⁻¹ CMI/MI	11.3 ± 0.2 gh
Fructose + 0.25 mL L ⁻¹ CMI/MI	10.0 ± 0.3 efg
Fructose + 0.5 mL L ⁻¹ CMI/MI	9.8 ± 0.3 ef
Fructose + 1 mL L ⁻¹ CMI/MI	10.5 ± 0.2 fgh
Sucrose + 0.25 mL L ⁻¹ CMI/MI	8.0 ± 0.2 cd
Sucrose + 0.5 mL L ⁻¹ CMI/MI	8.7 ± 0.3 de
Sucrose + 1 mL L ⁻¹ CMI/MI	10.2 ± 0.3 fg

Values represent the means of 3 replications ± SE., and those with the same letters are not significantly different (P<0.05) by the Tukey-Kramer’s multiple range test.

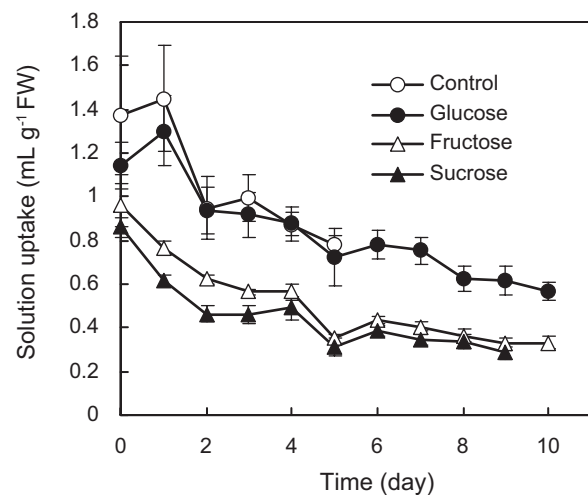


Fig. 2. Changes in vase solution uptake by cut ‘Rote Rose’ flowers treated with distilled water (Control), 10 g L⁻¹ glucose, fructose, or sucrose
 Sugar solution was supplemented with 0.5 mL L⁻¹ CMI/MI plus 30 mg L⁻¹ citric acid. Values are the means of 3 replications ± SE.

solutions by cut flowers. Similar results were obtained for CMI/MI concentrations of 0.25 and 1 mL L⁻¹ (data not shown). Solute analysis of the vase solution showed that the sugar uptake was proportional to solution uptake (data not shown). This suggests that the uptake of glucose was greatest among the three sugars.

We also investigated the effect of aluminum sulphate addition in glucose or fructose solutions with CMI/MI (0.5 mL L⁻¹) on the vase life of cut 'Rote Rose' flowers. Regardless of the type of sugar, the addition of aluminum sulphate increased the maximum fresh weight of cut flowers and delayed the time when fresh weight

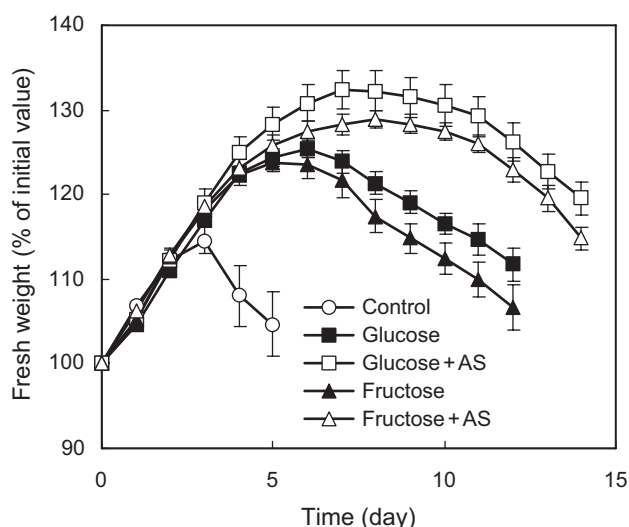


Fig. 3. Changes in fresh weight of cut 'Rote Rose' flowers treated with distilled water (Control), 10 g L⁻¹ glucose or fructose in combination with 50 mg L⁻¹ aluminum sulphate (AS)

Sugar solution was supplemented with 0.5 mL L⁻¹ CMI/MI plus 30 mg L⁻¹ citric acid. Values are the means of 3 replications ± SE.

Table 2. Effects of glucose or fructose in combination with aluminum sulphate on the vase life of cut 'Rote Rose' roses

Solution	Vase life (day)
Control (DW)	4.8 ± 0.3 a
Glucose + CMI/MI	12.8 ± 0.9 bc
Glucose + CMI/MI + Aluminum sulphate	14.0 ± 0.3 c
Fructose + CMI/MI	11.2 ± 0.2 b
Fructose + CMI/MI + Aluminum sulphate	13.3 ± 0.4 bc

Values represent the means of 3 replications ± SE., and those with the same letters are not significantly different (P<0.05) by the Tukey-Kramer's multiple range test.

started to decrease (Fig. 3). Furthermore, the glucose plus CMI/MI solution in combination with aluminum sulphate tended to extend the vase life of 'Rote Rose' flowers the most (Table 2). However, the addition of aluminum sulphate did not suppress transpiration from cut flowers (data not shown). Based on these results, the most effective formulation comprising 10 g L⁻¹ glucose, 0.5 mL L⁻¹ CMI/MI, 30 mg L⁻¹ citric acid and 50 mg L⁻¹ aluminum sulphate was designated as GLCA, which was taken from the first letters of glucose, CMI/MI (Legend MK), citric acid, and aluminum sulphate (Fig. 4).



Fig. 4. Cut 'Rote Rose' flowers treated with distilled water (Control) (left) and GLCA (right)

The photograph was taken 15 days after treatment.

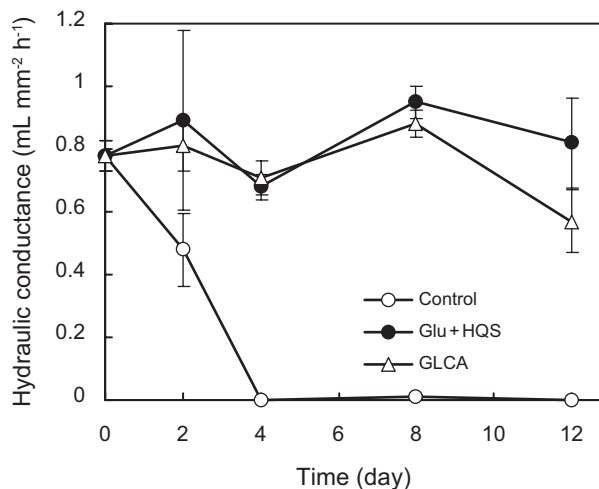


Fig. 5. Changes in hydraulic conductance of stem segments in cut 'Rote Rose' flowers treated with glucose plus HQS or GLCA

Values are the means of 3 replications ± SE.

Table 3. Effects of vase solution on the vase life of cut rose flowers

Cultivar	Vase life (day)		
	Solution		
	Control	Glucose + HQS	GLCA
Bridal Pink	4.6 ± 0.2 a	8.4 ± 0.4 b	12.1 ± 0.2 c
Gold Strike	8.6 ± 0.2 a	9.5 ± 0.2 a	16.0 ± 0.6 b
Noblesse	9.4 ± 0.2 b	6.8 ± 0.4 a	19.8 ± 0.9 c
Pareo ⁹⁰	6.0 ± 0.0 a	6.3 ± 0.1 a	14.8 ± 0.3 b
Pretty Woman	10.6 ± 0.6 a	10.1 ± 0.3 a	14.1 ± 0.4 b
Rote Rose	4.9 ± 0.4 a	4.4 ± 0.2 a	15.3 ± 0.1 b
Saphir	5.5 ± 0.3 a	8.3 ± 0.1 b	9.8 ± 0.8 b
Thineke	5.5 ± 0.2 a	8.9 ± 0.2 b	11.0 ± 0.4 c
Significance			
Cultivar		***	
Solution		***	
Cultivar × Solution		***	

Values represent the means of 4 replications ± SE., and those with the same letters are not significantly different ($P < 0.05$) by the Tukey-Kramer's multiple range test. ***: Significant at $P < 0.001$ by ANOVA.

2. Comparison of GLCA with glucose plus HQS on the vase life of various cultivars

Treatment with glucose plus HQS significantly extended the vase life of 3 cultivars, but significantly shortened that of 1 cultivar. On the other hand, treatment with GLCA significantly extended the vase life of all cultivars (Table 3). We confirmed that treatment with GLCA increased the maximum fresh weight and markedly delayed the start of the decrease in fresh weight in all cultivars (data not shown).

3. Effect of GLCA and glucose plus HQS on hydraulic conductance

Hydraulic conductance in the control segments gradually decreased and reached almost zero on the 4th day (Fig. 5). In contrast, hydraulic conductance of stem segments in both glucose plus HQS and GLCA remained nearly constant throughout the experiment period. When 'Rote Rose' flowers were continuously treated with 200 mg L⁻¹ HQS or 0.5 mL L⁻¹ CMI/MI alone, hydraulic conductance of stem segments on the 8th day were 1.3 and 0.8 mL mm⁻² h⁻¹, respectively.

Discussion

Most of the cut 'Rote Rose' flowers held in distilled water wilted before flower opening was complete. On the contrary, flowers in GLCA fully opened (Fig. 4). Furthermore, GLCA treatment markedly extended the vase life of cut rose flowers in all cultivars tested (Table

3). Thus, GLCA seems to be of practical use for generally improving the vase life of cut rose flowers. Vascular occlusion and the depletion of soluble carbohydrate are considered to be primarily responsible for shortened vase life in cut roses^{3,14,19}. In our study, additions of GLCA and CMI/MI maintained hydraulic conductance in stem segments (Fig. 5), suggesting that CMI/MI inhibits vascular occlusion. In addition, GLCA treatment improved the supply of glucose to cut flowers. Thus, the improvement of vase life by GLCA is, at least, attributed to its inhibition of vascular occlusion and improving the supply of soluble sugars.

Glucose, fructose and sucrose have been considered to similarly extend vase life⁹. However, glucose, followed by fructose, was the most effective for extending the vase life of cut 'Rote Rose' flowers, when CMI/MI was added in combination with the sugars (Table 1). On the other hand, Hu et al.¹⁰ reported that pulse treatment of fructose resulted in a greater extension of the vase life of 'Bridal Pink' rose than observed for glucose and sucrose. The difference in the results of our experiments and those of Hu et al. may be attributed to differences in the duration of treatment and/or the cultivars. In our study, the uptake of glucose was greater than that of fructose and sucrose. This suggests that glucose may have been more effective due to the larger amount of sugar absorbed by the cut flowers. In many plants, the uptake rates of sugars to tissue or protoplasts vary for various sugars^{17,27}. In parenchyma tissue of celery, uptake rates of glucose were the greatest, followed by those of fructose and sucrose⁵.

Since transporters of sugars have been found to be involved in the uptake of sugars², the activities of sugar transporters may be responsible for differences in sugar uptake in roses.

In our study, the extension of vase life for cut 'Rote Rose' was achieved by the addition of aluminum sulphate to solutions containing sugars plus CMI/MI (Table 2). Aluminum sulphate had been reported to inhibit transpiration from leaves²³, a main source of water loss in cut flowers. However, our measurements of transpiration rates from cut flowers showed that the addition of aluminum sulphate failed to suppress transpiration from cut flowers (data not shown). Although aluminum sulphate is known to be an antimicrobial compound, aluminum sulphate at concentrations of 350 mg L⁻¹ and 500 mg L⁻¹, which are much higher than concentrations used in our study, have been reported to have only weak antimicrobial activity^{22,25}. Thus, the positive effects of aluminum sulphate do not seem to be attributable to antimicrobial action. Further study is needed to clarify the role of aluminum sulphate in extending the vase life of cut rose flowers.

Continuous treatment with sucrose or glucose plus HQS is known to extend the vase life of cut rose flowers^{4,12,15}. However, treatment with sucrose plus HQS did not extend the vase life of 5 of 10 cultivars¹³ and glucose plus HQS did not extend the vase life of 5 of 8 cultivars in the present study (Table 3). However, hydraulic conductance did not decrease in the glucose plus HQS treatment, suggesting that HQS inhibits vascular occlusion (Fig. 5). This result agrees with previous reports^{7,12}. Thus, the ineffectiveness of HQS is not associated with inhibition of vascular occlusion. HQS seems to be toxic to flowers of some rose cultivars, causing a shortened vase life.

Of the 8 cultivars, the vase life of 3 cultivars was significantly extended, but 1 cultivar was shortened by glucose plus HQS (Table 3), suggesting cultivar variation for resistance to HQS. In contrast, GLCA extended the vase life of all cultivars more than did glucose plus HQS. While CMI/MI at the concentrations of 0.5 mL L⁻¹ or less were not toxic to cut rose flowers, it has been reported to be more suitable for some cut flowers, including carnation, *Alstroemeria* and *Gloriosa*^{16,21}. Thus, CMI/MI is likely to be suitable as an effective antimicrobial compound for many cut flowers.

In conclusion, continuous treatment with GLCA markedly extended the vase life of cut rose flowers. GLCA is effective at least in improving the supply of glucose and inhibiting of vascular occlusion. HQS, a well-known germicide, is toxic to some rose cultivars, leading to a shortening of vase life.

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