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Effect of Pre- and Post-transport Preservative Treatments to Extend Vase Life on Scent Emission of Tulip Cut Flowers

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Abstract

Tulipa gesneriana L. 'Sanne', a tulip cut flower cultivar, has a fruity scent derived from monoterpenes based on linalool. Treatment with a commercial preservative containing plant growth regulators, such as ethephon, before transportation or with a preservative containing glucose after transportation extends the vase life of various tulip cut flowers. However, the effects of preservatives on floral scents are unknown. In this study, pre-transport ethephon treatment did not affect the scent emission and increased the vase life of Sanne. However, post-transport glucose treatment increased terpenoid emissions and caused stalk softening. The combination of pre- and post-transport treatments increased terpenoid emissions but did not cause stalk softening. Comparative analysis with previous sensory evaluation of the Sanne scent revealed that the duration of the noticeable scent under the combination treatment was estimated to be twice than that under the control treatment. In Sanne, the vase life and scents are expected to be improved by the pre- and post-transport treatment combination.

Discipline: Horticulture
Additional key words: glucose, linalool, monoterpene, Sanne, *Tulipa gesneriana*

Introduction

Tulip, a perennial plant of the Liliaceae family, is a cut, potted, or garden flower having various colors and shapes. A survey of 30% of the Japanese flower market (Ichimura 2013) estimated that the annual production of tulip cut flowers is approximately 75 million. Cut flowers are harvested from winter to spring and shipped *via* dry transport.

Approximately 180 scent components classified into 9 groups based on their principal components have been identified in tulip flowers (Oyama-Okubo & Tsuji 2019). The various scents have the potential to add value to tulips. For example, Sanne has a sweet fruity scent with citrus note due to the monoterpene linalool and the carotenoid-derived sesquiterpene β-ionone (Oyama-Okubo & Tsuji 2019). The scent preferences of cut flowers in 20 scented tulip cultivars were evaluated by a total of 1,141 consumers on a three-point scale: "like," "neutral," and "dislike." When scent emission was sufficient, more than 70% of the consumers rated the scent of Sanne as "like" (Kishimoto et al. 2018). This percentage was the highest among all the tested cultivars. Therefore, its pleasant scent can add to the commercial value of Sanne cut flowers. However, daily changes in the quantity and composition of scent emissions during the anthesis have not been investigated in tulip cut flowers.

In Japan, to increase vase life, many tulip cut flowers are treated with commercial preservative containing plant growth regulators (PGRs), such as ethephon, 6-benzyladenine, and gibberellic acid, before shipping (Mason 2020). These PGRs suppress stalk elongation, senescence, and tepal abscission in tulip cut flowers (van Doorn et al. 2011). Furthermore, the continuous application of glucose and isothiazolinone germicide preservatives after dry transport increases the vase life of various tulip cultivars (Watanabe et al. 2013). However, the use of PGRs and chemicals in the preservatives may affect the scent emissions of the flowers.

This study demonstrated the changes in the emitted

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scent quantity and composition during the anthesis in Sanne cut flowers. In addition, it investigated the effects of the application of preservatives before and after dry transport on the scent emission of Sanne cut flowers. The retention period of noticeable scent in the cut flowers was also estimated under each preservative treatment by referring to the results of our previous work on the scent evaluation of Sanne (Kishimoto et al. 2018). On the basis of these results, this study proposes a preservative treatment suitable for retaining the scent of Sanne cut flower.

Materials and methods

1. Plant material

The plants were cultivated at the Horticultural Research Center, Niigata Agricultural Research Institute (37°59′N, 139°17′E), Kitakambara, Japan. First, the Sanne bulbs were maintained at 2°C in the dark for 2 months before planting in a 25-cm-deep plastic container (60 cm × 40 cm) with potting soil mixed with coconut husk and peat moss media. Next, the bulbs were kept in the dark at 15°C for 2 weeks for rooting. Finally, the rooted bulbs were planted in the containers and transferred to a glass greenhouse. The day and night temperatures of the greenhouse were maintained at 18°C and 13°C, respectively. To prevent the potting soil from drying, tap water was supplied daily by upper watering. Fertilizers were not used, and the cut flowers were harvested in March 2016 and 2017.

2. Pre-transport treatment

The pre-transport treatment used was Chrysal BVB & Plus (Chrysal Japan Co., Osaka, Japan), a preservative for tulip cut flowers containing ethephon (Mason 2020). First, the Sanne cut flower stems were cut to a length of approximately 40 cm and placed in a glass flask containing 0.5% Chrysal BVB & Plus diluted with tap water. Next, the cut flowers were kept in the dark at 5°C and 30% relative humidity for 3 h. Contrarily, the control group was treated with tap water alone. After the preservative application, 30 cut flowers were wrapped in dry newspaper, packed in a cardboard box (H 21 cm × W 49 cm × L 81 cm), and kept at 5°C and 30% relative humidity for approximately 1 day. One day after harvesting, the box containing cut flowers was courier-shipped to the Institute of Vegetable and Floriculture Science, NARO (36°02′N, 140°05′E), in Tsukuba City. The cut flowers during transportation encountered a temperature of 3°C-15°C and relative humidity of more than 80%, as recorded in the data logger. The time required for transport was approximately 19 h.

3. Post-transport treatment

The post-transport treatment was administered according to the method proposed by Watanabe et al. (2013). First, each flower stem was cut to approximately 30 cm in length and placed in a glass flask containing 500-mL distilled water with 1% glucose, 0.05% isothiazolinone germicide (CMIT/MIT; Rohm and Haas, Tokyo, Japan), and 0.005% aluminum sulfate. The control group was treated with distilled water alone. The cut flowers were then transferred into a growth chamber maintained at 23°C with a 12-h/12-h light/dark photoperiod under a light intensity of approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity of 60%.

4. Collection of emitted scents and their gas chromatography–mass spectrometry (GC-MS) analysis

The emitted tulip flower scent was collected using the dynamic headspace method (Kishimoto 2021). All samplings were performed at 09:30 a.m. to reduce the effects of circadian rhythms on the scent emissions of tulips (Yang et al. 2020). The tulip flowers were wrapped in 1-L Tedlar bags (GL Science Inc., Tokyo, Japan) and sealed with tape. A constant air stream (500 $mL \cdot min^{-1}$) was filtered through the activated charcoal and piped into the bags. Headspace volatiles were collected for 1 h using a Tenax-TA tube (180 mg, 60 × 80 mesh; Gerstel GmbH & Co, Mülheim, Germany).

The chemicals in the scents collected in the Tenax-TA tubes were analyzed *via* gas chromatography–mass spectrometry (GC-MS) with an Agilent 6890 N GC system connected to an Agilent 5930 N mass selective detector (both Agilent Technologies, Inc., Santa Clara, CA, USA). In the GC, a cooled injection system (CIS; Gerstel GmbH & Co. KG) and a DB-WAX capillary column (Agilent 122-7032; Agilent Technologies) were used. The cryofocusing of the CIS was set to −50°C, with an increasing temperature rate of $12°C \cdot s^{-1}$ and a final temperature of 300°C. Helium was used as the carrier gas. The temperature of the column oven was set to 40°C for 2 min, increased to 250°C at 5°C min^{-1}, and held at 250°C for 5 min. The injection, interface, and ion source temperatures were 250°C.

Each scent compound was identified using the Wiley Registry 9th Edition/NIST 2011 library search system (Agilent Technologies). The mass spectrum and retention time of each standard (purity > 90%) (Sigma-Aldrich, Co., LLC, Louis, MO, USA; Tokyo Chemical Industry, Co., Ltd., Tokyo, Japan; and Fujifilm Wako Pure Chemical, Co., Ltd., Osaka, Japan) were analyzed under similar conditions. The amount of each scent compound in each standard was calculated based

on the peak areas of 5, 25, 50, 250, and 1,000 ng on the ion chromatograms. The mean values of three independent plants are presented for each condition.

Results

Changes in the appearance of Sanne cut flowers under different preservative treatments are presented in Figure 1. Panels A and B were tested on different days, and each panel was provided with controls. In the control treatments, many tepals fell between the 7th and 9th days of anthesis, and those that had not fallen dropped upon slight disturbance, such as touching. The flower stalk elongation was also remarkable. Contrarily, pre- or post-transport treatment suppressed tepal abscission. The pre-transport treatment also suppressed stalk elongation, whereas the post-transport treatment did not. The stalks were softened by the post-transport treatment and often broke within the 6th to 9th day of anthesis. The pre- and post-transport treatment combinations suppressed stalk elongation and tepal abscission but did not cause stalk softening.

Fig. 1. Changes in the appearance of cut flowers of *T. gesneriana* 'Sanne' under different preservative treatments

"0 day" represents the day before the flower opening. "Control" represents the control (i.e., preservatives untreated). "Pre-transport treatment" represents treatment with a preservative containing ethephon before transport. "Post-transport treatment" indicates continuous glucose and isothiazolinone germicide treatment after transport. Panels A and B were tested on different days, and each panel was provided with different controls. Scale bars represent 4 cm.

The changes in the quantities of each emitted scent compound in Sanne cut flowers that were not treated with preservatives are presented in Table 1. The emitted scents were composed of benzenoid aromatic compounds, fatty acid derivatives, monoterpenes, general sesquiterpenes, and carotenoid-derived sesquiterpenes, each whose principal compound was 3,5-dimethoxytoluene, (*Z*)-3-hexenol, linalool, β-caryophyllene, and β-ionone, respectively. Monoterpenes were the most abundant and diverse component. In Table 1, the six principal monoterpenes are listed. Linalool was the most emitted compound among all the scent compounds.

The changes in the total scent emissions are presented in Figure 2 (upper panel). Because the analyses of the flowers in panels A and B were conducted on different days, each treatment condition was provided with separate controls. The scent emission on the 8th day of anthesis in the controls was not observed due to tepal abscission. Some of the flowers that received post-transport treatment did not emit scent from the 6th day of flower opening due to broken stalks. In all treatments, the lowest and highest emissions were observed 1 day before flower opening and on the 3rd day after flower opening, respectively. The patterns of change in total emission quantities under each treatment were similar. No significant differences were observed between the control and pre-transport treatment groups. However, the total scent emissions on the 3rd and 5th days in the post-transport treatment were significantly higher than those of the control. Compared with the controls, the pre- and post-transport treatment combinations had significantly higher total emissions on the 3rd, 5th, and 6th days.

Focusing on the scent composition, the proportion of fatty acid derivatives was high before flower opening (Fig. 1 and lower panels of Fig. 2). However, the proportion of monoterpenes rapidly increased after flower opening and was maintained at approximately 70% of the total emissions in all treatments. The changes in the composition ratios were similar between the treatments.

The emissions of benzenoid aromatic compounds and fatty acid derivatives on the same day for each treatment were almost the same (Fig. 3). Contrarily, the terpenoid emissions of the combination of pre- and post-transport treatments were significantly higher than those of the control on multiple days. The results of the post-transport treatment were similar to those of the combination treatment, except after 8 and 10 d when the stalks broke (Fig. 3).

Table 1. Changes in scent emission quantities (nmol h^{-1}/flower) of cut flowers of *T. gesneriana* 'Sanne'

Scent compound		Flower opening days					
		0[a]	1	2	3	5	6
Benzenoid aromatic compound	3,5-Dimethoxy toluene	0.2 ± 0.1[b]	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	Others	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.4 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Fatty acid derivative	(*Z*)-3-Hexenol	0.3 ± 0.2	0.9 ± 0.1	0.9 ± 0.0	1.1 ± 0.1	0.2 ± 0.1	–[c]
	(*Z*)-3-Hexenyl acetate	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	–	–
	Others	trace[d]	0.5 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.9 ± 0.2
Monoterpene	Geranyl acetone	–	–	0.6 ± 0.2	0.6 ± 0.1	1.0 ± 0.3	0.7 ± 0.3
	Linalool	0.5 ± 0.1	1.8 ± 0.6	8.4 ± 0.5	9.3 ± 0.6	6.5 ± 0.5	5.7 ± 0.5
	Linalool oxide	–	trace	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.3 ± 0.0
	β-Myrcene	–	trace	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
	β-Ocimene	–	0.1 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
	α-Terpineol	–	trace	0.3 ± 0.1	0.4 ± 0.0	trace	–
	Others	trace	0.3 ± 0.1	2.1 ± 0.2	2.4 ± 0.0	1.5 ± 0.1	1.4 ± 0.2
Sesquiterpene	β-Caryophyllene	–	–	0.3 ± 0.0	2.7 ± 0.1	2.2 ± 0.1	1.3 ± 0.1
	β-Ionone	0.1 ± 0.0	0.5 ± 0.2	2.7 ± 0.1	2.2 ± 0.1	1.3 ± 0.1	0.6 ± 0.1
	Others	–	trace	0.2 ± 0.0	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.1
Total		1.4 ± 0.5	4.9 ± 1.5	18.5 ± 0.4	21.7 ± 0.4	15.6 ± 1.2	12.3 ± 1.2

[a] Indicates the day before the flower opening day
[b] mean ± SE, n = 3
[c] not detected
[d] trace < 0.05

Discussion

In the scent emission of Sanne cut flowers, the proportion of fatty acid derivatives was high before flower opening (Figs. 1, 2). However, the scents derived from Sanne were not particularly detectable due to their low quantity. After flower opening, linalool-based monoterpenes were found to be dominant (Figs. 1, 2). In several flowers, the composition of scent emissions is known to change during anthesis (Pragadheesh et al. 2017, Robertson et al. 1995), but it was relatively stable in Sanne cut flowers.

In Japan, before shipping, tulip cut flowers are treated with one preservative, Chrysal BVB & Plus, which contains various PGRs, such as ethephon (Mason 2020). Ethylene generated from ethephon is known to reduce the emissions of various floral scents (Schade et al. 2001, Sexton et al. 2005, Underwood et al. 2005). However, this preservative, which contains ethephon, did not significantly change the scent emission of Sanne (Fig. 2 panel A).

By contrast, continuous treatment with 1% glucose and 0.05% isothiazolinone germicide after dry transport increased terpenoid emissions (Fig. 2 panel B). An increase in terpenoid emissions due to sucrose treatment has also been reported in sweet pea cut flowers (*Lathyrus odoratus* L.) (Ikeura et al. 2013). An exogenously applied glucose might act as a substrate for terpenoid. Previous

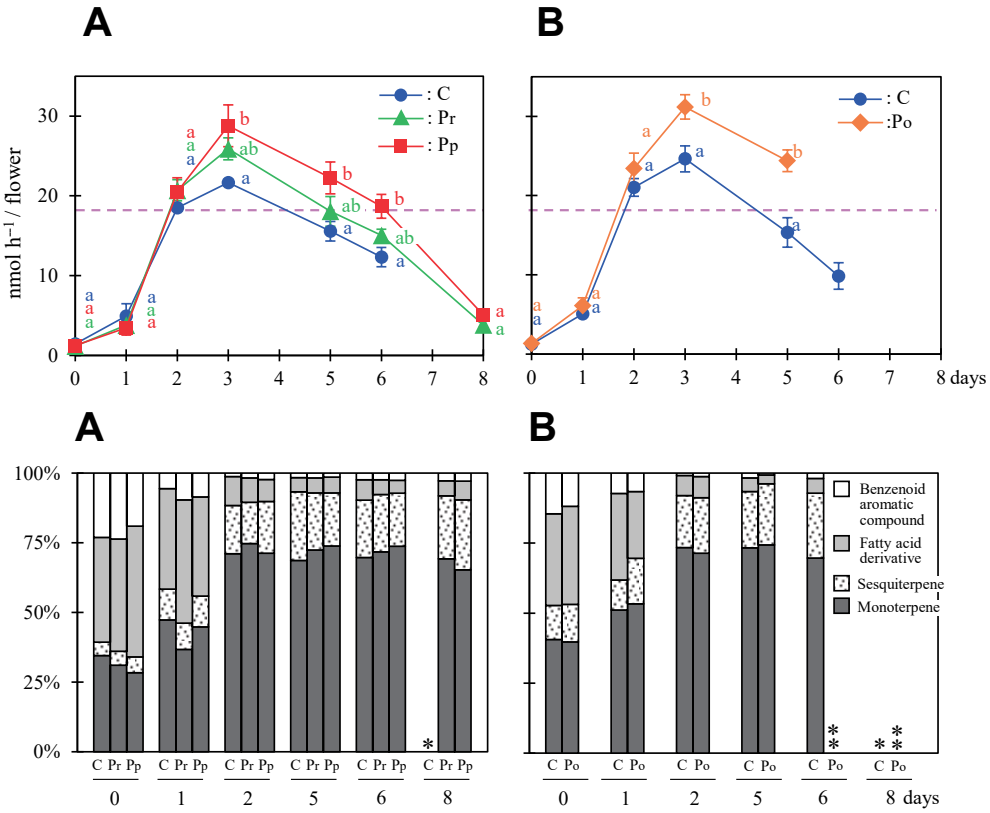

Fig. 2. Changes in quantities (upper panels) and composition rates (lower panels) of scent emission in cut flowers of *T. gesneriana* 'Sanne' under different preservative treatments
"0 day" represents the day before the flower opening. "C" denotes the control (i.e., preservatives untreated). "Pr" represents pre-transport treatment with a preservative containing ethephon. "Po" denotes post-transport treatment with a preservative containing glucose and isothiazolinone germicide. "Pp" represents pre- and post-transport treatment combinations. The mean values of the three independent cut flowers are presented for each day. Panels A and B were analyzed on different days, and controls were provided for each. In the upper panels, different lowercase letters on the same measurement day indicate significant differences in the scent emissions (Tukey's test, $P < 0.05$, $n = 3$). The flower scent intensities were categorized by 718 consumers as "very scented," "scented," "slightly scented," and "unscented." The dotted lines in the upper panels indicate the lowest limit at which more than 70% of consumers gave positive evaluations of "very scented" or "scented" (Kishimoto et al. 2018). The single and double asterisks in the lower panels indicate that scent emission could not be measured because of tepal abscission and broken stalks, respectively.

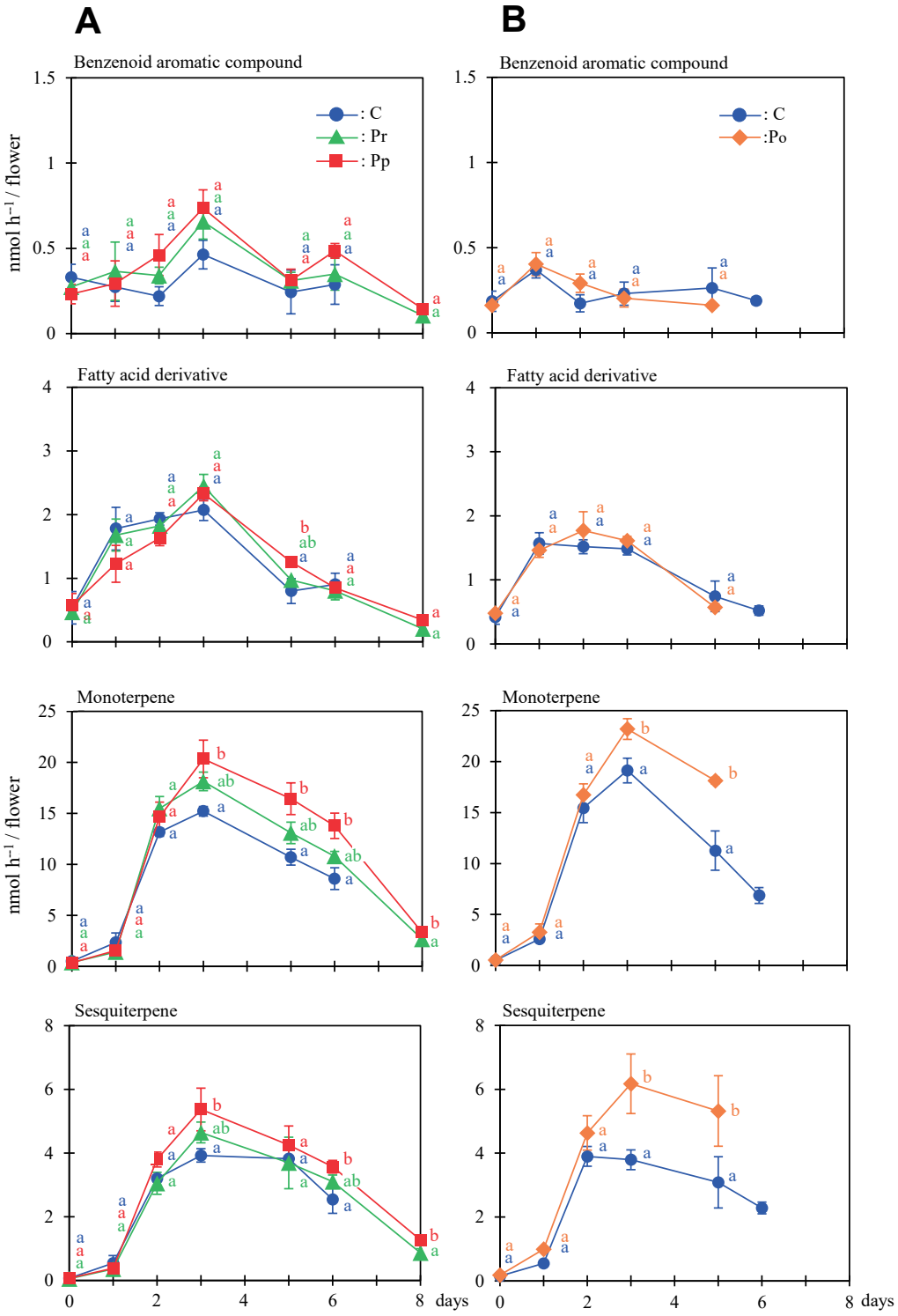

Fig. 3. Changes in the emission quantities of each scent group in cut flowers of *T. gesneriana* 'Sanne' under different preservative treatments

"0 day" represents the day before flower opening. "C" indicates the control (i.e., preservatives untreated). "Pr" represents pre-transport treatment with a preservative containing ethephon. "Po" indicates post-transport treatment. "Pp" represents pre- and post-transport treatment combinations. Post-transport treatment comprised a preservative containing glucose and isothiazolinone germicide after transport. Panels A and B were analyzed on different days, and controls were provided for each. The mean values of the three independent cut flowers are presented for each treatment. The different lowercase letters on the same measurement day indicate significant differences in the scent emissions (Tukey's test, $P < 0.05$, $n = 3$).

studies have demonstrated that isothiazolinone germicide suppresses the emission of organic sulfur compounds from ornamental cabbage cut flowers (*Brassica oleracea* L. var. *acephala* DC. f. *tricolor* Hort.), though the underlying mechanism was not elucidated (Kishimoto et al. 2014). However, this germicide did not affect the scent emission of the Sanne cut flower.

Softening of cut flowers by post-transport treatment has been reported in other several tulip cultivars and was found to be caused by glucose or other sugars, not isothiazolinone (Watanabe et al. 2013). In rose cut flowers (*Rosa* × *hybrida* L.), excessive absorption of exogenous sugars has been reported to induce cell dehydration and tissue damage (Markhart III & Harper 1995). Therefore, Sanne may be sensitive to exogenous sugar stress. Furthermore, softening was suppressed by the combination of pre- and post-transport treatment. The suppression of flower stalk elongation by the combination treatment may have strengthened the stem tissue.

In seven independent scent sensory tests using Sanne cut flowers, the strength of the scent was evaluated by a total of 718 consumers as "very scented," "scented," "slightly scented," or "unscented" (Kishimoto et al. 2018). The dotted lines in Figure 2 indicate the lowest limit of scent emission, at which more than 70% of consumers gave a positive evaluation of "very scented" or "scented." In this study, we evaluated the scent intensity by considering the lowest limit as the lowest limit of the noticeable scent. As a prerequisite for this evaluation, it was critical for the composition of the scent emission to not change remarkably. Because the composition of the scent emission of Sanne is stable (Fig. 2), we believe that the above evaluation is possible. In the control group, a noticeable scent was maintained for 2 days (Fig. 2). However, in the pre- and post-transport treatment combinations, a noticeable scent was maintained for 4 days. Therefore, treatment combination was found to increase the duration of the noticeable scent of Sanne cut flowers by two times.

In conclusion, pre-transport treatment with a commercial preservative, which is widely used in Japan to improve the vase life of tulip cut flowers, did not affect the scent emission of Sanne cut flowers. Moreover, continuous treatment with 1% glucose after transport increased the scent emission and induced stalk softening. Therefore, the combination of pre- and post-transport treatments can improve the scent and vase life of Sanne cut flowers.

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