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Effect of Temperature on Scent Emission from Carnation Cut Flowers

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Abstract

Temperature, an environmental factor affecting cut flowers' physiological state, is expected to affect scent emission. We investigated scent emission from carnation cut flowers of two scent types (*Dianthus caryophyllus* L.), exhibiting fruity (because of methyl benzoate) and spicy (because of eugenol) scents, at various temperatures (10°C, 15°C, 23°C, and 28°C). Cut flowers harvested on the day of flower opening were used for analysis. Scent emission was significantly higher at 23°C and 28°C than at 10°C and 15°C until 1 or 2 days but was significantly lower at 8 and 10 days postharvest. Methyl benzoate emissions decreased faster than eugenol emissions. Considering the lower limit of noticeable scent, as per previous sensory evaluations of carnations, scent lasted the longest at 10°C and 15°C. After ~5 days of pretreatment at 10°C, scent emission was slightly improved at 23°C than at 23°C. Such cut flower management at 10°C before sale may contribute to the persistence of scent at room temperature in consumers' homes after sale. Various factors, including the suppression of scent substrate consumption, regulation of scent emission from the cuticle, and influence on the expression of scent emission–related genes, may affect the retention of scent emission because of low temperature.

Discipline: Horticulture
Additional key words: benzenoid/phenylpropanoid, eugenol, methyl benzoate, postharvest

Introduction

Carnation (*Dianthus caryophyllus* L.), a perennial plant of the family Caryophyllaceae, is mainly used as cut flowers. In Japan, approximately 206 million carnation cut flowers were produced in the fiscal year 2020, the second highest following that of chrysanthemum (*Chrysanthemum* × *morifolium* R.) (MAFF, Japan 2020).

The scents emitted from carnations include benzenoids/phenylpropanoids, terpenoids, and fatty acid derivatives (Clery et al. 1999). Specifically, volatile benzenoids/phenylpropanoids (VBPs) are the key components of the scents. Most carnation cultivars for cut flowers distributed in Japan have a fruity scent because of benzenoid methyl benzoate (Kishimoto et al. 2019). The methyl benzoate scent type is considered a characteristic of modern cut flower cultivars (Clery et al. 1999). Several cultivars also have a spicy scent because of the presence of benzenoid eugenol (Kishimoto et al. 2019). Conventional carnation cultivars grown in Southern Europe, from which essential oils are obtained for perfume production, belong to the eugenol scent type (Anonis 1985).

Typically, the amount and composition of scents change during flower opening, and scents decrease with flower senescence (Pragadheesh et al. 2017, Robertson et al. 1995). In petunias (*Petunia* × *hybrida* Vilm. 'Mitchell') and snapdragons (*Antirrhinum majus* L.), VBP emission decreases with flower senescence induced by ethylene (Negre et al. 2003, Underwood et al. 2005). In carnation cut flowers, the ethylene action inhibitor silver thiosulfate (STS) inhibits the decrease in VBP emission following the onset of flower senescence, indicating that ethylene contributes to this decrease at the later stage of flower opening (Kishimoto et al. 2019).

Temperature is an environmental factor that significantly affects the physiological state of cut flowers (Gupta & Dubey 2018). In general, the senescence of cut

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flowers progresses faster at higher temperatures (Gupta & Dubey 2018). In carnation cut flowers, the progression of senescence at 32°C was faster than that at 24°C (Yangkhamman et al. 2005). Moreover, low temperatures suppress the progression of senescence in many cut flowers (Ichimura 2018). Additionally, fluctuating temperatures alter ethylene production in carnations (Yangkhamman et al. 2005).

Scent emission is also affected by temperature. At temperatures ranging between 20°C and 30°C, VBP emission from wild petunia (*P. axillaris*) increased with increasing temperature (Sagae et al. 2008). Scent emission from white clover (*Trifolium repens* L.) at 10°C was significantly lower than that at 20°C (Jakobsen & Olsen 1994). Nonetheless, the effect of temperature on scent emission from cut flowers has rarely been investigated. In a *Narcissus* line, "double flower" scent emission from cut flowers varied at different temperatures (Terry et al. 2021). Based on these findings, scent emission from carnation cut flowers can be expected to be affected by temperature.

To this end, the present study investigated the effect of temperature on scent emission from carnation cut flowers. A temperature of 23°C is a common condition in the quality confirmation test of cut flowers (Ichimura et al. 2015, Pun et al. 2016, Shimizu-Yumoto et al. 2020), and it is almost the same as the average room temperature in Japan (Katsuno & Rijal 2011). Therefore, a temperature of 23°C was used as the standard for comparison in this study, and other temperature conditions, including 10°C, 15°C, and 28°C, were tested. The lowest temperature of 10°C corresponds to the temperature in the common display refrigerator for cut flowers. The temperatures of 15°C and 28°C correspond to the average room temperatures without heating and cooling in the winter and summer, respectively, in Japan (Katsuno & Rijal 2011). This study revealed the effects of temperature on scent emission from carnation cut flowers and proposed temperature conditions suitable for the long-term retention of scent.

Materials and methods

1. Plant material

Young *D. caryophyllus* 'Komachi' and 'Milky Way' plants were purchased from a private seed company (Japan Agribio, Shizuoka, Japan). The plants were grown in culture soil containing red clay, peat moss, and vermiculite in a glass greenhouse in Tsukuba (36°02′N, 140°05′E), which was heated when the temperature fell below 15°C and opened when the temperature rose above 25°C. The plants were fertilized weekly with 1,500-fold diluted OKF-1 fertilizer (Oat Agrio, Tokyo, Japan).

Generally, carnation cut flowers are treated with STS, which is a mixture of 0.2-mM silver nitrate and 1.6-mM sodium thiosulfate/pentahydrate, as an ethylene action inhibitor at the time of shipment to improve their vase life. In this study, cut flowers were treated with STS according to the method described by Yangkhamman et al. (2005). On the day of opening, the flowers were harvested, cut to a stem length of ~40 cm, and placed in glass flasks containing 500 mL STS. The flowers were moved to a growth chamber at 23°C under a 12-h light/12-h dark photoperiod, ~10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, and 70% relative humidity. The light period was from 0600 to 1800 h. The STS treatments were performed from 0900 to 2100 h. STS was replaced with 500 mL of fresh distilled water 12 h after the onset of treatment. The flowers were further cut to 30 cm and moved to a growth chamber at 10°C, 15°C, 23°C, or 28°C under a 12-h light/12-h dark photoperiod, ~10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, and 70% relative humidity. The light period was from 0600 to 1800 h.

2. Collection and gas chromatography-mass spectrometry (GC-MS) of the emitted scent

The scent emitted from carnation flowers was collected using the dynamic headspace method (Oka et al. 1999). All collections were performed at 0900 h to avoid the effects of circadian rhythms on scent emission. The flowers were wrapped in 1-L Tedlar bags (GL Science, Tokyo, Japan). A constant stream of air (500 $mL \cdot min^{-1}$) was filtered through activated charcoal and piped into the bags. Volatiles were collected for over an hour using a Tenax TA tube (Gerstel GmbH & Co. KG, Mülheim, Germany).

The scents collected in the Tenax TA tubes were analyzed by GC-MS using the Agilent 6890 N GC System with the Agilent 5930 N Mass Selective Detector (Agilent Technologies, Santa Clara, USA). The GC equipment was equipped with a cooled injection system (CIS) (Gerstel GmbH & Co. KG) and the DB-WAX capillary column (Agilent 122–7032; Agilent Technologies). CIS was set to the splitless mode, cryofocusing was set at −50°C, the temperature setting was $12°C \cdot s^{-1}$, and the final temperature was 300°C. Helium was used as the carrier gas at a flow rate of 1 $mL \cdot min^{-1}$. The temperature program of the column oven was set to 40°C for 2 min, raised to 250°C at $5°C \cdot min^{-1}$, and held at 250°C for 5 min. The injection, interface, and ion source temperatures were 250°C. The mass scan range was 30-300 $m \cdot z^{-1}$, and the electron potential was set to 70 eV.

Each scent compound was identified using the Wiley 9th/NIST 2011 library search system (Agilent

Technologies). The mass spectrum and retention time of each standard (purity > 90%) (Sigma-Aldrich, St. Louis, USA) were analyzed under the same conditions. The amount of each scent compound was calculated based on the peak areas of 5, 50, 250, and 1,000 ng of each standard on the ion chromatograms. The mean values of three independent plants are presented for each condition.

Results

Changes in the flower appearance of the methyl benzoate scent type 'Komachi' and the eugenol scent type 'Milky Way' following harvest are shown in Figure 1(A), and changes in the scent emission of these cultivars are shown in Figure 2. Immediately postharvest, all cut flowers were treated with STS at a constant temperature (23°C) for half a day. The drooping of flower petals progressed faster at higher temperatures. In 'Komachi,' the color fading of petals was remarkable at the highest temperature (28°C). In 'Milky Way,' browning, whose position is hidden by healthy petals in Figure 1, at the base of the petals was observed at 28°C at 10-14 days postharvest. Thus, visible flower senescence progressed faster at high temperatures, particularly at 28°C.

The total scent emission from most carnation cut flowers was the highest on the day of harvest, and it decreased over time. Exceptionally, the total scent emission at 28°C transiently increased. Following this transient increase, the total scent emission rapidly decreased and reached its lowest value under all temperature conditions during the study period. The total scent emission at 10°C and 15°C was not significantly different throughout the study period, with the lowest under all temperature conditions at 1 day after harvest and the highest at 2 or 4 days after harvest and thereafter. The total scent emission at 23°C was significantly higher or comparable with that at 10°C and 15°C until 6 or 8 days after harvest but was significantly decreased at later days.

The emission of most scent compounds decreased over time, similar to the total scent emission. Methyl benzoate in 'Komachi' showed the highest emission rate (~70%-80%) almost throughout the study period. Exceptionally, at 23°C and 28°C, terpenoid emissions were the highest (~40%) at 10 days after harvest. In 'Milky Way,' eugenol emission remained the highest (~50%-90%) regardless of the temperature. Initially, the proportion of methyl benzoate in total emissions was also high, accounting for approximately 40%, but it rapidly decreased over time. The decrease in methyl benzoate emission was faster than that in the eugenol emission.

In our previous sensory test of the scent of 'Komachi' and 'Milky Way' carnation cut flowers, 80 subjects evaluated the intensity of scent as "very scented," "scented," "slightly scented," or "unscented" (Kishimoto et al. 2019). In Figure 2, dotted lines indicate the emitted amount of the lowest limit at which over 70% of the subjects evaluated the flowers to be "very scented" or "scented." In this study, the noticeable intensity of the scent was compared using the lowest limit as a guide. In 'Komachi,' the total scent emission remained above the lowest limit for 2 days postharvest at 10°C, 15°C, and 23°C but not at 28°C. In 'Milky Way,' the total scent emission at 10°C and 15°C remained above the lowest limit for 14 days postharvest, whereas that at 23°C remained above the lowest limit for 10-12 days postharvest. However, scent emission at 28°C remained above the lowest limit for only 6 days postharvest.

The effects of temperature transition on scent emission from 'Milky Way' flowers were also investigated. The STS treatment of cut flowers was performed under the same conditions as described above. Following STS treatment, the cut flowers were maintained at 10°C for four and a half days after harvest and then transferred to 23°C. Changes in flower appearance and scent emission are shown in Figures 1(B) and 3, respectively. The cut flowers of the control plants were maintained at 23°C. Following the transition from 10 to 23°C, the drooping of the petals rapidly progressed. At 14 days postharvest, the appearance of flowers under fluctuating temperature (from 10 to 23°C) was not significantly different from that under constant temperature (23°C). Meanwhile, at 10 days postharvest, the decrease in scent emission under fluctuating temperature was slower than that under constant temperature.

Discussion

Scent emissions from carnation cut flowers varied under different temperature conditions. The decrease in scent emission over time slowed down at 10°C and 15°C and accelerated at 28°C compared with that at 23°C (Fig. 2). VBPs are biosynthesized from sugars (Widhalm & Dudareva 2015). In carnation cut flowers, the sugar content of petals rapidly decreases over time (Kondo et al. 2020, Minakuchi et al. 2007), indicating a shortage of substrates for VBP biosynthesis in petals following harvest. Generally, sugar consumption during respiration in cut flowers increases with increasing temperature (Ichimura 2018). At 28°C, the total scent emissions rapidly decreased (Fig. 2). One possible explanation is that a high temperature (28°C) may have accelerated

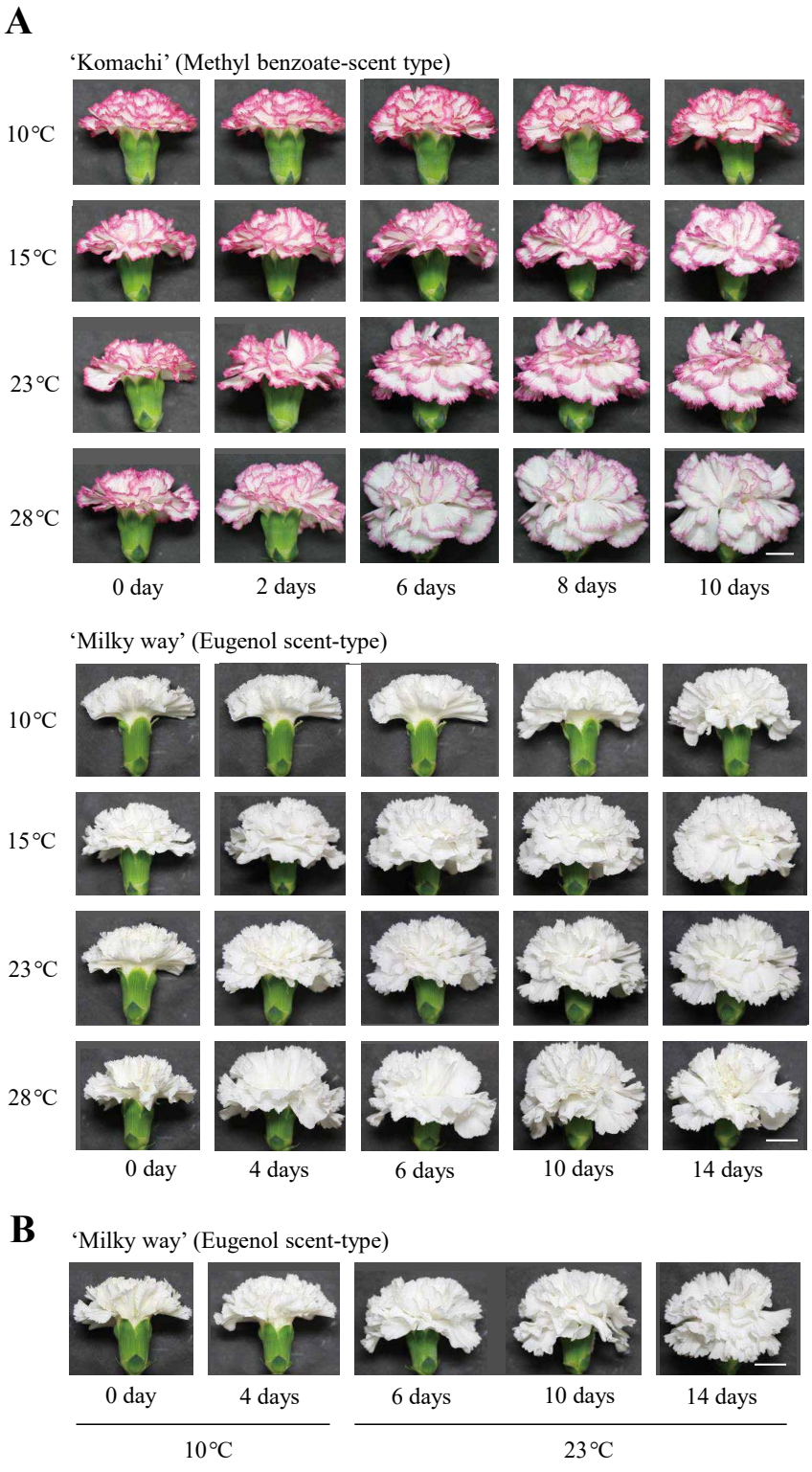

Fig. 1. Changes in flower appearance in the carnation cut flower cultivars 'Komachi' and 'Milky Way' from the day of harvesting under different temperatures

A typical appearance in the test with three independent cut flowers is shown. STS treatment was performed on harvest day, indicated as "0 d." Following STS treatment, the cut flowers were exposed to each of the tested temperatures (A). Following STS treatment, the cut flowers were incubated at 10°C for four and a half days and then transferred to 23°C (B). Scale bar = 1.5 cm

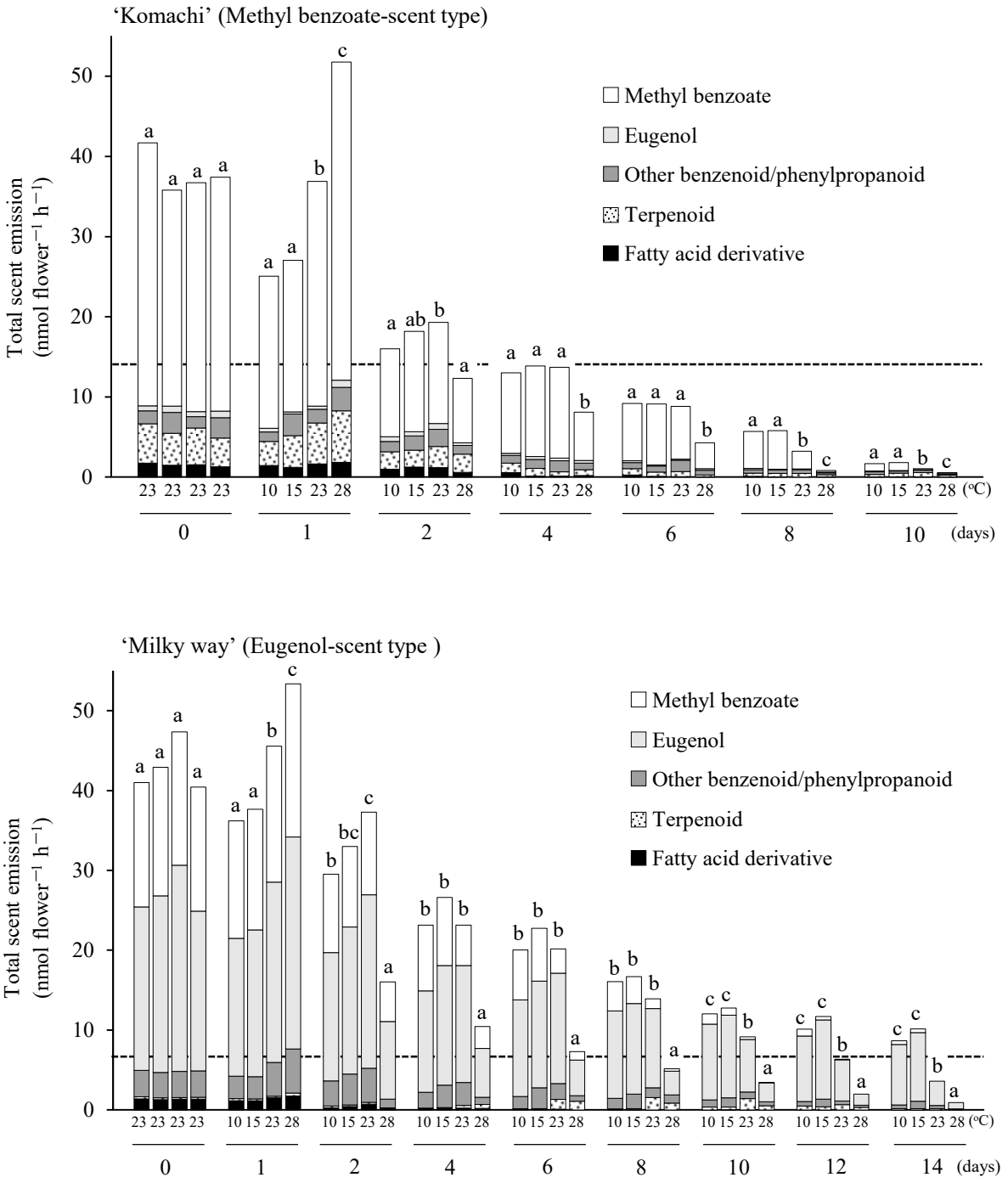

Fig. 2. Changes in the emission quantity and composition of scents in the carnation cut flower cultivars 'Komachi' and 'Milky Way' from the day of harvesting under different temperatures

The mean values of three independent cut flowers are presented for each condition. STS treatment was performed on harvest day, indicated as "0 d." Following STS treatment, the cut flowers were exposed to each of the tested temperatures. Lowercase letters indicate significant differences in total scent emissions on different survey dates (Tukey's test, $P < 0.05$, n = 3). The intensity of flower scent was evaluated by 80 subjects as "very scented," "scented," "slightly scented," or "unscented." Dotted lines indicate the level at which over 70% subjects evaluated the flowers to be "very scented" or "scented" (Kishimoto et al. 2019).

sugar consumption during respiration and rapidly decreased substrate availability for the biosynthesis of scent components. Contrarily, as reported for the VBPs of petunia (*P.* × *hybrida*) flowers, a high temperature of 28°C may decrease the catalytic activity of the biosynthesis of these scent compounds through a decrease in the expression of their biosynthetic genes (Cna'ani et al. 2015).

In a recent study on petunia, the emitted scents were found to accumulate in the cuticle of the petals (Liao et al. 2021). The amount of scent components in the cuticle accounts for approximately half of the total amount in the whole petal. Generally, the vaporization efficiency of scent compounds increases with increasing temperature. Temperature is also expected to increase the emission of scent from the cuticle into the atmosphere via vaporization. In this study, the acceleration of vaporization by high temperature may have contributed to the transient increase in scent emission at 28°C and its subsequent rapid decrease due to the depletion of scent components in the cuticle. Meanwhile, low temperatures of 10°C and 15°C may have decreased both the vaporization of scent compounds from the cuticle and the consumption of sugars by respiration. Consequently, the consumption of scents and their substrates may have reduced, contributing to continuous scent emissions. After approximately 5 days of pretreatment at 10°C, scent emission at 23°C was slightly improved compared with that at 23°C (Fig. 3). This result also suggests that the conservation of scents and their substrates at low temperatures (10°C) contributed to the subsequent scent emission at 23°C.

Ethylene is a negative regulator of VBP emissions (Negre et al. 2003, Underwood et al. 2005). In this study, all test flowers were treated with the ethylene action inhibitor STS; therefore, inward rolling of petals, a typical symptom of ethylene-dependent senescence (Kim et al. 1998), was not observed throughout the study period.

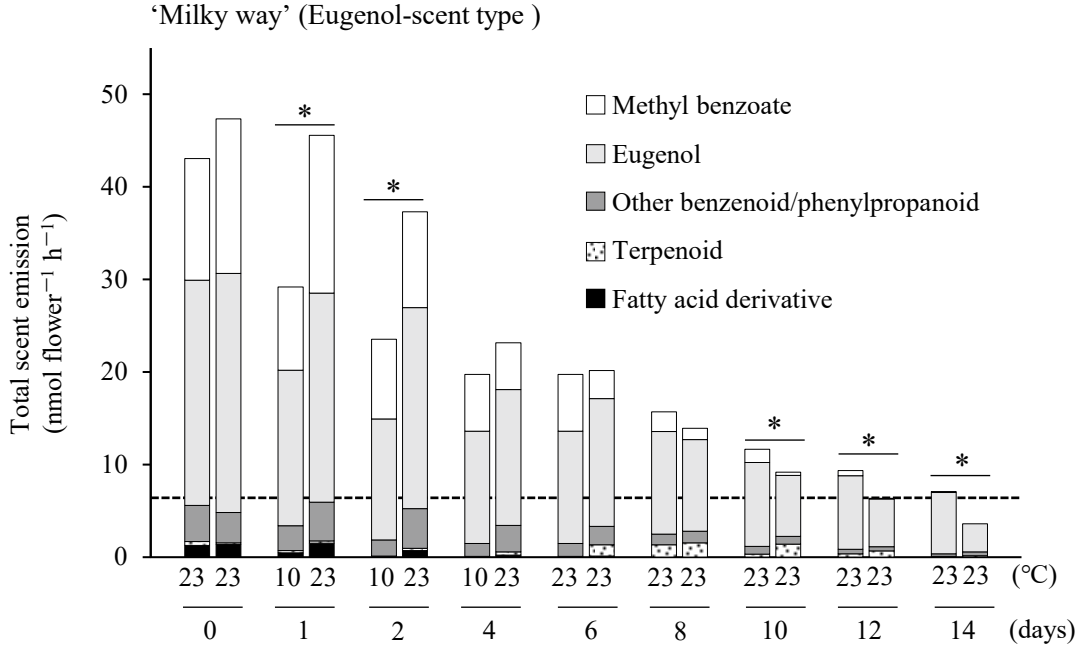

Fig. 3. Changes in the carnation cut flower cultivar 'Milky Way' from the day of harvesting under temperature transition from 10 to 23°C

STS treatment was performed on harvest day, indicated as "0 d." Following STS treatment, the cut flowers were incubated at 10°C for 4 days and then transferred to 23°C. The mean values of three independent cut flowers are presented for each condition. The right graphs show changes in scent emission under a constant temperature (23°C), which represents data from Figure 2 for comparison. Asterisks indicate significant differences in emission at a constant temperature (23°C) (Student's *t*-test, $P < 0.05$, n = 3). The intensity of flower scent was evaluated by 80 subjects as "very scented," "scented," "slightly scented," or "unscented." Dotted lines indicate the level at which over 70% subjects evaluated the flowers to be "very scented" or "scented" (Kishimoto et al. 2019).

Therefore, ethylene was not largely involved in the decrease in scent emission with increasing temperature under the conditions of this study.

Based on the sensory evaluation of carnations (Kishimoto et al. 2019), low temperatures of 10°C-15°C were suitable for the retention of noticeable scents perceptible to humans. Meanwhile, the retention of the noticeable scent at 28°C, which corresponds to the average room temperature in the summer in Japan (Katsuno & Rijal 2011), was clearly shorter than that at low temperatures (Fig. 2). Therefore, the noticeable scent in carnation cut flowers may be rapidly impaired in hot seasons. Low-temperature management at 10°C-15°C during trade can be easily achieved with common display refrigerators for cut flowers. Such management of cut flowers at low temperatures before sale is expected to contribute to the persistence of scent at room temperatures in consumers' homes after sale.

The effect of retaining the noticeable scent at low temperatures was remarkable in the eugenol scent type cultivar 'Milky Way,' but not in the methyl benzoate scent type cultivar 'Komachi,' Based on the findings of the present and previous studies, eugenol-based scent emissions tend to decrease more slowly than methyl benzoate-based scent emissions (Kishimoto & Shibuya 2021). Additionally, the aroma threshold of eugenol is lower than that of methyl benzoate (Burdock 2010). Therefore, eugenol scent type carnations are thought to be superior to methyl benzoate scent type carnations in terms of retaining scent and realizing the effects of low-temperature management.

In summary, low temperatures of 10°C-15°C are suitable for the retention of noticeable scent in carnation cut flowers. Particularly, the retention of eugenol-type scents is expected in low-temperature management. Premaintaining the temperature at 10°C at the time of sale may also help retain the scent at room temperature after sale. Various factors, such as the suppression of scent substrate consumption, regulation of scent emission from the cuticle, and influence on the expression of scent emission–related genes, are presumed to affect the retention of scent emission due to low temperature.

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