JARQ 59 (3), 219-226 (2025) https://www.jircas.go.jp

Detailed Analysis of Scent Emissions in Potted Carnations Using *Dianthus caryophyllus* 'HINAARARE'

Kyutaro KISHIMOTO*, Kenichi SHIBUYA, Masafumi YAGI and Hiroyasu YAMAGUCHI

Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, Tsukuba, Japan

Abstract

Although the scent of flowers generally changes during anthesis, this phenomenon has not been investigated in potted carnations. In this study, we examined the scent emissions of potted carnations using the fragrant *Dianthus caryophyllus* 'HINAARARE.' The plants were maintained under a 12-h light/dark cycle, and immediately after the flowers had opened, scent emissions were investigated at 2-day intervals for 15 days. The predominant scent components detected were isoeugenol, a type of phenylpropanoid/benzenoid, and the sesquiterpene β-caryophyllene. The unique sweet scent of this cultivar is believed to be attributable mainly to these two compounds, along with the fatty acid derivative1-octen-3-ol, which has a relatively low aroma threshold. The total scent emissions were characterized by an apparent diurnal rhythm, being lower during the light period and higher during the dark period. This rhythmicity was mainly associated with the emission of isoeugenol and β-caryophyllene, thereby suggesting that the strength of the scent in potted carnations, as perceived by humans, varies between day and night. In addition, we investigated the relationship between an increase in petal in-rolling, a typical symptom of flower senescence, and scent emission. However, no clear correlation was detected. To the best of our knowledge, this is the first study that has provided evidence of a diurnal rhythm in scent emissions from carnations.

Discipline: Horticulture
Additional key words: caryophyllene, diurnal rhythm, in-rolling, isoeugenol, volatile

Introduction

Carnations (*Dianthus caryophyllus* L.) are perennial plants in the family Caryophyllaceae that are widely cultivated as ornamental flowers. A survey of approximately 900 general consumers in Ibaraki Prefecture, Japan, revealed that carnations are among the flowers with the highest demand for scent (Kishimoto 2012). In Europe, the essential oil derived from carnation flowers is used as a perfume, and its scent has long been cherished (Anonis 1985).

Although carnations are primarily used as cut ornamental flowers, it has been reported that having been cut, the flowers rapidly lose their scent (Kishimoto & Shibuya 2021). In addition, the flowers of many recently developed carnation cultivars may have lost their scent (Clery et al. 1999). Therefore, to utilize scent as an added-value trait for cut ornamental flowers, it is necessary to select fragrant cultivars and develop techniques that contribute to preserving the scent. In this regard, providing potted carnations can prevent the loss of scent that occurs when stems are cut. Furthermore, it has been shown that potted carnation cultivars have a greater variety of scents than cut-flower cultivars (Kishimoto 2020). Consequently, compared with cut flowers, the retention of scent can be regarded as an important selling point of potted carnations.

At present, however, there is comparatively little basic information available regarding the scent emissions from potted carnations. For example, the chemical composition and/or quantity of the scent of cut carnation flowers have been reported to change with flower senescence and over time (Schade et al. 2001, Kishimoto & Shibuya 2021), as also illustrated by petunias (*Petunia* × *hybrida* 'Mitchell Diploid'), in which scent emission has been shown to be negatively regulated by ethylene, a

*Corresponding author: kishimoto.kyutaro748@naro.go.jp
Received 27 August 2024; accepted 6 November 2024; J-STAGE Advanced Epub 30 April 2025.
https://doi.org/10.6090/jarq.24S21

floral senescence-inducing hormone (Underwood et al. 2005). However, this has not been investigated in potted carnation cultivars. It has also been shown that the floral scents emitted by wild species of plants in the genus *Dianthus* have a diurnal rhythm (Erhardt 1991, Balao et al. 2011). Consequently, it is assumed that similar diurnal rhythms in scent emission would occur in carnations. Accordingly, gaining a comprehensive understanding of the diurnal cycles and senescence of plants and how the emissions of floral scent change over time is important for exploiting scent as an added-value trait.

Dianthus caryophyllus 'HINAARARE' is a potted carnation cultivar that was bred jointly by Snow Brand Seeds Co., Ltd. (Sapporo, Japan) and the National Agriculture and Food Research Organization (NARO, Tsukuba, Japan). This cultivar has light pink petals and a uniquely sweet scent, in recognition of which, the cultivar recently received a special award in Japan's most prestigious flower competition (Japan Flower Selection 2024). However, the chemical components of this scent have yet to be investigated.

In this study, using the 'HINAARARE' cultivar as a model, we investigated changes in the quantity and chemical composition of scent emissions during the anthesis stage of potted carnations. In addition, to assess the circadian rhythm of scent emissions, we compared the emission of floral scent during the day and immediately after sunset. Furthermore, we also focused on the relationship between petal in-rolling and scent emissions. In carnations, petal rolling is a typical symptom of ethylene-induced senescence (Mayak et al. 1977). Hence, we reasoned that it may be possible to identify a correlation between floral scent emissions, which are predicted to be negatively regulated by ethylene, and petal rolling, which is positively regulated by ethylene. On the basis of the findings of this study, we present basic knowledge of the scent emissions of potted carnations and discuss future research topics regarding the utilization of scent as an added-value trait.

Materials and methods

1. Materials

Young plants of *D. caryophyllus* 'HINAARARE' produced by Snow Brand Seed Co., Ltd. were planted in pots containing approximately 1.2 L of a general gardening soil (Royal Baiyōdo; Tachikawa Heiwanoen, Co., Ltd., Kanuma, Japan), and then cultivated in the glass greenhouse of NARO (36° 02′ N, 140° 05′ E) in Tsukuba. The temperature of greenhouse was set at a nighttime minimum of 12°C. Plants were watered when the topsoil had dried, and fertilization was performed once a week using a 1,500-fold diluted OKF-1 fertilizer (Oat Agrio Co., Ltd., Tokyo, Japan).

A few days before the flowers opened, the pots were transferred to an incubator at 20°C and 70% relative humidity under a 12-h light/dark cycle. These temperature and humidity conditions were based on the averages in May when potted carnations are commercially available in Tokyo, Japan (Japan Meteorological Agency 2024). Lights were turned on at 04:00 h and off at 16:00 h, with a light intensity of approximately 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the light period. Owing to experimental constraints, the light cycle ended 2 h in advance of the actual sunset in Tokyo in May. During this period, the plants were provided with approximately 100 mL of distilled water once daily, although they were not fertilized. All investigations in this study were conducted in this environment.

2. Evaluation of the degree of in-rolling in flowers

Flower rolling was evaluated based on the flower senescence evaluation method described by Kishimoto & Watanabe (2023). Flowers were grouped into one of the following four stages: stage 1, from the time a flower opened until the outermost petal reached the horizontal plane; stage 2, when the expansion of the outermost petal exceeded the horizontal plane and petal in-rolling could not be visually confirmed; stage 3, when both in-rolled and unrolled petals were observed; and stage 4, when all petals were in-rolled. Three flowers were selected from each of four independent pots, and the stages of the 12 flowers were examined every other day. For each of the assessed flowers, observations continued for 15 days after the flowers opened.

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

The emitted flower scent was collected using the dynamic headspace method (Oka et al. 1999), using different plants to those that had been used to evaluate the degree of petal in-rolling. Each flower was wrapped in a 500-mL Tedlar bag (GL Science Inc., Tokyo, Japan) and sealed. A constant stream of air (500 mL min^{-1}) was filtered through activated charcoal and piped into the bags. Volatile headspace gases were collected from the flowers using a Tenax-TA tube (180 mg, 60 × 80 mesh; Gerstel GmbH & Co. KG, Mülheim, Germany) twice daily for 2 h every alternate day. Of the two daily collected samples, the first was obtained during the daytime (09:30 h), 5.5 h after the commencement of the light period, and the second was taken 0.5 h after sunset

(16:30 h) at the start of the dark period. Sampling was conducted over the same period as the in-rolling observations described previously.

The samples were analyzed by GC-MS using an Agilent 6890N GC system equipped with an Agilent 5930 N mass-selective detector (Agilent Technologies, Santa Clara, CA, USA). The temperature of the Thermal Desorption System 2 (Gerstel GmbH & Co., KG) was increased from an initial temperature of 30°C at a rate of 60°C·min^{-1} until reaching a final temperature of 250°C, at which it was maintained for 10 min. The GC instrument was equipped with a cooled injection system (CIS; Gerstel GmbH & Co.) and a DB-WAX capillary column (30 m length, 0.25 mm inner diameter, and 0.25 µm film thickness; Agilent Technologies). The CIS was set to the splitless mode; cryofocusing was set at –50°C, with a temperature increase of 12°C s^{-1} and a final temperature of 300°C. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The temperature settings used for the GC-MS analysis were based on those described by Kishimoto & Shibuya (2021).

The detected compounds were identified using a Wiley 9th/NIST 2011 library search system (Agilent Technologies) and the mass spectra of standard compounds (purity > 90%) (Sigma-Aldrich, St. Louis, USA; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan; FUJIFILM Wako Pure Chemical Co., Osaka, Japan). The quantity of each scent compound was calculated based on calibration curves derived from the peak areas of each standard in the ion chromatograms. Data represent the mean values of three independent flowers for each temperature treatment.

4. Statistical analysis

The statistical significance of scent emissions was evaluated using GraphPad Prism software version 10.1.2 (GraphPad, San Diego, CA, USA) and a one-way ANOVA with Tukey's test.

Results

1. Changes in the degree of petal in-rolling

Changes in the degree of petal in-rolling after the opening of *D. caryophyllus* 'HINAARARE' flowers were examined at 2-day intervals (Fig. 1). On the first day of the investigation, all flowers were assessed to be at stage 1. From the third to the fifth day, all flowers advanced to stage 2, and in-rolling was observed from the seventh day onwards. At this time, we recorded a mixture of stage 2 and stage 3 flowers, the former of which were predominant. From day 9 to day 13, stage 3 flowers were the predominant type, with stage 4 flowers being observed from the 11th day, reaching a proportion of 50% on day 15.

2. Changes in the chemical composition of scent

The scent of *D. caryophyllus* flowers was found to comprise phenylpropanoids/benzenoids, terpenoids, and

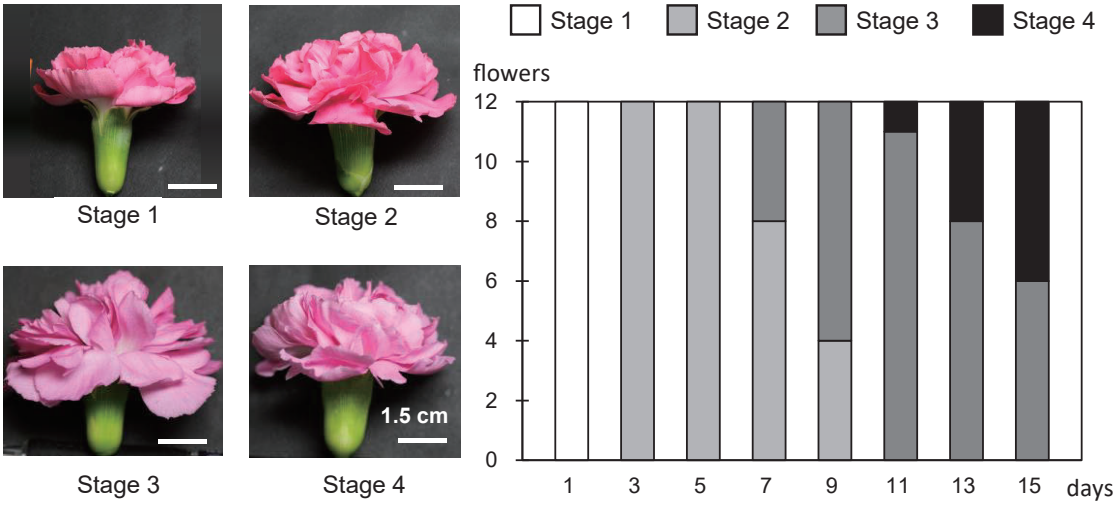

Fig. 1. Changes in the degree of petal in-rolling

To assess the degree of petal in-rolling, flowers were grouped into one of the following four stages: stage 1, from the time the flower opens until the outermost petal reaches the horizontal plane; stage 2, when the expansion of the outermost petal exceeds the horizontal plane and petal in-rolling cannot be visually confirmed; stage 3, when both in-rolled and unrolled petals are observed; and stage 4, when all petals are in-rolled. The potted plants were maintained under a 12-h light/dark cycle, with temperature and relative humidity being maintained at 20°C and 70%, respectively.

fatty acid derivatives. With the exception of the light period on day 1, the percentage of phenylpropanoids/benzenoids was invariably greater than 50% (Fig. 2). In particular, among the phenylpropanoids/benzenoids, isoeugenol showed the highest percentage, accounting for 21% to 46% of all scent emissions. In contrast, eugenol and benzaldehyde were also found to have relatively high contents, accounting for 13% to 33% of all scent emissions, with the exception being during the dark period on day 1. The next most abundant scent compound after isoeugenol was the sesquiterpene β-caryophyllene, which accounted for 19% to 32% of the total, with the exception of the light period on day 1. Among the terpenoids, caryophyllene oxide had the next highest percentage, and 1-octen-3-ol was identified as the most abundant fatty acid derivative, accounting for 20% of the total during the light period on the first day and 4% to 10% during the other periods. In addition, (*Z*)-3-hexenyl acetate was identified as the second most prominent fatty acid derivative, accounting for approximately 5% of the total up to the third day, whereas from the fifth day onwards, the proportion dropped to between 0% and 2%.

A unique composition comprising a high percentage of fatty acid derivatives was observed only during the light period on the first day (Fig. 2). During the remainder of the assessment period, the composition of the scent remained relatively constant, with isoeugenol and β-caryophyllene accounting for approximately half (48% to 65%) of the total scent constituents.

3. Changes in the quantity of scent emission

Figure 3 shows the daily changes in the total quantity of scent emissions and the number of major scent components, including phenylpropanoids/benzenoids, terpenoids, and fatty acid derivatives, as well as changes in the sum of other minor phenylpropanoids/benzenoids, terpenoids, or fatty acid derivatives. The only sesquiterpenes detected were β-caryophyllene, caryophyllene oxide, and humulene (Fig. 3). All other minor terpenoids were monoterpenes (3-carene, D-limonene, sabinene, and α-terpineol).

Notably, total scent emissions were higher during the dark period than during the light period. A similar rhythmicity was observed for the individual constituents eugenol, isoeugenol, and β-caryophyllene. Humulene also showed similar rhythmicity, although the difference was only marginally statistically significant. We also detected tendencies of differences in the quantities of

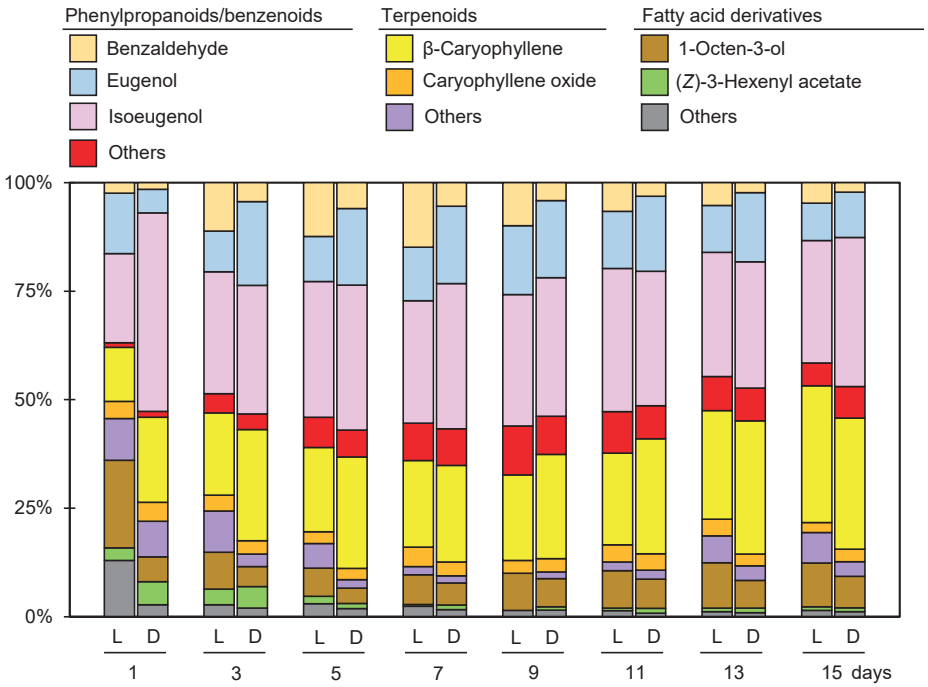

Fig. 2. Changes in the composition of scent emissions

Scent constituents are shown as the percentages of each compound in the average (n= 3) total scent emissions (nmol h^{-1}/flower). The first and second samplings on each measurement day were performed during the light (L) and dark (D) periods, respectively. The potted plants were maintained under the same conditions as described in Figure 1.

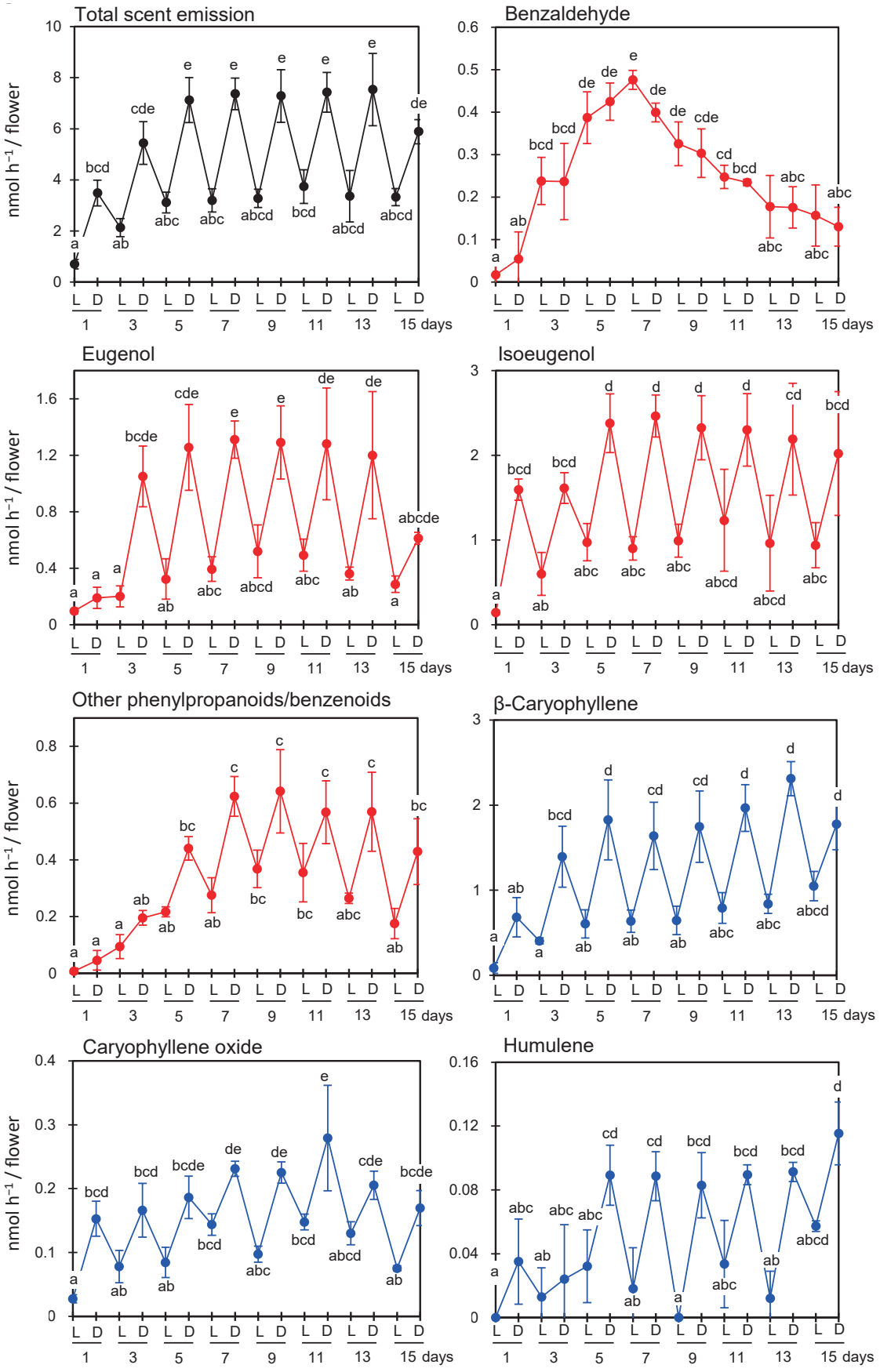

Fig. 3. Changes in the quantity of scent emissions

(Continued on next page)

(Continued)

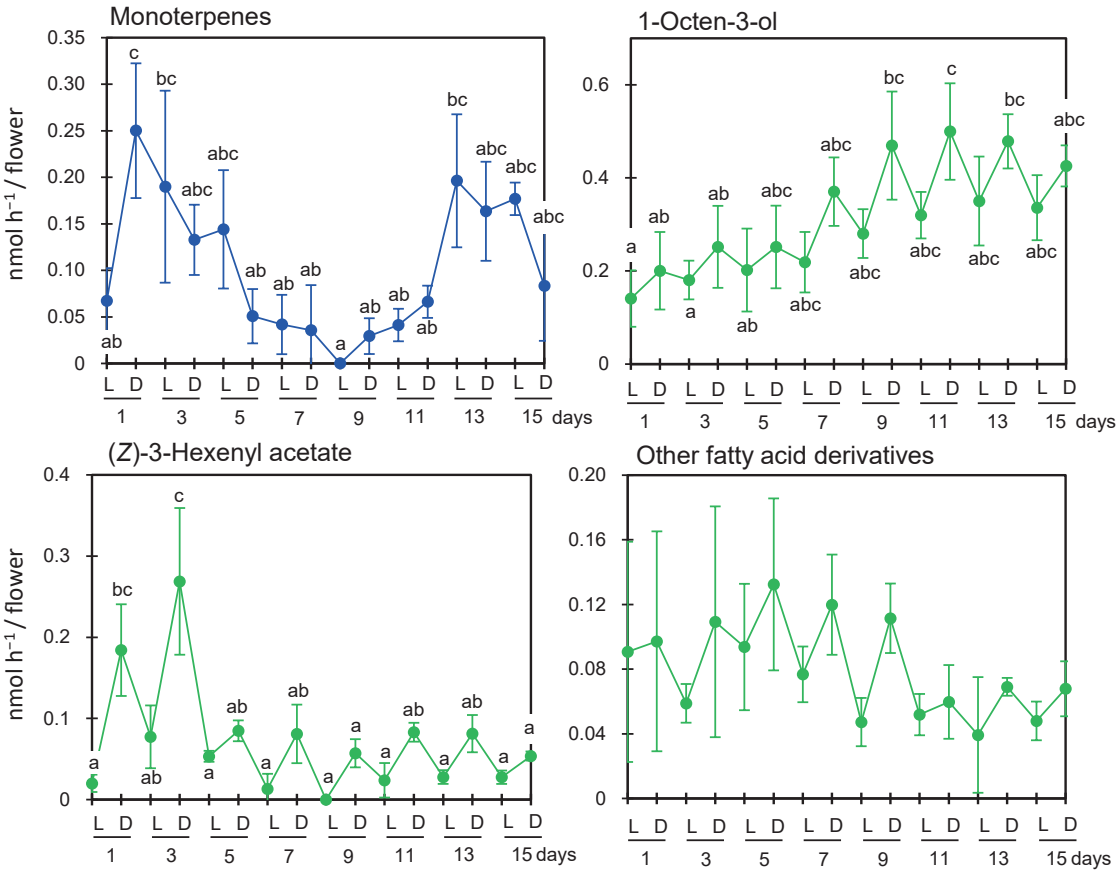

Fig. 3. Changes in the quantity of scent emissions

The presented results were obtained from the same investigation as described in Figure 2. L: light period; D: dark period. The black, red, blue, and green lines represent the total scent compounds, phenylpropanoids/benzenoids, terpenoids, and fatty acid derivatives, respectively. Values are presented as the means ± SD (n = 3). Different lowercase letters shown above and below bars indicate significant differences ($P < 0.05$; one-way ANOVA with Tukey's test).

other phenylpropanoids/benzenoids, (*Z*)-3-hexenyl acetate, 1-octen-3-ol, and caryophyllene oxide during the light and dark periods; however, the results were not statistically significant. In contrast, we found no evidence to indicate rhythmicity of benzaldehyde or monoterpene emissions during the light and dark periods, nor for the emissions of other fatty acid derivatives.

Although not always statistically significant, we also detected tendencies indicating the peaking of light-dark rhythms for benzaldehyde or monoterpene emissions by day 3 or 7, which were thereafter maintained at constant levels (Fig. 3). (*Z*)-3-Hexenyl acetate was found to show the highest emissions on days 1 and 3 and thereafter remained at low levels. In contrast, benzaldehyde emissions increased until day 7 and then began to decline.

Discussion

1. Characteristics of the scent of *D. caryophyllus* 'HINAARARE'

Our analyses in this study revealed phenylpropanoids/benzenoids (eugenol, isoeugenol, and benzaldehyde), a sesquiterpene (β-caryophyllene), and a fatty acid derivative (1-octen-3-ol) to be the major components of the scent of the 'HINAARARE' cultivar (Figs. 2, 3). Among these, isoeugenol and β-caryophyllene accounted for a notably high percentage. The aroma threshold for 1-octen-3-ol has been established to be approximately one-third to one-half that of the other scent components (Burdock 2010), thereby indicating that the average person perceives the aroma of 1-octen-3-ol as stronger than the aromas of the same amounts of isoeugenol or β-caryophyllene. Consequently, we conclude that the

main scent-contributing components of this cultivar are isoeugenol, β-caryophyllene, and 1-octen-3-ol.

Isoeugenol is often detected as a scent component in carnations (Kishimoto & Shibuya 2021), and many respondents in sensory evaluations of floral scents based on this compound have described the scent as having a sweet or vanilla-like aroma (Kishimoto et al. 2015). β-Caryophyllene has been detected in the flowers of at least 320 species (including several subspecies) of angiosperms, including carnations (Knudsen et al. 2006), and its scent is described as having a spicy-woody or clove-like aroma (Burdock 2010). 1-Octen-3-ol is the main component of mushroom scents, such as matsutake (Cho et al. 2008), and has a characteristic powerful, sweet, and earthy aroma (Burdock 2010). Collectively, it is assumed that these scents make a major contribution to the unique sweet scent of *D. caryophyllus* 'HINAARARE.'

Although the scent components detected in 'HINAARARE' have also been found in other cultivars of carnation, the compositional ratios are unprecedented (Clery et al. 1999, Schade et al. 2001, Lavy et al. 2002, Kishimoto et al. 2015, Kishimoto et al. 2019). Notably, 1-octen-3-ol has never been identified as a major component of carnations. It has been shown that potted carnation cultivars have greater scent diversity than that of cut flower cultivars (Kishimoto 2020), and the results support this.

2. Rhythmicity of scent emission

Apparent circadian rhythms were observed for some of the identified scent constituents, namely, phenylpropanoids/benzenoids and β-caryophyllene, the emissions of which were higher in the dark period and lower in the light period, and a similar rhythmicity in the total scent emission is also primarily attributable to the behavior of these compounds (Fig. 3). To the best of our knowledge, this is the first study in which the occurrence of a diurnal rhythm in the scent emissions of carnations has been demonstrated. Similar to the phenylpropanoids/benzenoids and sesquiterpenes observed in this study, the findings for other flowers have indicated that scent compounds derived from different biosynthetic pathways show synchronous circadian rhythms (Fenske & Imaizumi 2016). However, there are also known examples in which the emissions of a phenylpropanoid/benzenoid (methyl benzoate) and a terpenoid (linalool) show different rhythms in the same flower species (Matile & Altenburger 1988). In this study, however, we only investigated a single period during each of the light and dark phases. We thus cannot exclude the possibility that examining multiple periods during these phases may reveal differences in the rhythms of these compounds. Consequently, further studies will be necessary to determine whether the benzenoid isoeugenol and sesquiterpene β-caryophyllene are characterized by synchronous diurnal rhythm.

Contrastingly, we obtained no evidence to indicate that benzaldehyde or monoterpene emissions show diurnal rhythms (Fig. 3). However, although on the basis of our findings, we cannot categorically conclude that these emissions lack diurnal rhythms, it is presumed that they are under a control that differs from that of isoeugenol or β-caryophyllene. Furthermore, although we detected a certain rhythmicity in the behavior of fatty acid derivatives, the observed changes were not statistically significant. A more detailed analysis would enable us to provide a more definite conclusion. Indeed, although the compounds differ, rhythmic emissions of fatty acid derivatives have been observed in wild species of the genus *Dianthus* (Balao et al. 2011).

Nevertheless, despite the preliminary nature of our observations, the findings of this study do indicate that the strength of carnation scent varies depending on the time of the day. This is an important consideration from the perspective of ornamental flower consumers and should be further investigated using other cultivars.

3. Changes in scent with flower development

A clear change in scent composition was observed only between the light and dark periods on the first day (Fig. 2). The cause of this change was a decrease in the proportion of fatty acid derivatives. On the first day of flower opening, the proportion of fatty acid derivatives also tends to be higher in carnation cultivars of cut flowers (Kishimoto & Shibuya 2021), and this may be a common change in many cultivars. In this study, we also focused on the relationship between petal in-rolling and scent emissions. In this regard, it has been reported that when carnation cultivars for cut flowers are maintained in pots, there is a reduction in scent emissions, with an increase in the proportion of sesquiterpenes as the flowers gradually senescence (Kishimoto & Shibuya 2021). However, even when the proportion of in-rolling increased in *D. caryophyllus* 'HINAARARE,' the quantity and composition of the scent emissions appeared to remain relatively stable, at least during this observation period (Figs. 1, 3). The fact that scent emissions were maintained at a high level despite visible signs of petal senescence can be seen as an advantage in utilizing scent as an added-value trait. Accordingly, it will be necessary to determine whether this phenomenon is common to multiple potted carnation cultivars.

Acknowledgements

We thank Akira Yokofuji (Chiba Research Station, Snow Brand Seed Co., Ltd., Chiba, Japan) for providing the young plants of *D. caryophyllus* 'HINAARARE.'

References

Anonis, D. P. (1985) The application of carnation in perfumery. *Flavour Fragr. J.*, **1**, 9-15.

Balao, F. J. et al. (2011) Spatial and temporal patterns of floral scent emission in *Dianthus inoxianus* and electroantennographic responses of its hawkmoth pollinator. *Phytochemistry*, **72**, 601-609.

Burdock, G. A. (2010) *Fenaroli's handbook of flavor ingredients*-6th edition. CRC Press, Boca Raton, USA. pp. 134-1537.

Cho, I. H. et al. (2008) Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.). *Food Chem.*, **106**, 71-76.

Clery, R. A. et al. (1999) An investigation into the scent of carnations. *J. Essent. Oil Res.*, **11**, 355-359.

Erhardt, A. (1991) Pollination of *Dianthus superbus* L. *Flora*, **185**, 99-106.

Fenske, M. P. & Imaizumi, T. (2016) Circadian rhythms in floral scent emission. *Front. Plant Sci.*, **7**, 462.

Japan flower selection (2024). *JFS2024 Hachimonobumon Haru Sinsakai*. https://jf-selections.net/results/. Accessed on 1 November 2024 [In Japanese].

Japan Meteorological Agency (2024) Past weather data search. https://www.jma.go.jp/jma/menu/menureport.html. Accessed on 1 November 2024 [In Japanese].

Kishimoto, K. (2012) Characteristics of floral scent components in *Dianthus*; Evaluation as a breeding material for fragrant carnations. *SHOKUCHO*, **46**, 291-299 [In Japanese].

Kishimoto, K. (2020) The characteristics of flower scents in carnations. *In* Onozaki, T. & Yagi, M. (eds) The carnation genome. Springer Nature Singapore Pte. Ltd., Singapore, pp. 147-157.

Kishimoto, K. & Shibuya, K. (2021) Scent emissions and expression of scent emission-related genes: a comparison between cut and intact carnation flowers. *Sci. Hortic.*, **281**, 109920.

Kishimoto, K. & Watanabe, Y. (2023) Analysis of scent emission in cut flowers of *Tulipa gesneriana* L. 'Niigata 13 go,' known as Koshiharuka. *JARQ*, **57**, 217-224.

Kishimoto, K. et al. (2015) Analysis and sensory evaluation of emitted scent compounds of pot carnation flowers. *Bull. Natl. Inst. Flor. Sci.*, **15**, 1-13 [In Japanese with English abstract].

Kishimoto, K. et al. (2019) Component analysis and sensory evaluation of scent emitted from cut carnation flowers. *Bull. NARO Crop Sci.*, **3**, 29-40 [In Japanese with English abstract].

Knudsen, J. T. et al. (2006) Diversity and distribution of floral scent. *Bot. Rev.*, **72**, 1-120.

Lavy, M. et al. (2002) Linalool and linalool oxide production in transgenic carnation flowers expressing the *Clarkia breweri* linalool synthase gene. *Mol. Breed.*, **9**, 103-111.

Matile, P. & Altenburger, R. (1988) Rhythms of fragrance emission in flowers. *Planta*, **174**, 242-247.

Mayak, S. et al. (1977) Regulation of senescence in carnation (*Dianthus caryophyllus*) by ethylene: mode of action. *Plant Physiol.*, **59**, 591-593.

Oka, N. et al. (1999) Aroma evolution during flower opening in *Rosa damascena* Mill. *Z. Naturforsch., C* **54**, 889-895.

Schade, F. et al. (2001) Fragrance volatiles of developing and senescing carnation flowers. *Phytochemistry*, **56**, 703-710.

Underwood, B. A. et al. (2005) Ethylene-regulated floral volatile synthesis in petunia corollas. *Plant Physiol.*, **138**, 255-266.