Effect of Post-harvest Management on Scent Emission of Carnation Cut Flowers

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Many cut flowers are treated with an ethylene action inhibitor, silver thiosulfate (STS), to delay senescence and are shipped by dry transport that involves relatively easy loading. In addition, cut flowers are often treated with a mixture of sugar and germicide to improve their vase lives. Exogenous treatments of these compounds or drying by transport are thought to have various effects on cut flowers. This study investigated the effects of these post-harvest management methods on the scent emission of carnation (Dianthus caryophyllus L.) cut flowers. Under all the management conditions, the total scent emissions of cut flowers were highest on harvest days and then decreased, but major changes in their compositions were not observed. The decreases in scent emissions were thought to occur earlier than the known ethylene induction in carnation cut flowers, which is equivalent to the 4th day after harvest. The STS treatment had no effect on the scent emissions for some time after harvest, but suppressed the decreases in scent emission 4–8 days after harvest under the wet transport condition. It is likely that the decreases in scent emissions in carnation cut flowers occur in an ethylene-independent manner, but ethylene induction a few days after harvest further promotes decreases under wet transport conditions. STS may have suppressed the promotion of decreases due to ethylene. On the other hand, the treatment that assumed dry transport for one day dramatically promoted the decreases in scent emissions. Since the promoting effects were not affected by the STS treatment, they were considered to be ethylene-independent. A common sugar treatment with 1% glucose, sucrose, or fructose did not affect the scent emissions in the cut flowers. An isothiazolinonic germicide, which is a common cut flower germicide, did not affect the treatment. Considering the current post-harvest process, the duration of a noticeable scent in carnation cut flowers can be expected to be extended by adopting wet transport instead of dry transport.

Key Words: Dianthus caryophyllus, dry transport, eugenol, methyl benzoate, silver thiosulfate.

Introduction

Carnation (Dianthus caryophyllus), a perennial of the family Caryophyllaceae, is mainly used as cut and potted flowers. Approximately 234 million cut flowers were produced in the fiscal year 2018 and this was the third highest in Japan following production of chrysanthemum (Chrysanthemum × morifolium R.) and rose (Rosa × hybrida L.) (Statistics of Agriculture, Forestry and Fisheries in Japan <https://www.maff.go.jp/j/tokei/kouhyou/ana_sangyo/>). It is estimated that approximately 8 million potted flowers are produced annually in Japan (Ichimura, 2013).

The scent components emitted from carnations are composed of benzenoids/phenylpropanoids, terpenoids, and fatty acid derivatives (Clery et al., 1999; Kishimoto et al., 2015, 2019). In particular, the floral volatile benzenoids/phenylpropanoids (FVBPs) are important principal components (Clery et al., 1999; Kishimoto et al., 2015, 2019). Recently, in an investigation of 35 carnation cultivars used as cut flowers in Japan, we found that most cultivars had a fruity scent based on the benzenoid methyl benzoate (Kishimoto et al., 2019). The methyl benzoate scent-type is considered to be a characteristic of modern cut flower cultivars (Clery et al., 1999). Several cut flower cultivars have a spicy scent based on the benzenoid eugenol (Kishimoto et al., 2019). Traditional carnation cultivars that are cultivated in Southern Europe to obtain essential oils for perfumes belong to the eugenol scent-type (Anonis, 1985; Clery et al., 1999). In carnation cultivars for potted flowers,
various FVBPs such as eugenol, isoeugenol, and methyl salicylate, have been detected as the principal scent components (Kishimoto et al., 2015). On the other hand, the methyl benzoate scent-type seems to be extremely rare in potted cultivars (Kishimoto et al., 2015).

Many cut flowers are placed under unique management after harvest. For carnation cut flowers, silver thiosulfate (STS), an ethylene action inhibitor, is used to delay ethylene-induced flower senescence. In addition, dry transport, which does not supply water, is used for the transport of cut flowers. Thus, cut flowers are placed under specific management conditions, but the effects of these conditions on the scent emission of cut flowers are unknown. FVBP emission in petunia (Petunia × hybrida Vilm. ‘Mitchell’) is known to be inhibited by pollination-induced ethylene and exogenous ethylene treatment (Colquhoun et al., 2010; Underwood et al., 2005). Recently, the ethylene-responsive R2R3-MYB transcription factor ETHYLENE RESPONSE FACTOR6 was shown to suppress the expression of the transcription factor and enzyme genes that contribute to FVBP emission (Liu et al., 2017). Exogenous ethylene treatment has also been reported to reduce FVBP production in cut carnation flowers (Schade et al., 2001). Therefore, STS treatment, which inhibits ethylene action, may affect the scent emissions of cut flowers. Drying by transport is also known to affect cut flowers. The vase lives of several cut flowers, such as rose and gypsophila (Gypsophila paniculata L.), have been reported to be reduced by dry transport (Hu et al., 1998; Miyamae et al., 2007). Therefore, drought stress from transport may also affect the scent emissions of cut flowers.

Cut flowers are often treated with mixtures of sugar and germicide to improve their vase lives after transport. Common treatment with 1% glucose and 0.05% isothiazolinonic germicide, has been reported to improve the vase life of more than 20 species of cut flowers (Ichimura et al., 2006, 2015; Watanabe et al., 2013). Exogenous sugar treatment is thought to contribute to the vase life of cut flowers by acting as a respiratory substrate, a synthetic substrate for cell walls, and as an osmoregulator (Abdul-Wasea, 2012; Dung et al., 2017; Hussen and Yassin, 2013; Kesta and Boonrote, 1990; Pun and Ichimura, 2003). Sugar treatments have also been reported to delay ethylene production or reduce ethylene sensitivity in several cut flowers (Ichimura and Suto, 1999; Ichimura et al., 2000; Yanagisawa et al., 2003). Similar effects of sugar treatment have been reported in carnation cut flowers (Pun et al., 2016; Verlinden and Garcia, 2004). Sugars are also essential substrates for FVBP biosynthesis (Colquhoun and Clark, 2011). Germicides suppress the growth of microbes and keep the water in the vase clean. Isothiazolinonic germicides, which are common germicides for cut flowers, have been shown to suppress the odor emission of ornamental cabbage (Brassica oleracea var. acephala f. tricolor) cut flowers (Kishimoto et al., 2014). Therefore, post-harvest sugar and germicide treatments may also affect the scent emissions of cut flowers.

This study investigated the effects of common STS, sugar and germicide treatments, and dry transport on the scent emissions of carnation cut flowers. Furthermore, in order to confirm the universality of our results in carnations, four cultivars of two different scent types, namely, the methyl benzoate and eugenol-scent types, were used in this study. Based on the results, the effects of these post-harvest managements on scent emissions and their causes are discussed, and post-harvest management suitable for scent emission is proposed.

Materials and Methods

Plant materials

Young plants of Dianthus caryophyllus L. ‘Komachi’ (Japan Agribio, Co., Ltd., Hamamatsu, Japan), ‘Mandisa’ (Miyoshi, Co., Ltd., Tokyo, Japan), ‘Milky Way’ (Japan Agribio) and ‘Misty’ (Inochio Fujiplants, Co., Ltd., Aichi, Japan) were purchased from private seed companies. The young plants were grown in commonly cultivated soil containing red clay, peat moss, and vermiculite in a glass green house, which was heated below 15°C and opened above 25°C, in Tsukuba (36°02’ N, 140°05’ E). The young plants were fertilized weekly with 1500-fold diluted OKF-1 fertilizer (OAT Agrio Co., Ltd., Tokyo, Japan) and were replanted every year. The cultivation period was from 2015 to 2016.

Cut flower treatments

1. STS treatment

Carnation flowers were treated with an ethylene action inhibitor, a mixed solution of 0.2 mM silver nitrate and 1.6 mM sodium thiosulfate/pentahydrate (STS), according to the methods of Yangkhamman et al. (2005). On the day of flower opening (day 0 in Fig. 1), the flowers were harvested at 0800 h, cut back to a stem length of about 40 cm, and placed in a glass flask containing 500 mL of distilled water (DW) or STS. The cut flowers were moved into a growth chamber at 23°C with a 12 h light-dark photoperiod under a light intensity of approximately 10 μmol·m⁻²·s⁻¹ and 70% relative humidity. Twelve hours after the onset of STS treatment, the cut flowers were re-cut to 30 cm, and the DW or STS was replaced with new DW.

2. Dry transport treatment

The treatments that mimicked dry transport for about a day were carried out according to the method of Yangkhamman et al. (2005). The cut flowers after DW or STS treatment were packed with dry newspaper in a box and stored at 23°C. One day later, each cut flower was re-cut to 30 cm, placed in a glass flask containing 500 mL DW, and maintained under the above environ-
mental conditions. The controls that mimicked wet transport were only re-cut to 30 cm and were maintained under the same conditions.

3. Sugar and germicide treatment

Carnation cut flowers were treated with sugars and isothiazolinonic germicide according to the methods of Ichimura et al. (2015). After STS treatment, each cut flower was placed in a glass flask containing 500 mL of DW with 1% sugar comprised of glucose, sucrose, or fructose. In order to prevent the growth of microbes in the flask, 0.05% isothiazolinonic germicide (CMIT/MIT; Rohm and Haas Japan, Tokyo, Japan), composed of 1.15% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.35% 2-methyl-4-isothiazolin-3-one, was added. The controls were only treated with isothiazolinonic germicide. The cut flowers were maintained under the same conditions as described above.

Collection and GC-MS analysis of emitted scents

The emitted scents from carnation flowers were collected using the dynamic headspace method (Oka et al., 1999). All the collections were performed at 0900 h to avoid any effects of circadian rhythm (Matile and Altenburger, 1988) on scent emissions. The carnation flowers were carefully wrapped in 1-L Tedlar bags (GL Science Inc., Tokyo, Japan) that were then completely sealed with tape. A constant stream of air (500 mL·min⁻¹) that was controlled by a flow meter (Kofloc Co., Ltd., Kyoto, Japan) was filtered through activated charcoal and then piped through the Tedlar bags, and volatiles were collected with a Tenax-TA tube (180 mg, 60 × 80 mesh; Gerstel GmbH & Co. KG, Mülheim, Germany).

The collected scent compounds in the Tenax-TA tubes were directly analyzed by gas chromatography-mass spectrometry (GC-MS) (Kishimoto et al., 2019).
GC-MS was performed using an Agilent 6890 N GC System coupled to an Agilent 5930 N mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The GC was equipped with a cooled injection system (CIS; Gerstel GmbH & Co. KG) and a DB-WAX capillary column (Agilent 122–7032; Agilent Technologies). CIS was set to splitless mode, the cryofocusing was −50°C, the temperature setting was 12°C·s⁻¹, and the final temperature was 250°C. Helium was used as the carrier gas, and the flow rate was 1 mL·min⁻¹. The temperature program of the column oven was set to 40°C for 2 min, 5°C·min⁻¹ up to 250°C, and held at 250°C for 5 min. The injection, interface, and ion source temperatures were 250°C. The mass scan range was m·z⁻¹ 30–300, and the electron potential was set to EI 70 eV.

Each scent compound was identified using a Wiley 9th/NIST 2011 library search system (Agilent Technologies). The mass spectrum and retention time of each standard (purity > 90%) (Sigma-Aldrich Corp., St. Louis, MO, USA; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were analyzed under the same conditions. The amounts of each scent compound were calculated by the area rate of the ion chromatogram obtained from 5, 25, 50, 250, and 1,000 ng of each standard. The mean values of three independent plants were presented for each condition.

Results

1. Effect of STS and dry transport on scent emissions

The changes in flower appearance of methyl benzoate scent-type cultivars ‘Komachi’ and ‘Misty’ and eugenol scent-type cultivars ‘Mandisa’ and ‘Milky Way’ after harvesting are shown in Figures 1 and 2 shows the changes in the scent emissions. Inward rolling of petals, one of the typical symptoms of ethylene-dependent senescence (Soo et al., 1998), was not observed under the STS treatment conditions, while inward rolling of petals was observed in all cultivars under the control condition 8 days after harvest. In ‘Komachi’, the fading of petals was suppressed by the STS treatment. In ‘Mandisa’ and ‘Milky Way’, the browning of base of petals was suppressed by the STS treatment. Therefore, the STS treatment markedly suppressed the progression of visible flower senescence. On the other hand, no apparent differences in flower appearance under dry and wet transport-mimicking treatments were observed. In ‘Komachi’ and ‘Misty’ cultivars, methyl benzoate was the principal scent component throughout the experimental period, accounting for 36–87% of the total emissions (Fig. 2). In ‘Mandisa’ and ‘Milky Way’ cultivars, eugenol was the principal scent component throughout the experimental period, accounting for 49–90% of the total emissions. Therefore, the principal scent components did not change under these conditions. The total scent emissions from the cut flowers were highest on the day of flower harvesting and decreased over time. Most of the emissions of each scent compound also continued to decrease from the day of harvesting. Especially under STS treatment, it was clear that the decrease in scent emission occurred prior to visible flower senescence (Figs. 1 and 2).

The total scent emissions retained with the wet treatment were higher than those with the dry treatment (Fig. 2). The STS treatment only suppressed the scent emission decreases with the wet treatment. This suppressive effect was not observed immediately after harvest but was detected 4–8 days after harvest. The total scent emissions were highest under the combination of STS and wet treatment.

This study also investigated whether the duration of noticeable scent of carnation cut flowers differed under each condition. In our sensory test of scent using cut carnation flowers, the strength of the scent was evaluated by 80 subjects as “very scented”, “scented”, “slightly scented”, or “unscented” (Kishimoto et al., 2019). The dotted lines in Figure 2 indicate the lowest limit of scent emission, at which more than 70% of subjects gave a positive evaluation of “very scented” or “scented”. In the methyl benzoate scent-type cultivars, scent emissions under the wet treatment remained above the lowest limit for 2–4 days after harvesting, which was more than twice as long as that period under the dry treatment (Fig. 2). In the eugenol scent-type cultivars, scent emissions under the combination of STS and wet treatment remained above the lowest limit for at least 8 days after harvesting, while the scent emissions under the dry treatment remained above the lowest limit for 2–6 days after harvesting.

2. Effect of sugar and germicide on scent emission

The effects of exogenous sugar and isothiazolinonic germicide on the scent emissions in carnation cut flowers were also investigated. Figures 3 and 4 show the changes in flower appearance and scent emissions in ‘Komachi’ cut flowers treated with 1% glucose and 0.05% isothiazolinonic germicide, respectively. Treatment with the germicide alone and treatment with glucose, sucrose, and fructose, mixing each with the germicide, did not affect the flower appearance or scent emissions of ‘Komachi’. Treatment with the germicide alone and treatment using glucose mixed with the germicide did not affect the flower appearance or scent emissions of ‘Misty’, ‘Mandisa’, and ‘Milky way’ either (Figs. S1 and S2).

Discussion

Ethylene is considered to be a negative regulator of floral scent emissions (Colquhoun et al., 2010; Schade et al., 2001), and the decrease in scent emissions is synchronized with ethylene-induced flower senescence (Negre et al., 2003; Underwood et al., 2005). Ethylene
production in carnation cut flowers in other studies has been detected approximately 4 days after harvesting (Borochov and Woodson, 1989; Kondo et al., 2020; Shibuya et al., 2000; ten Have and Woltering, 1997). In this study, the scent emission decreases seemed to occur earlier than the timing of ethylene production. Although STS treatment suppressed the visible senescence of cut flowers (Fig. 1), the scent emission decreases were only suppressed under the wet transport condition (Fig. 2). Interestingly, this suppressive effect was not observed immediately after harvest, but was detected 4–8 days after harvest (Fig. 2). The timing of the suppression in scent emissions decrease under the STS treatment seemed to coincide with the known ethylene production timing (Borochov and Woodson, 1989; Kondo et al., 2020; Shibuya et al., 2000; ten Have and Woltering, 1997). These findings suggest that the initial decrease in scent emissions from cut carnation flowers occurs in an ethylene-independent manner, but subsequent ethylene production further promotes its reduction. Therefore, STS treatment is thought to contribute to the suppression of scent emission decrease in carnation cut flowers in the late flower opening stage.

The treatment which mimicked the dry transport of cut flowers for about a day enhanced the decrease in scent emissions and duration of noticeable scent in

Fig. 2. Changes in emission quantities and compositions of scents in carnation cut flower cultivars ('Komachi', 'Misty', 'Mandisa', and 'Milky way') from the day of harvesting. Mean values of three independent cut flowers are presented for each condition. STS indicates a mixed solution of 0.2 mM silver nitrate and 1.6 mM sodium thiosulfate pentahydrate. ‘D’ represents dry treatment, ‘W’ represents wet treatment, ‘C’ represents the control (i.e., STS untreated), and ‘S’ represents treatment with STS. The dry and wet treatments mimicked dry and wet transport of cut flowers for about a day, respectively. Lower case letters indicate significant differences in the total scent emission on each survey date (Tukey’s test, \( P < 0.05, n = 3 \)). Intensities of flower scents were evaluated by 80 subjects as “very scented”, “scented”, “slightly scented”, or “unscented”. Dotted lines indicate the level at which more than 70% of subjects gave the positive evaluations “very scented” or “scented” (Kishimoto et al., 2019).
carnation-cut flowers (Fig. 2). Based on this result, drought stress by transport is considered to promote a decrease in the scent emissions of carnation cut flowers. Induction of abscisic acid is a typical drought stress response in plants (Vishwakarma et al., 2017). Abscisic acid is also induced in carnation cut flowers with a decrease in water content in the petals (Eze et al., 1986). In addition, abscisic acid is thought to activate ethylene production and promote ethylene-induced senescence in carnation flowers (Nomura et al., 2013; Shibuya et al., 2000). However, the promotion of scent decrease by drying is thought to be ethylene-independent because it was not affected by the STS treatment (Fig. 2). The relationship between drought stress or abscisic acid, and floral scent emissions in plants is unknown. Since various cut flowers are transported by dry transport, a similar phenomenon may occur with other cut flowers. In carnation cut flowers, the combination of wet transport and STS treatment is expected to significantly suppress the decrease in scent emissions due to dry transport.

In carnations, the sugar content of the petals decreases rapidly after harvest (Kondo et al., 2020; Minakuchi et al., 2007). Since sugar is an essential substrate for FVBP, its deficiency in the petals is expected to cause a decrease in scent emissions. However, treatment with sugar at a general concentration (Ichimura et al., 2015) did not contribute to scent emissions in carnation cut flowers. Minakuchi et al. (2007) treated carnation cut flowers with sugars and investigated the sugar content in the petals. In order to maintain the sugar content of cut flowers at the same level as intact flowers, a high concentration sugar treatment of 5% or more was required. In fact, the accumulation of anthocyanins in the petals of carnation cut flowers was improved by treatment with high concentrations of

![Fig. 3. Changes in flower appearance in cut flowers of the ‘Komachi’ carnation cultivar from the day of harvesting. Typical flowers selected from three independent cut flowers are shown. ‘C’ represents the control, ‘I’ represents treatment with isothiazolinonic germicide, ‘GI’ represents treatment with glucose and isothiazolinonic germicide, ‘FI’ represents treatment with fructose and isothiazolinonic germicide, and ‘SI’ represents treatment with sucrose and isothiazolinonic germicide. Isothiazolinonic germicide was composed of 1.15% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.35% 2-methyl-4-isothiazolin-3-one. The concentrations of each sugar and isothiazolinonic germicide were 1% and 0.05%, respectively. Scale bars represent 1.5 cm.](image1)

![Fig. 4. Changes in emission quantities and compositions of scents in cut flowers of the ‘Komachi’ carnation cultivar from the day of harvesting. Mean values of three independent cut flowers are presented for each condition. ‘C’ represents the control, ‘I’ represents treatment with isothiazolinonic germicide, ‘GI’ represents treatment with glucose and isothiazolinonic germicide, ‘FI’ represents treatment with fructose and isothiazolinonic germicide, and ‘SI’ represents treatment with sucrose and isothiazolinonic germicide. Isothiazolinonic germicide was composed of 1.15% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.35% 2-methyl-4-isothiazolin-3-one. The concentrations of each sugar and isothiazolinonic germicide were 1% and 0.05%, respectively. Lower case letters in indicate significant differences in the total scent emissions on each survey date (Tukey’s test, $P < 0.05, n = 3$). Intensities of flower scents were evaluated by 80 subjects as “very scented”, “scented”, “slightly scented”, or “unscented”. Dotted lines indicate the level at which more than 70% of subjects gave the positive evaluations “very scented” or “scented” (Kishimoto et al., 2019).](image2)
sucrose (more than 5%, Minakuchi et al., 2008). This suggests that sugars are important for the accumulation of anthocyanins that share the biosynthetic pathway with FVBP in carnation cut flowers. Therefore, trying sugar treatment at higher concentrations than typical in cut flowers could be effective.

Exogenous isotiazolinone germicide treatment suppresses the emission of organic sulfur compounds such as dimethyl disulfide from cut flowers of ornamental cabbage (Kishimoto et al., 2014). The suppression mechanism for the emission of organic sulfur compounds has not been clarified, but it is thought to have no effect on the FVBP emissions of carnation cut flowers.

The scent composition of carnation cut flowers does not change dramatically under general post-harvest management. The methyl benzoate scent-type is the most typical modern carnation cultivar used for cut flowers and it has a fruity fragrance (Clery et al., 1999; Kishimoto et al., 2019). The duration of a noticeable scent from cut flowers was estimated to be less than 6 days after harvest (Fig. 2). According to a questionnaire survey of 870 visitors at the open campus of NARO in Ibaraki, Japan, less than 8% of the respondents knew the scent of carnation flowers (Kishimoto, 2012). This rate was very low compared with the number of respondents who knew the scents of chrysanthemums, roses, and lilies (about 20%, 70%, and 66%, respectively) (Kishimoto, 2012). The fact that the scent of carnations is not well recognized in Japan may be related to the short duration of noticeable scent from cut carnation flowers that are methyl benzoate scent-type cultivars. The eugenol scent-type cultivars that have a spicy fragrance were estimated to have a relatively long duration of noticeable scent (Fig. 2). In particular, the scent emissions under wet transport with STS treatment retained a noticeable scent for at least 8 days after harvesting (Fig. 2). Eugenol has a lower aroma threshold than methyl benzoate (Burdock, 2010); therefore, flowers of this scent-type may have an advantage for noticeable scent retention compared with methyl benzoate scent-type flowers.

In conclusion, it is expected that the duration of noticeable scent of carnation cut flowers can be extended by adopting wet transport instead of dry transport, which is a more common transport method. The effect of STS treatment appears to result in higher scent emissions a few days after harvest under a wet transport system. Eugenol scent-type cultivars, which are relatively less common compared with methyl benzoate scent-type cultivars, have better characteristics to retain noticeable scent for a longer time.

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