

The influence of the cultivation environment on the fragrance of cyclamens

NAOE SHIBUSAWA^{1*}, SHIGERU MATSUYAMA², RYO OHSAWA³

¹*Blight and Harmful Insects Prevention Office, Tokyo, Japan*

²*Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*

³*Institute of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan*

*Corresponding author: Naoe_Shibusawa@member.metro.tokyo.jp

Abstract

Shibusawa N., Matsuyama S., Ohsawa R. (2018): The influence of the cultivation environment on the fragrance of cyclamens. Hort. Sci. (Prague), 45: 145–155.

The scent of scented cyclamen flowers weakens when the plants are displayed for long periods, and this phenomenon is affected by the environment in which the plants are displayed. Counteracting environmental effects on scent intensity requires an understanding of floral scent emission during display. Here, we used gas chromatography-mass spectrometry and sensory analysis to evaluate the influence of light intensity on floral scent emission from scented cyclamens kept indoors and in a greenhouse. For the greenhouse cyclamen, odour intensity was nearly constant throughout the study period. In contrast, the odour intensity of the indoor plants had decreased by 13 days after flowering, and the odour intensities of the indoor and greenhouse cyclamen differed significantly. Rank correlation analysis showed a positive correlation between odour intensity as determined by sensory analysis and the total amount of volatile compounds released as determined by gas chromatography-mass spectrometry. That is, the sensory analysis results could be explained in terms of the emission amounts of odour compounds.

Keywords: cyclamen; effect of light; odour compound; sensory analysis; volatile

Cyclamens are popular flowering plants for indoor display in homes and large facilities during autumn and winter. However, anecdotal evidence suggests that the scent of cyclamens becomes weaker over time during a period of long exhibition. Reduction of the scent during exhibition markedly lowers the product value. However, there has been no validation of whether the decrease in the scent of exhibited cyclamens is a real phenomenon. Therefore, we hypothesised that sensory tests should determine whether fragrance weakens during exhibition. In general, decreased scent is considered to be influenced by the cultivation conditions, such as light intensity or temperature. Therefore, if sensory testing proves a decrease in scent, elucidating the effects of environmental conditions on the volatile odour compounds of cyclamens will allow us to overcome this problem.

It has been well established that the composition of plant volatiles is influenced by various abiotic factors, including soil and air humidity, temperature, light intensity and fertilisation rate (DUDAREVA

et al. 2006). Specifically, it has been observed that emissions from *Trifolium repens* L. flowers (JAKOBSEN, OLSEN 1994) and *Petunia axillaris* flowers (SAGAE et al. 2008) increase with temperature, and the amounts of floral scent compounds released by *Lilium* 'siberia' (HU et al. 2013) and some Mediterranean plants (FARRÉ-ARMENGOL et al. 2014) have been reported to be positively correlated with temperature in a certain range. In contrast, CNA'ANI et al. (2015) reported that floral scent production by *Petunia* × *hybrida*, decreases with increasing ambient temperature. With regard to the influence of light, emission from *Trifolium repens* L. flowers is the most intense at high irradiances (JAKOBSEN, OLSEN 1994). HU et al. (2013) found that both the number and amounts of volatile compounds released from *Lilium* 'siberia' increase with increasing light intensity in a certain range. DUDAREVA et al. (2003) reported that the amounts of (*E*)- β -ocimene and myrcene released from continuously irradiated *Antirrhinum majus* are higher than the amounts released from plants

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kept continuously in the dark. Further, the amounts of released volatile compounds depend on flowering stage. For example, distinct changes in scent composition and concentration across flowering stages have been reported for snapdragon (KOLOSOVA et al. 2001), rose (SHALIT 2003; RUSANOV 2011), *Luculia pinceana* (LI et al. 2016), *Cananga odorata* (QIN et al. 2014) and *Vanda Mimi Palmer* (MOHD-HAIRUL et al. 2010). Since scented cyclamens have been only recently bred, there is no knowledge of the influence of the cultivation environment on the strength and sustainability of their scents. Clarifying this relationship is not only significant for horticultural science but also in terms of plant physiology.

In this study, we first established that fragrance becomes weak during exhibition using a sensory test. Subsequently, we determined the influence of light intensity and temperature on emissions of volatile odour compounds from the flowers of scented cyclamens using gas chromatography-mass spectrometry (GC-MS). Based on the results of the sensory test and the analysis of volatile substances, we discuss the influence of the cultivation environment on the fragrance of cyclamens.

MATERIAL AND METHODS

Plant material. The main purpose of this study was to evaluate the influence of light intensity on volatile odour emission. Therefore, it was necessary to eliminate the effect of temperature. First, we evaluated the effects of temperature, and, based on the result, we established conditions to investigate the influence of light intensity on the emission of volatile odour compounds. It was difficult to conduct both experiments at the same time; therefore, we used two interspecific cyclamen hybrid cultivars: 'Sawayaka midi' for temperature experiments and 'Odayaka' for light intensity experiments, and ensured that the flowering time of each peaked during the respective experiment. These cultivars were bred to add fragrance to the popular cyclamen, *C. persicum*. 'Sawayaka midi' was produced by crossing the cyclamen cultivar 'Schubert' with the scented wild species *Cyclamen purpurascens* and then culturing the ovules (ISHIZAKA, UEMATSU 1995). 'Sawayaka midi' leaves were propagated by tissue culture (KANDA et al. 1997), and the resulting clone plantlets were cultured in a greenhouse at an air temperature of >10°C. Plantlets with two or three leaves were cultured in 9-cm-diameter plastic pots,

and when the plants had approximately ten leaves, they were transplanted into 15-cm-diameter pots. We used a planting medium composed of tuff loam, leaf mould and peat (5:3:2), and we watered the plants with fertiliser solution (N:P:K 50:100:50 ppm) at 7–10-day intervals. 'Odayaka' was produced by crossing the cyclamen cultivar 'Akebono' with the scented wild species *C. purpurascens* and then culturing the ovules (ISHIZAKA, UEMATSU 1995). Cultivation management was the same as for 'Sawayaka midi' described above. Since these plants grow by vegetative propagation, individuals used in this experiment can be said to be genetically uniform with other individuals from the variety.

Investigation of temperature exchange effect

To evaluate the effects of temperature, we used the interspecific cyclamen hybrid 'Sawayaka midi', which was produced by crossing the cyclamen cultivar 'Schubert' with the scented wild species *Cyclamen purpurascens* and then culturing the ovules (ISHIZAKA, UEMATSU 1995). 'Sawayaka midi' leaves were propagated by tissue culture (KANDA et al. 1997), and the resulting clone plantlets were cultured in a greenhouse at an air temperature of >10°C. Plantlets with two or three leaves were cultured in 9-cm-diameter plastic pots, and when the plants had approximately ten leaves, they were transplanted into 15-cm-diameter pots. We used a planting medium composed of tuff loam, leaf mould and peat (5:3:2), and we watered the plants with fertiliser solution (N:P:K 50:100:50 ppm) at 7–10-day intervals.

Investigation of light intensity influence. To evaluate the effects of light intensity, we used the cyclamen interspecific hybrid 'Odayaka', which was produced by crossing the cyclamen cultivar 'Akebono' with the scented wild species *C. purpurascens* and then culturing the ovules (ISHIZAKA, UEMATSU 1995). Cultivation management was as described above for 'Sawayaka midi'.

Investigation of temperature exchange effect. Temperature experiments were carried out in a climate chamber at 15, 25 and 35°C. The plants were continuously irradiated at a light intensity of 200 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. Three plants of 'Sawayaka midi' with about ten flowers each were used for each temperature. Three days after flowering (DAF), headspace volatiles were collected from the flowers over a continuous 24-h period (see below); the collected volatiles were analysed using GC-MS (see below). Fig. 1

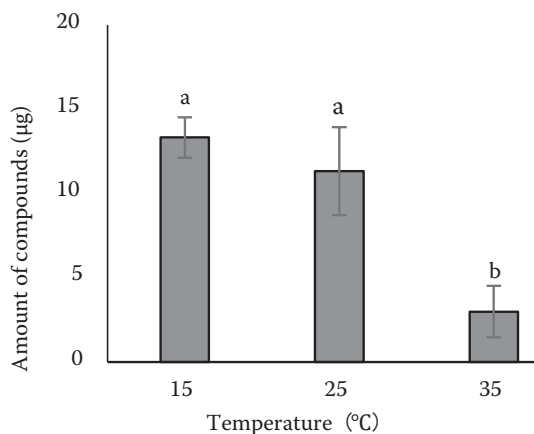


Fig. 1. Effect of temperature on total amount of floral scent compounds emitted from single flower of 'Sawayaka midi' statistically significant differences (Tukey's test, $P < 0.05$) are indicated by different lowercase letters; bars indicate standard error

shows the total amount of floral scent compounds per flower. Because the total amount of volatiles was significantly lower at 35°C than at the other two temperatures and because the results obtained at 15 and 25°C did not differ significantly from each other (Fig. 1), we kept the air temperature during the light intensity experiments within a range from 15 to 25°C to eliminate the influence of temperature.

Investigation of light intensity influence

Sensory evaluation of cyclamens housed indoors and in a greenhouse. We housed two blooming 'Odayaka' plants with about twenty flowers indoors. Every 2–3 days during the period from 10.30 a.m. to

12.30 p.m., panellists were asked to smell the flowers at a distance of 20–30 cm from the corollas and to rate the overall intensity of odour on a four-point scale: 0, not detected; 1, weak; 2, medium; 3, strong. As a control experiment, we also placed two blooming 'Odayaka' plants with about twenty flowers in a greenhouse and asked a different group of panellists to smell the flowers and rate the overall intensity of odour using the same four-point scale. For both experiments, the panellists consisted of a mixed-sex group consisting of at least ten people between the ages of 20 and 50. The temperature, humidity and light intensity values at the time of each sensory evaluation are shown in Table 1. The odour intensity scores were analysed using two-way factorial analysis of variance.

Sensory evaluation of cyclamen flowers housed in an incubator. In an incubator, we established a high-light-intensity (300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) space and, using shade netting, a low-light-intensity (10 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) space. The incubator was illuminated for 12 h (from 7.00 a.m. to 7.00 p.m.). Immediately after flowering, four pots containing a plant with a single flower were placed in the incubator. We used a single flower from each pot and cut off the other flowers. Panellists (one men and four women) were asked to smell each flower at a distance of 20–30 cm from the flower, at 1, 4, 8, 11, 15, 18 and 22 DAF (sampling time, 10.30 a.m. to 12.30 p.m.) and to evaluate the overall intensity of odour on a four-point scale (0 – not detected; 1 – weak; 2 – medium; 3 – strong). The temperatures at the time of each sensory evaluation are shown in Table 2. The odour intensity scores

Table 1. Conditions in the greenhouse and indoors during the study period

		Days after flowering								
		1	3	6	8	10	13	15	17	21
Temperature (°C)	greenhouse	21.3	25.5	21.2	19.8	19.4	18.1	19.4	17.9	13.7
	indoors	18.5	19.3	18.9	17.8	17.3	16.2	16.1	15.8	15.7
Humidity (%)	greenhouse	38	18	30	58	20	31	15	38	80
	indoors	52	41	55	65	36	46	35	55	68
Light intensity ($\text{mmol}/\text{m}^2\cdot\text{s}$)	greenhouse	275	302	359	212	290	365	301	288	63
	indoors	4	8	6	1	3	5	5	4	2

Table 2. Temperature in the incubator during the study period

	Light intensity ($\text{mmol}/\text{m}^2\cdot\text{s}$)	Days after flowering						
		1	4	8	11	15	18	22
Temperature (°C)	300	23.0	24.2	23.7	23.3	23.6	23.9	23.2
	10	21.4	21.9	22	22.3	21.4	22	21.9

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(sensory score) were analysed using two-way factorial analysis of variance.

Floral scent collection

Investigation of temperature exchange effect. To collect the volatiles from the flowers, we covered flowers from potted plants with a Tedlar® bag (GL Sciences, Tokyo, Japan), the upper part of which was equipped with a glass column containing Tenax® TA (400 mg, GL Sciences, Tokyo, Japan) as the volatile trap. A portable air sampler was used to pump air into the bag at a flow rate of 500 ml/min for 24 hours. The adsorbed volatiles were eluted from the column with 5 ml of diethyl ether. Dibutyl hydroxyl toluene (8 µg) was added to each sample as an internal standard, the solvent was removed by evaporation, and the residue was analysed using GC on an instrument equipped with a flame ionisation detector.

Investigation of light intensity influence. To collect the volatiles from the flowers, we covered single flowers from potted plants with a Tedlar® bag, the upper part of which was equipped with an absorbent cartridge (ORBO 42-small, Supelco, USA) containing a porous polymer as the volatile trap. A portable air sampler was used to pump air filtered through a charcoal-filled bottle into the bag at a flow rate of 500 ml/min for 24 hours. The cartridge was extracted with 2 ml of redistilled hexane. Ethyl *n*-decanoate (20 µg) was added as an internal standard, the solvent was removed by evaporation, and the residue was analysed using GC-MS for identification of the compounds and then using GC for quantification of the compounds.

Floral scent analysis using GC-MS

Investigation of temperature exchange effect. For GC-MS, we used a Shimadzu GC-MS-QP2010 system fitted with a split injector and a BC-WAX capillary column (50 m length, 0.25 mm i.d., 0.15 µm film thickness). Each sample (2 µl) was injected at 250°C in split mode and split ratio was 20:1. The column oven temperature program was as follows: increase from 70°C to 220°C at 4°C/min and then hold for 10 min. The carrier gas was helium with constant pressure mode at 100 kPa. The injection temperature was 250°C, interface and mass spectrometer source temperatures were all 200°C. The identification of volatile compounds was achieved by comparing and

matching the retention times and mass spectra with those of standard chemicals.

For GC, we used a HP 6890N gas chromatograph equipped with a flame ionisation detector. The analytical conditions were the same as those used for GC-MS. The carrier gas was helium with a constant pressure mode at 185 kPa. The injection and FID temperatures were 250°C. The amount of each volatile compound was calculated by comparing its peak area with that of an internal standard.

Through the conversion, we were able to estimate the total volatiles from a single flower (Fig. 1).

Investigation of light intensity influence. For GC-MS, we used an Agilent Technologies 6890N gas chromatograph fitted with a split/splitless injector and a DB-5MS column (25 m × 0.25 mm, 0.25 µm film thickness, Agilent Technologies). Each sample (1 ml) was injected at 280°C in splitless mode (sampling time, 0.75 min). The column oven program was as follows: hold at 45°C for 1 min, ramp to 280°C at 10°C/min, and then hold for 0.5 minutes. The carrier gas was helium at a flow rate of 1.0 ml/min in the constant-flow mode. The column was introduced through an interface (280°C) into the ion source (210°C) of the mass spectrometer (JEOL, MS-600H). Mass spectra were obtained by electron ionisation at 70 eV and analysed with the TSS2000 software package (ver. 2.00.0062, Shrader Analytical and Consulting Laboratories) using the NIST mass spectra library (ver. 1.6).

For GC, we used an Agilent 6890 gas chromatograph equipped with a DB-WAX capillary column (30 m × 0.25 mm, 0.25 µm film thickness) and a flame ionisation detector. The analytical conditions were the same as those used for GC-MS except that the injector and detector temperatures were both 250°C. Chromatograms were processed with the ChemStation software package (ver. A.10.01, Agilent Technologies), and the amount of each volatile compound was calculated by comparison of its peak area with that of the internal standard (ethyl *n*-decanoate).

Statistical analysis. Tukey's test was employed for analysis of the amounts of volatile compounds emitted from 'Sawayaka midi' flowers at different temperatures. The sensory scores and the amounts of volatile compounds released at every stage under each light intensity were analysed using variance analysis. We conducted Spearman rank correlation tests to assess the relationships between the amounts of volatile compounds as analysed by GC-MS and the sensory scores. The amounts of volatile compounds

released at every stage under each light intensity were analysed using principal component analysis. Furthermore, Spearman rank correlation tests were conducted to assess the relationships between the scores obtained using principal component analysis and the sensory scores. For these statistical analyses, we used Bell Curve for Excel (ver. 2.00, Social Survey Research Information Co., Ltd., Japan).

RESULTS AND DISCUSSION

Emission of volatile compounds from cyclamen flowers during display

The sensory scores of the cyclamens housed in the greenhouse did not vary significantly during the study period. In addition, up to 10 DAF, the sensory scores of plants housed indoors did not differ significantly from those of the cyclamens housed in the greenhouse. However, by 13 DAF, the sensory scores of the indoor cyclamens was significantly lower than that of the greenhouse cyclamens (Fig. 2). The numbers of flowers on the indoor and greenhouse cyclamens did not differ significantly during the study period, and the total numbers of flowers were almost the same at the start and end of the period (Fig. 2).

Effect of light intensity on emission of volatile compounds from single cyclamen flowers housed in an incubator

At 1 DAF, the sensory scores of single flowers were low regardless of light intensity, and the sensory

scores at the two light intensities did not differ significantly (Fig. 3). At a light intensity of 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, sensory scores were significantly elevated from 4 DAF until 11 DAF. By 15 DAF, sensory scores had decreased almost to the level at 1 DAF. In contrast, at 10 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, sensory scores during the period from 1 DAF to 11 DAF did not differ significantly. By 15 DAF, sensory scores had decreased substantially, and by 22 DAF, the scent could not be detected by sensory evaluation. From 4 DAF to 18 DAF, the sensory scores at 10 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ were consistently lower than those at 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$.

CNA'ANI et al. (2015) reported that in *Petunia* \times *hybrid* flowers, an increase in day/night temperatures from 22/16 to 28/22°C was associated with a decrease in production and emission of most of the scent compounds originating from the phenylpropanoid pathway. The changes in scent production were attributed not only to decreases in transcript levels of enzymes that catalyse the final steps of the pathway but also to downregulation of the expression of genes involved in substrate availability and maintenance of metabolic flux. We found that the emission of volatile compounds from cyclamen flowers was lower at 35°C than at 15 or 25°C. Therefore, exhibition of cyclamens at temperatures >35°C should be avoided.

With regard to the influence of light, HU et al. (2013) and JAKOBSEN and OLSEN (1994) found that the amounts of volatile compounds that are released increase with increasing light intensity. These results suggest that the emission of volatile compounds can be expected to decrease when plants are housed indoors, where light intensity is lower than it is outdoors. In this study, we also

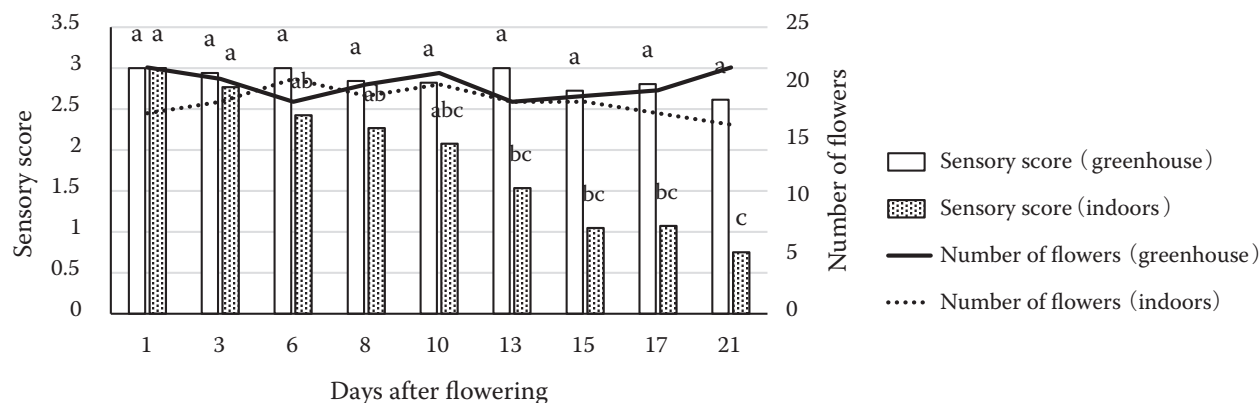


Fig. 2. Temporal variation of sensory score and number of flowers for scented cyclamen housed in a greenhouse and indoors

statistically significant differences are indicated by different lowercase letters

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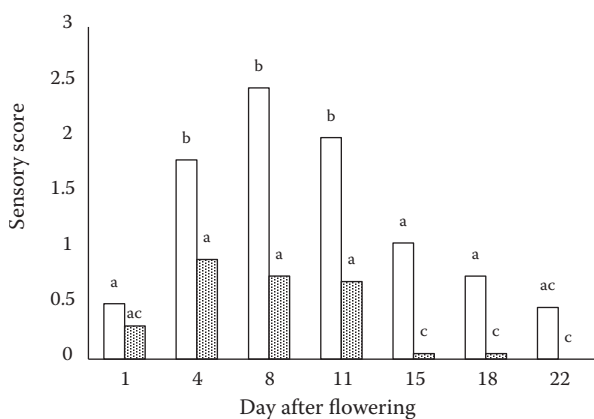


Fig. 3. Effect of light intensity on temporal variation of odor intensity of scented cyclamen flowers housed in an incubator. Statistically significant differences are indicated by different lowercase letters

found that both scent intensity as scored by sensory analysis and emission of volatile compounds as analysed by GC-MS decreased with decreasing light intensity. HU et al. (2015) contended that light plays a critical role in the emission of floral scent. To explore the mechanisms of volatile compound emission in response to different light intensities, HU et al. investigated the expression of *Li-mTPS*, a monoterpene synthase gene in *Lilium 'siberia'* petals, and concluded that perception of the light signal is the initial step in the light-induced signalling pathway. Photoreceptors reportedly mediate the recognition of light signals in plants (VAN BUSKIRK et al. 2012). Although progress is being made, the details of the influence of light on the mechanism of emission of volatiles remain unclear.

We found that at 13 DAF, the sensory scores of indoor-housed cyclamens were significantly lower than at 1 DAF and that the sensory scores of indoor cyclamens differed significantly from those of cyclamen housed in a greenhouse. In experiments with individual flowers, we found that at 4 DAF, the sensory scores of flowers exposed to low light intensity was significantly lower than those of flowers exposed to high light intensity. At 22 DAF, no scent was detected by sensory evaluation of the flowers exposed to low light. These results show that the odour intensity of the indoor cyclamens diminished gradually because the amounts of odour compounds emitted from new flowers was low under low light, and therefore the total emitted amounts of odour compounds diminished gradually under low light. Because the numbers of flowers at the start and end of the study pe-

riod did not differ drastically, we conclude that the influence of the number of flowers on the emission of odour compounds was small.

GC-MS analysis of volatile compounds

The results of the GC-MS analysis of volatile emissions from single flowers housed under low and high light intensities are shown in Fig. 4. The amounts of citronellol, geraniol, (*E*)-2,3-dihydrofarnesol, (*Z,E*)-farnesol, (*E,E*)-farnesol, undecan-2-one, (*E*)- β -farnesene and α -terpineol, as well as the total amount of volatile compounds released from a single flower under low light intensity were consistently lower than the amounts released under high light intensity. In contrast, the amount of caryophyllene was higher under low light intensity than under high light intensity. The amounts of linalool, methyl geranate and (*E,E*)- α -farnesene released were unaffected by differences in light intensity.

In their study of *Lilium 'siberia'*, HU et al. (2013) found that an increase in light intensity significantly increased the release of α -ocimene, linalool 2-ethyl-1-hexanol and methyl benzoate but not the amount of (*E*)- β -ocimene. These investigators reported that the response to light intensity depended on the kinds of the emitted compounds. In the case of cyclamen, we found that the emission amounts of some of the volatile compounds were drastically affected by light intensity but the emission amounts of other volatile compounds were unaffected; furthermore, low light intensity did not result in decreased levels of all compounds. HU et al. (2013) found that the amount of linalool released from *Lilium 'siberia'* was significantly higher at 300 $\mu\text{mol}/\text{m}^2\text{s}$ than at 100 $\mu\text{mol}/\text{m}^2\text{s}$, whereas we found that the amount of linalool released by cyclamens was unaffected by a change in light intensity. This interspecies difference is likely related to differences between the biosynthesis pathways in the two species, and additional work will be necessary to clarify the mechanism of light sensitivity in detail.

We found that the released amounts of citronellol, geraniol, (*E*)-2,3-dihydrofarnesol and (*E,E*)-farnesol were higher than the released amounts of the other volatile compounds (Fig. 4). The amount of geraniol released was highest at 4 DAF, and the amounts of citronellol released from 4 DAF to 15 DAF were nearly identical. These results show that the effect of flowering stage on the amounts of volatiles release differed for the various volatile compounds.

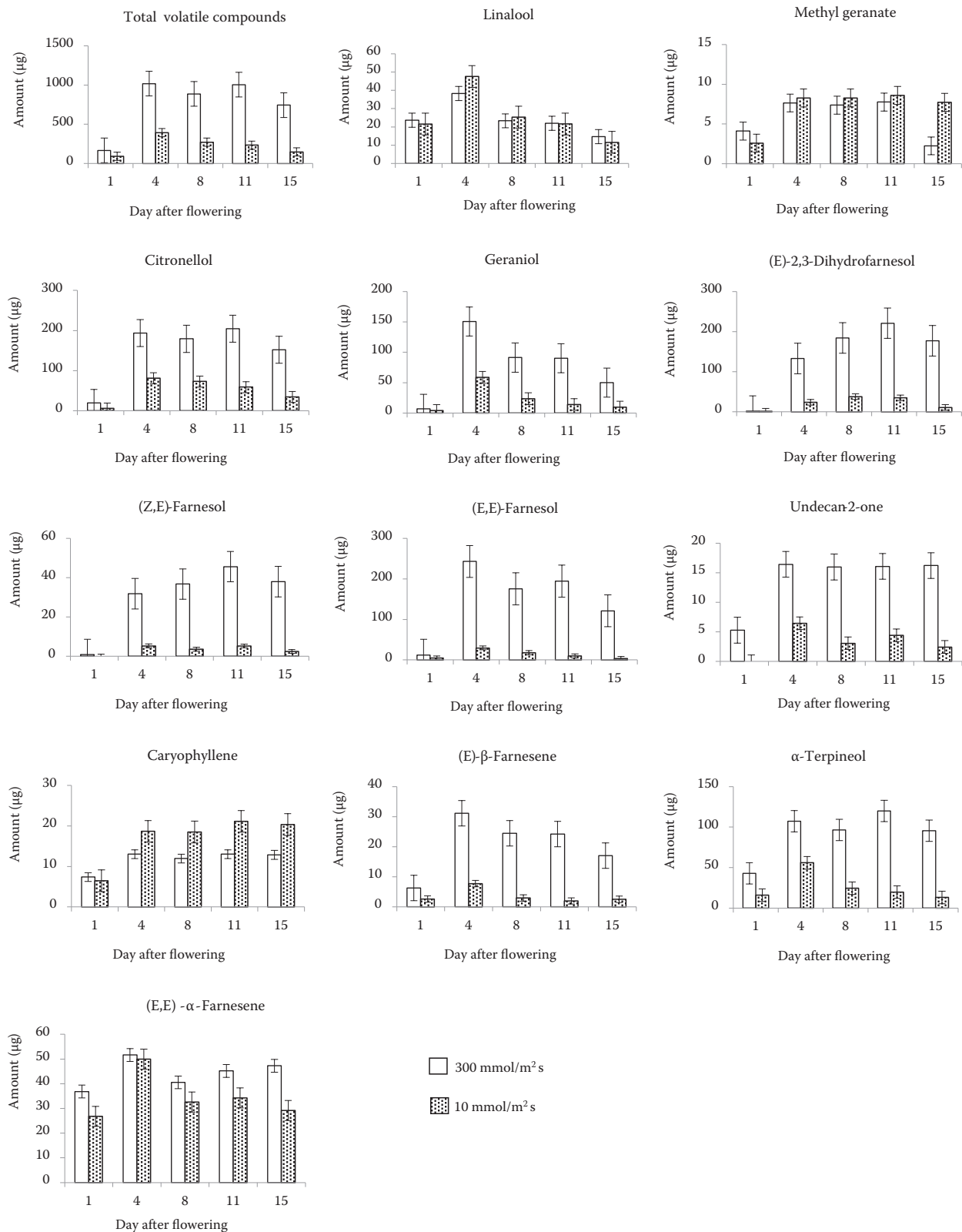


Fig. 4. Effect of light intensity on amounts of volatile compounds emitted from individual cyclamen flowers, as determined by GC-MS

bars indicate standard errors

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Other researchers have also found that the amounts of volatile compounds released depend on flowering stage. For example, SCHADE et al. (2001) found that the steady-state levels of 10 volatile compounds emitted by carnation flowers changed independently as the flowers developed and senesced, suggesting that the synthesis of the compounds was developmentally regulated. We found that at $300 \mu\text{mol}/\text{m}^2 \text{ s}$, the odour intensity of individual cyclamen flowers was high from 4 DAF to 11 DAF and then decreased at 15 DAF. At $10 \mu\text{mol}/\text{m}^2 \cdot \text{s}$, the scent intensity had decreased remarkably by 15 DAF. These results indicate that emissions from cyclamen flowers depended on flowering stage and that the fluctuation pattern was affected by light intensity. Furthermore, as was observed for carnations, the fluctuations in emission pattern with flowering stage differed for the various volatile compounds.

Relationship between GC-MS results and sensory analysis results

Spearman rank correlation analysis revealed a significant correlation between the total amount of volatile compounds released and sensory score ($p < 0.01$, $r_s = 0.9394$; Fig. 5). Similarly, significant correlations were observed between sensory score and the amounts of citronellol, geraniol, (*E*)-2,3-dihydrofarnesol, (*Z,E*)-farnesol, (*E,E*)-farnesol, undecan-2-one, (*E*)- β -farnesene, α -terpineol and (*E,E*)- α -farnesene that were released (Table 3).

The results of principal component analysis of the amounts of volatile compounds released at every stage under each light intensity are shown in Fig. 6. The score for principal component 1 was positively correlated with the floral-scented compounds citronellol and (*E*)-2,3-dihydrofarnesol and negatively correlated with the waxy-scented compound undecan-2-one and the green floral-scented compound methyl geranate. The score for principal component 2 showed positive correlations with the green-scented compound (*E,E*)- α -farnesene and the floral-scented compound linalool, whereas there were negative correlations with the floral-scented compounds (*E*)-2,3-dihydrofarnesol and (*E,E*)-farnesol. At both high and low light intensities, the score at 1 DAF was plotted on the low position of principal component 1, indicating that the contribution of floral-scented compounds was small at 1 DAF. At 4 DAF and later (except in the case of 15 DAF at low light intensity), the scores at both high and low

light intensities were plotted in similar areas of principal component 1. In contrast, the score at low light intensity was positive for principal component 2, whereas the score at high light intensity was negative. This result shows that the proportion of green floral compounds was higher at low light intensity than at high light intensity, whereas the score of the floral scent compound was almost the same under the two light intensities. Furthermore, the correlation between principal component 2 and sensory score was statistically significant ($p < 0.001$, $r_s = -0.891$, Fig. 7).

DU et al. (2011) comprehensively evaluated the relationship between GC-MS results and sensory analysis in strawberry fruits and specified the main compounds that affected sensory evaluation. In the current study, we found that the rank correlations between sensory score and the amounts of citronellol, geraniol, (*E*)-2,3-dihydrofarnesol, (*Z,E*)-farnesol, (*E,E*)-farnesol, undecan-2-one, (*E*)- β -farnesene, α -terpineol and (*E,E*)- α -farnesene, as well as the total amount of volatile compounds, were significant. These results indicate that the amounts of these compounds affected the sensory scores.

Because the amounts of citronellol, geraniol, (*E*)-2,3-dihydrofarnesol and (*E,E*)-farnesol were correlated significantly with sensory score, and, because their emission amounts were higher than those of other compounds, these compounds can be expected to strongly affect scent impression. From 4 DAF to 11 DAF, during which period the sensory score was high, principal component analysis showed that under low light intensity, the contributions of the floral-scented compounds (*E*)-2,3-dihydrofarnesol and (*E,E*)-farnesol were lower and the contributions of the green-scented compound (*E,E*)- α -farnesene and

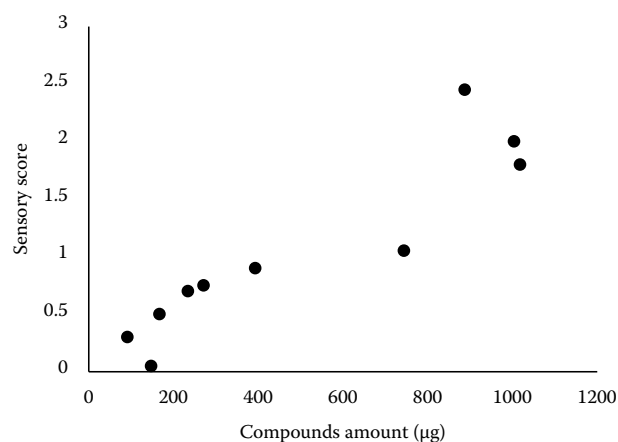


Fig. 5. Relationship between total volatile compound emission amount and sensory score

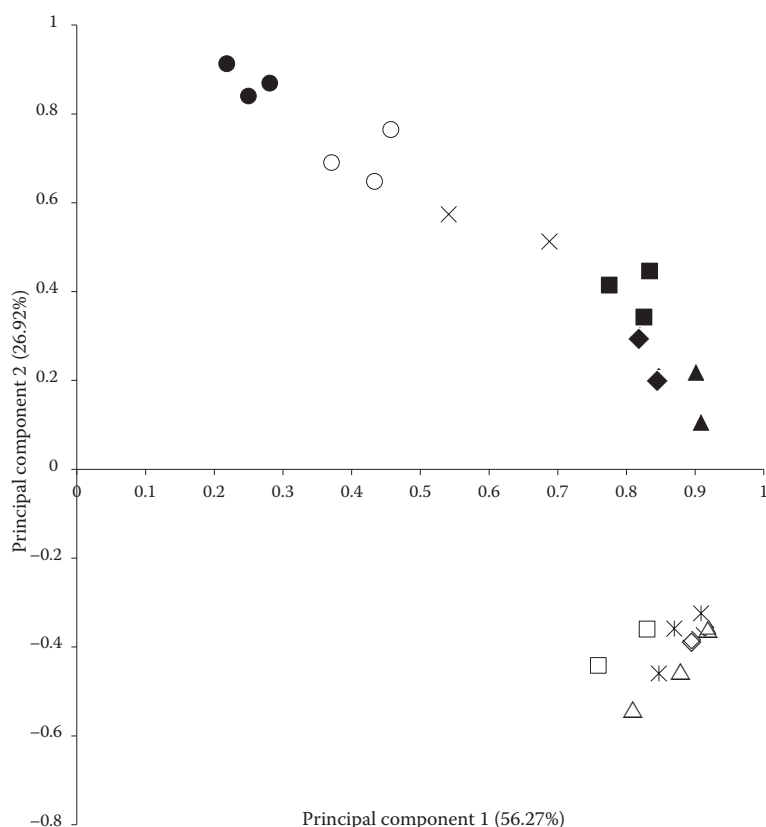
Table 3. Rank correlation coefficients for the relationship between volatile compound amount and sensory score^a

Volatile compound	rs	Significance level
Linalool	0.394	
Methyl geranate	-0.006	
Citronellol	0.927	**
Geraniol	0.915	**
(E)-2,3-Dihydrofarnesol	0.903	**
(Z,E)-Farnesol	0.891	**
(E,E)-Farnesol	0.939	**
Undecan-2-one	0.830	**
Caryophyllene	-0.127	
(E)- β -Farnesene	0.867	**
α -Terpineol	0.927	**
(E,E)- α -Farnesene	0.733	*

^ars, Spearman's rank correlation coefficient; ** $\alpha = 0.001$, * $\alpha = 0.05$

the floral-scented compound linalool were higher than the corresponding contributions under high light intensity. Furthermore, there was a significant rank correlation between principal component 2 and sensory score. This result shows that when the score for principal component 2 was positive and the contribution of floral-scented compounds decreased, the scent score and odour intensity decreased. We assume that the weakening of the scent under low light intensity can be attributed not only to a decrease in the amount of floral emission but also to a change in the relative amounts of green floral-scented compounds and floral-scented compounds, which affected the scent impression.

Our results indicate that when cyclamens are kept indoors under low light intensity, their scent intensity decreases gradually, thus reducing their ornamental value. We suggest that 10 days is the limit for exhibition indoors from the point of view of scent emission,



○ 1 day after flowering (DAF), high light intensity; □ 4 DAF, high light intensity; △ 8 DAF, high light intensity; ◇ 11 DAF, high light intensity; * 15 DAF, high light intensity; • 1 DAF, low light intensity; ■ 4 DAF, low light intensity; ▲ 8 DAF, low light intensity; ◆ 11 DAF, low light intensity; × 15 DAF, low light intensity

the correlations for principle component 1 were citronellol (7.831), (E)-2,3-dihydrofarnesol (3.764), undecan-2-one (-4.231), and methyl geranate (-4.130)

Fig. 6. Principal component analysis of amount of compounds after flowering at high and low light intensities

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because odour intensity decreases significantly after 10 days. KOMAGATA et al. (2002) reported that after 10 days of display under low light intensity, the quality of cyclamens begins to degrade: the leaves turn yellow, and the number of flowers decreases. That is, the time limit for indoor exhibition of cyclamens in terms of scent emission corresponds to the time limit in terms of morphological quality.

Some studies have shown that photoperiod and light quality affect flowering period, plant growth and number of flowers in cyclamens. Specifically, RHIE et al. (2006) reported that a 16-h-light photoperiod promotes flowering and plant growth better than an 8-h-light photoperiod. HEO et al. (2003) found that the use of blue plus red LEDs results in more flowers than the use of fluorescent light. Cyclic lighting and night interruption have been used as methods for long-day treatment of cyclamens (KANG et al. 2008), and the use of night interruption lighting to accelerate cyclamen flowering has been reported (OH et al. 2008, 2013). It would be interesting to determine whether light quality and photoperiod could improve scent emission from cyclamens, and if so, which method

of light supplementation would best extend the scent emission and exhibition period.

GUTIERREZ (2009) showed that in roses, phenylpropanoids, lipid derivatives and terpenoids are produced in different amounts during development and that the production pattern varies among cultivars. In addition, the amounts and types of flower volatiles vary widely among citrus types and during blooming. For example, AZAM et al. (2013) found that in some citrus types, the amount of linalool decreases during blooming, whereas the amount of the same compound increases during flowering in other citrus types. Similarly, in some cultivars, the amounts of limonene and β -pinene are higher in unopened flowers than in fully opened flowers, but the opposite is true of other cultivars. Because the duration of floral emission varies among cultivars, breeding with cultivars that release volatiles for a longer time can be expected to be effective for extending the display period.

In summary, we found that in scented cyclamens, the emission of volatile compounds from the flowers was affected by temperature, light and flowering stage, as has been reported for other plants. Emissions of volatiles from cyclamen flowers kept indoors under low light intensity were lower than emissions from cyclamen flowers kept under high light intensity. Furthermore, sensory analysis indicated that 10 days is the limit for indoor display from the point of view of scent emission. Light supplementation and the development of new cultivars may extend the display period, but further study is required.

Acknowledgement

We thank Yoshihiro Yaguchi, Hisae Kasuga and Yumi Kusano at Takasago International Corporation Corporate Research and Development Division for supporting this study.

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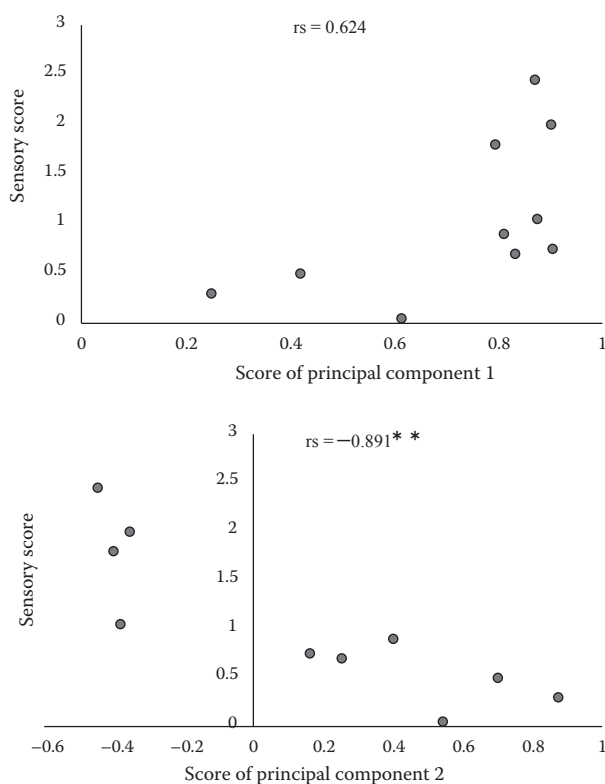


Fig. 7. Correlation between sensory score and score of principal components (a) 1 and (b) 2. r_s , Spearman's rank correlation coefficient; ** statistically significant at $\alpha = 0.001$

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Received for publication June 21, 2017

Accepted after corrections October 20, 2017