Characteristics of Unpleasant Odor Emitted by *Gypsophila* Inflorescences

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Summary

Characteristics of unpleasant odor emitted by inflorescences of four gypsophila cultivars (*Gypsophila elegans* Bied. ‘Covent Garden Market’ and *G. paniculata* L. ‘Bristol Fairy’, ‘Golan’ and ‘Yukinko’) were investigated by headspace adsorption/gas chromatographic (HA/GC) analysis. The major volatile compounds emitted from ‘Bristol Fairy’ inflorescences were ocimene, 3-methylbutyric acid, 2-methylbutyric acid, ethanol and n-hexanol. Of these volatile compounds, the two methylbutyric acids were identified as the compounds that characterize the unpleasant odor of gypsophila inflorescences. The composition of volatiles varied among cultivars; ocimene was the most abundant and common constituent detected in all gypsophila cultivars. Methylbutyric acids was the highest in ‘Bristol Fairy’ and ‘Golan’ followed by ‘Yukinko’; they were undetectable in non-scented ‘Covent Garden Market’. Methylbutyric acids were not detected in the volatiles emitted from ‘Bristol Fairy’ inflorescences at the bud stage; the emissions increased gradually with bud opening and reached the highest level at the full open stage on day 4.

Key Words: gypsophila, headspace absorption analysis, methylbutyric acids, unpleasant odor.

Introduction

Flower scent has been recognized as a modulating factor in plant–insect interactions and plays an important role in successful pollination. People appreciate the aesthetic value in certain types of floral scents, as well as the ornamental value, so that the presence of floral scents has contributed to the cultivation of specific plant species. Roses, narcissuses and jasmines from which perfumes are produced are examples. The volatile components released from flowers with pleasant scent that have been identified in many species (Knudsen et al., 1993) are often a mixture of low molecular weight compounds; the characteristic scent of the flower is determined by the relative abundance and interactions among the constituents. Most of these compounds are terpenoids, phenylpropanoids, benzenoids, and/or esters (Dudareva et al., 2000; Knudsen et al., 1993). The floral scent varies widely among species in number, identity, and relative amounts of constituent volatile compounds (Dudarera and Pichersky, 2000). However, only a few researchers have identified the volatile compounds that characterize the unpleasant odors (Erhardt, 1993; Kite and Smith, 1997; Stransky and Valterova, 1999).

Perennial gypsophila (*Gypsophila paniculata* L.) is a worldwide major floricultural crop that is commonly used as a filler in flower arrangements and bouquets. Its common name, “Baby’s Breath”, indicates the unpleasant odor emitted by the inflorescences to make it undesirable for an indoor floral arrangement. ‘Perfecta’ and ‘Bristol Fairy’ have been the leading cultivars for the last two decades, and new cultivars, such as ‘Golan’, ‘Yukinko’, and ‘Million Stars’ are increasing in popularity. However, these cultivars still have, more or less, an unpleasant odor, which may presumably be emitted to attract pollinators, although they are double‐flowered and stamen‐less.

Our preliminary sensory evaluation revealed that ‘Bristol Fairy’ and ‘Golan’ have a strong unpleasant scent, whereas ‘Yukinko’ has a weak odor. *G. elegans* ‘Covent Garden Market’ is a non-scented cultivar. In this study, the characteristics of the odor emission from four gypsophila cultivars with varying unpleasant scent levels were analyzed by a HA/GC analysis. Furthermore, the changes in the odor emission levels of gypsophila inflorescences were monitored during the post-harvest period.

Materials and Methods

1. Plant materials

*Gypsophila elegans* Bied. ‘Covent Garden Market’ and *G. paniculata* L. ‘Bristol Fairy’, ‘Golan’, and
'Yukinok' were grown from 2001 to 2003 in a greenhouse at the experimental field of Osaka Prefecture University. Lateral inflorescences with about 20% of the florets open, 20–30 cm in length, were harvested in the morning, transferred to our laboratory, and immediately placed in a solution containing 2% sucrose and 100 mg·L⁻¹ 8-hydroxyquinoline sulfate as a biocide. To obtain inflorescences having florets at a uniform opening stage, we removed open florets from inflorescences immediately after harvest (bud stage), replaced the inflorescences in the same solution for 24 h, removed the unopened buds and left only open florets. We assigned this stage as day 1. Cut inflorescences were kept in the laboratory at 20℃.

2. Headspace volatile adsorption (HA)

Floral volatiles were collected by enclosing 12–15 g cut inflorescences (about 8 cm in length) with 350–400 florets in a 1.5 L desiccator fitted with a column packed with 500 mg Tenax TA (60–80 mesh, GL Science, Tokyo). The stem bases were immersed in a solution in the desiccator. Air (21% O₂ and 79% N₂), which was filtered through charcoal and Molecular Sieve 5A (30–60 mesh, Shimadzu, Kyoto) columns, was sent into the desiccator; the volatile compounds, emitted to the headspace by inflorescences, were trapped in Tenax TA for 3 h. The flow rate of air was maintained at 60 mL·min⁻¹ with a flow controller (GL Science, Tokyo). After volatile compounds were released from Tenax TA by heat (see GC analysis), the columns were regenerated by flushing them with helium gas at 220℃ for at least 2 h. The adsorption of volatiles was carried out from 10.00 to 13.00 at light and room temperature (20 ± 1℃).

3. Gas chromatographic (GC) analysis

The oven of a flush sampler (FLS-1, Shimadzu, Kyoto) was connected to the injection port of GC (GC-17A, Shimadzu, Kyoto) and previously heated to 200℃ before analysis. The Tenax TA column was placed in the oven with the syringe needle inserted into the GC injector. The volatile compounds were released and flushed out from the Tenax TA column with helium gas as carrier. The GC was equipped with a fused silica capillary column coated with 0.25 μm film of polyethylene glycol (0.25 mm i.d. × 60 m; DB-WAX, J&W Scientific, USA) and flame ionization detector (FID). The column was maintained at 70℃ for 5 min, raised to 220℃ at a rate of 5℃·min⁻¹, and then maintained at 220℃ for 5 min. The injection and detector block temperatures were set at 200℃ and 300℃, respectively. The split ratio was set automatically at 1:110 with nitrogen as the make-up gas. The peak area was measured with Chromatopak Integrator (C-RSA, Shimadzu, Kyoto). Volatile components were identified by comparing their retention times (RT) to those of the authentic standards and by co-chromatography, according to the result of GC-MS measurement described by Sakai et al. (1993). The standard peak area of methylbutyric acid was obtained by injecting 1 μL n-hexane solution which contained 5 mL·L⁻¹ 3-methylbutyric acid. The analysis was repeated thrice.

4. Varietal differences in volatile emission

GC tracings of the volatiles, emitted from cut inflorescences of four cultivars, were compared at anthesis when the volatile emissions reached the maximum in all cultivars. The triplicated volatile trappings taken around noon were made on day 2 for 'Covent Garden Market' and on day 4 for 'Bristol Fairy', 'Golan', and 'Yukinok'.

5. Time course analysis during flower opening and senescence

Volatile emissions from cut inflorescences of 'Bristol Fairy' were monitored over 6 days, starting from the bud stage immediately after harvest. Headspace was collected for 3 h around noon and the inflorescences were weighed simultaneously.

Results

1. HA/GC analysis of four cultivars

A typical GC tracing (Fig. 1A) obtained by HA/GC analysis of the volatile compounds from the strongly scented 'Bristol Fairy' inflorescences at anthesis (day 4) showed 5 dominant peaks. The largest peak (peak b, RT:}

![Fig. 1. Gas chromatographic tracings of the volatile compounds from flowering inflorescences of 'Bristol Fairy' (A), 'Covent Garden Market' (B), and unopened inflorescences of 'Bristol Fairy' (C). Day 0 is immediately after harvest. Peaks a, b, c, d, and e were identified as ethanol, ocimene, n-hexanol and methylbutyric acids, respectively. Peak d was not identified.](image-url)
Table 1. Composition of major volatile compounds emitted from four gypsophila cultivars analyzed by HA/GC at full open stage.

<table>
<thead>
<tr>
<th>Volatile compound</th>
<th>Covent Garden Market</th>
<th>Bristol Fairy</th>
<th>Golan</th>
<th>Yukinko</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>2.4</td>
<td>6.8</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Ocimene</td>
<td>65.3</td>
<td>43.2</td>
<td>50.0</td>
<td>46.9</td>
</tr>
<tr>
<td>n-Hexanol</td>
<td>1.1</td>
<td>5.2</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Unidentified volatile*</td>
<td>ND</td>
<td>2.2</td>
<td>2.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Methylbutyric acids</td>
<td>ND</td>
<td>17.8</td>
<td>20.3</td>
<td>22.1</td>
</tr>
<tr>
<td>Others</td>
<td>31.2</td>
<td>24.9</td>
<td>24.5</td>
<td>23.7</td>
</tr>
</tbody>
</table>

*The unidentified volatile is peak d in Fig. 1.
ND: not detected.

Fig. 2. Varietal difference in the level of methylbutyric acids emitted from flowering inflorescences of four gypsophila cultivars at anthesis. ND: not detected. Vertical bars represent SE (n = 3).

23.8 min) corresponded to ocimene; the second largest (peak e, RT: 43.4 min) was made by methylbutyric acids which consisted of 3-methylbutyric acid (isovaleric acid) and 2-methylbutyric acid. In addition to these two major peaks, n-hexanol (peak c, RT: 28.5 min), ethanol (peak a, RT: 12.5 min), and an unidentified compound (peak d, RT: 33.1 min) were detected. These major peaks were not detected at the bud stage (Fig. 1C). The tracing of fully opened ‘Covent Garden Market’ (day 2), which does not have an unpleasant odor, displays peaks of ethanol, ocimene and n-hexanol, but none for methylbutyric acids (Fig. 1B).

Ocimene, the most abundant compound detected in the volatile compounds from both cultivars occupied 43% and 65% of the total peak area of the chromatograms derived from ‘Bristol Fairy’ and ‘Covent Garden Market’, respectively (Table 1). Methylbutyric acids occupied 18% of the total peak area of volatiles emitted from ‘Bristol Fairy’, but they were not detected in the volatiles from ‘Covent Garden Market’. Methylbutyric acids were detected abundantly in the volatiles from

Fig. 3. Changes in inflorescence fresh weight and the level of methylbutyric acids emitted from ‘Bristol Fairy’ inflorescences during the bloom period. Vertical bars represent SE (n = 3).

‘Golan’ and ‘Yukinko’ (Fig. 2), sharing more than 20% of the total peak area (Table 1).

2. Time course analysis during flower opening and senescence

We detected large changes in the level of methylbutyric acids during the flower development and senescence of ‘Bristol Fairy’ (Fig. 3). Buds did not emit methylbutyric acids; the level increased with bud opening and reached the maximum on day 4 when the florets fully opened, and the fresh weight of the inflorescences was also at its maximum level. The level of methylbutyric acids subsequently decreased during the senescence of the flowers. The levels of ocimene and n-hexanol showed similar change, whereas that of ethanol increased with time during the postharvest period of 6 days (data not shown).

Discussion

Five dominant peaks were present on the GC tracing of volatile compounds from flowering ‘Bristol Fairy’ inflorescences (Fig. 1A). Four of these peaks were identified as ocimene, methylbutyric acids, n-hexanol, and ethanol. Ikemoto and Nagai (1997) reported similar chromatograms of volatile compounds from ‘Bristol Fairy’ inflorescences. Our results show that ocimene is
the most abundant and common compound detected in all gypsophila cultivars. Ocimene is a monoterpenoid known as one of the common major volatiles that impart the aroma to many flowers, such as Narcissus pseudonarcissus (Mettal et al., 1988) and Laelia anceps (Kaiser, 1993).

Results of sniffing the reference compounds by a panel suggested that methylbutyric acids are the constituents responsible for the unpleasant odor of gypsophila inflorescences, because they have a strong pungent rancid odor. Whereas ocimene and n-hexanol have emissions reminiscent of freshly cut wood and greens, respectively. The chromatograms of the volatile compounds from flowering 'Covent Garden Market' and unopen 'Bristol Fairy' inflorescences, whose odors were undetected by sniffing, did not display a peak of methylbutyric acids (Fig. 1B, C). Contrarily, methylbutyric acids were detected at the level of nearly 18-22% of the total volatiles that were emitted from inflorescences of scented cultivars at anthesis. Methylbutyric acids are known to be the responsible compounds of sweaty smell of animals, to which the sensibility threshold of humans is as low as 1 ppb, indicating that methylbutyric acids are primary responsible for the unpleasant odor. However, it is unknown whether 3-methylbutyric acid or 2-methylbutyric acid is dominant, because these two compounds were not separated by our GC analysis on account of their similarity in molecular weight and polarity. Methylbutyric acids are rarely detected in flowers from most species; their presence has been reported in Senecio articulatus (Kite and Smith, 1997), Theophrasta americana (Knudsen and Tollsten, 1993), Deherania smaragadina (Knudsen and Tollsten, 1993), Masdevallia striatella (Kaiser, 1993) and Leontopodium alpinum (Erhardt, 1993).

Differences in the level of methylbutyric acid emission among cultivars (Fig. 2) are consistent with the result of sensory evaluation, so that our data support the hypothesis that methylbutyric acids are the main constituents responsible for the unpleasant odor of gypsophila inflorescences.

Changes in the level of methylbutyric acid emission during bud opening, full bloom, and at senescence of 'Bristol Fairy' are also consistent with the results of our sniffing panel; the open florets emitted the most disagreeable smell. The cut inflorescences of gypsophila bear numerous tight buds, as well as open florets. Cut inflorescences are treated commercially with a preservative that contains sugars and silver thiosulphate to promote bud opening and prolong the longevity of individual florets. However, this treatment may also promote the emission of odor from the inflorescences when they are arranged. Therefore, an effective treatment to suppress odor emission is desired. Ikemoto and Nagai (1997) succeeded in lowering the level of methylbutyric acids by a treatment with phenylethyl D-glucoside, although this compound is difficult to use commercially.

Our time course analysis of volatile emission shows that the odor collected around noon. Volatile emission is often correlated with the corresponding temporal activity of their pollinators (Dobson, 1994). Such day-night alternations in volatile emissions have been detected in many flowers (Loughrin et al., 1990, 1991; Jacobsen and Olsen, 1994). We observed that flowering 'Bristol Fairy' inflorescences attract the common diurnal insects Meranostoma spp, Sphaerophoria sp, and Anthomyiidae sp., and this suggests that gypsophila flowers emit odor more abundantly during daytime.

We conclude from our results of HA/GC analysis that methylbutyric acids are the constituents that characterize the unpleasant odor emitted from most gypsophila cultivars. We clarified the characteristics of methylbutyric acids emission; now we wish to elucidate the metabolic pathway that leads to the synthesis and accumulation of methylbutyric acids. Such study may lead to the elimination of the unpleasant odor from Gypsophila species.

Literature Cited


Loughrin, J. H., T. R. Hamilton-Kemp, R. A. Anderson and D. F. Hildebrand. 1990. Volatiles from flowers of Nicotiana sylvestris, N. otophora and Malus domestica: headspace components and day/night changes in their
Gypsophila属花序からの悪臭の発散特性

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摘要

Gypsophila属4品種（Gypsophila elegans Bied. 'コレントガーデンマーケット',  'G. paniculata L.' 'プリストルフェアリー', 'ゴラン'および'ユキノキ')の花序から発散される悪臭をヘッドスペース吸引/ガスクロマトグラフィー(HA/GO)法により分析した。'プリストルフェアリー'の花序から発散されている主な揮発性物質としては、オシメン、メチルアルデヒド、2-メチルエタノール、3-メチルエタノール、4-メチルエタノール、4-メチルイソプロピルアルコール、2-メチルイソプロピルアルコール、2-メチルイソプロピルエチルアルコール、2-メチルイソプロピルエチルエタノール、および2-メチルエチルアルコールが検出された。各品種の揮発性物質組成は異なっていた。いずれの品種ともオシメンが検出され、その比率が最も大きかった。メチルアルデヒドの発散量は'プリストルフェアリー', 'ゴラン'で最も多く、'ユキノキ'で少なかった。一方、悪臭のしない'コレントガーデンマーケット'からは、メチルアルデヒドの発散は認められなかった。また、メチルアルデヒドは、'プリストルフェアリー'の未開花数段階の花序では発散がなく、開花に伴って徐々に発散量が増加し、開花時（開花4日後）に最大の発散量に達した。

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