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## A Practical Method of Deodorizing Cut Gypsophilas (*Gypsophila paniculata* L.)

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### Abstract

When about 20% of the cut gypsophila inflorescences were open, 2-phenylethylalcohol ( $500 \mu\text{l}\cdot\text{l}^{-1}$ ) added to the vase solution reduced the emission of methylbutyric acids, which are responsible for their unpleasant odor, to one-third. Benzyl alcohol ( $500 \mu\text{l}\cdot\text{l}^{-1}$ ), titanium tetrachloride ( $19 \text{ mg}\cdot\text{l}^{-1}$ ) and abietic acid ( $30 \text{ mg}\cdot\text{l}^{-1}$ ) also decreased the emission of methylbutyric acid to nearly half, while ethanol ( $500 \mu\text{l}\cdot\text{l}^{-1}$ ) had no effect. The amount of methylbutyric acids emitted coincided well with the results of a sensory test.

**Key Words:** abietic acid, alcohols, cut gypsophila, deodorizing, methylbutyric acid, titanium tetrachloride

### Introduction

*Gypsophila* (*Gypsophila paniculata* L.) is a worldwide major floricultural crop that is commonly used as a filler in flower arrangements and bouquets. 'Bristol Fairy' has dominated commercial production in Japan for the last two decades. However, this cultivar emits a strong unpleasant odor during flower opening and this is a disadvantage for indoor use. Recently introduced cultivars such as 'Golan' and 'Yukinko' also have, more or less, an unpleasant odor. The major volatile compounds emitted from gypsophila are ocimene, methylbutyric acid [2-methylbutyric acid and 3-methylbutyric acid (isovaleric acid)] and ethanol. Methylbutyric acids are components in the smell of animal sweat and are responsible for the unpleasant odor of gypsophila (Nimitkeatkai et al., 2004). According to Ikemoto and Nagai (1997), treatment with phenylethyl D-glucopyranoside synthesized from 2-phenylethyl alcohol and D-glucose lowered the levels of methylbutyric acids. However, this compound has not been applied commercially because of its high cost.

We examined the effects of commercially available alcohols, titanium tetrachloride and abietic acid, on the unpleasant smell of cut gypsophila flowers.

### Materials and Methods

#### *Plant materials*

*Gypsophila paniculata* L. 'Bristol Fairy' plants were grown in a greenhouse at the experimental field of Osaka Prefecture University in 2003. Lateral inflorescences with about 20% of the florets open, were harvested and immediately transferred to our laboratory.

#### *Chemical treatment*

The vase solution containing 2% sucrose and  $100 \text{ mg}\cdot\text{l}^{-1}$  8-hydroxyquinoline sulfate was supplemented with  $500 \mu\text{l}\cdot\text{l}^{-1}$  (10.9 mM) ethanol,  $500 \mu\text{l}\cdot\text{l}^{-1}$  (4.6 mM) benzyl alcohol,  $500 \mu\text{l}\cdot\text{l}^{-1}$  (4.1 mM) 2-phenylethyl alcohol,  $19 \text{ mg}\cdot\text{l}^{-1}$  (0.1 mM) titanium tetrachloride or  $30 \text{ mg}\cdot\text{l}^{-1}$  (0.1 mM) abietic acid. To make aqueous solutions of abietic acid, we used ethanol as a solvent (final concentration was  $500 \mu\text{l}\cdot\text{l}^{-1}$ ). We trimmed the cut inflorescences to 25 cm and put the stem base in these solutions. We kept the vases at  $20^\circ\text{C}$  for 4 days until they reached the fully-open stage.

#### *Analysis of volatile compounds*

Fifteen grams of cut inflorescences with 350-400 open florets were sampled 4 days after keeping them at  $20^\circ\text{C}$ . The stem bases were immersed in deionized water in a 50 ml beaker

and placed in a 1.5 liter desiccator with air inlet and outlet. The volatiles in the headspace volatiles were adsorbed by the method reported previously (Nimitkeatkai et al., 2004). Volatiles were analyzed by gas chromatography (GC-17A, Shimadzu, Kyoto) equipped with a polyethylene glycol capillary column (DB-Wax; 60 m x 0.25 mm i.d.; J&W Scientific, USA). The injection and detector block temperatures were set at 200°C and 300°C, respectively. The column was maintained at 70°C for 5 min and then the temperature was raised at 3°C · min<sup>-1</sup> steps to 220°C. The volatile compounds were tentatively identified by GC retention time against authentic standards and by co-chromatography. The compounds were also analyzed by GC-MS measurement as described by Sakai et al. (1993).

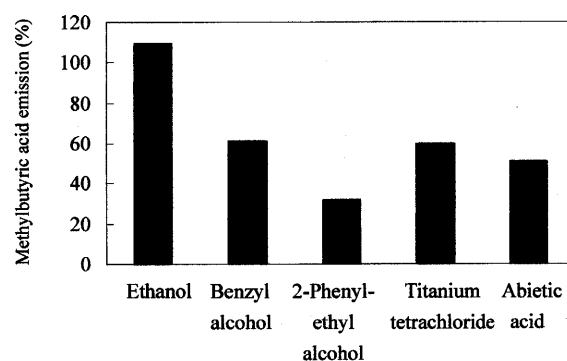
#### Sensory evaluation

Seven grams of inflorescences, treated with the chemicals for 4 days were placed in a flask containing the vase solution. They were subjected to a sensory evaluation (sniffing). The test panel members consisted of 7 males and 5 females. The panelists compared the odor of the inflorescences treated with individual chemicals and with that of the untreated control inflorescences by the paired comparison test, and rated the odor as stronger than, the same as or weaker than the control.

### Results

#### Effect of chemical compounds on the volatile emission from cut *gypsophilas*

Treatment with benzyl alcohol and 2-phenylethyl alcohol reduced the emission of methylbutyric acids to 61% and 32% of the control,



**Fig. 1. Emission of methylbutyric acid from *gypsophila* inflorescences after continuous treatment with ethanol, benzyl alcohol, 2-phenylethyl alcohol, titanium tetrachloride or abietic acid, for 4 days. Vertical axis indicates percentage of the methylbutyric acid emission against untreated control *gypsophila* inflorescences.**

respectively (Fig. 1). Treatment with titanium tetrachloride and abietic acid reduced the emission of methylbutyric acids to 51% and 60%, respectively. However, treatment with ethanol did not decrease methylbutyric acid emission at all.

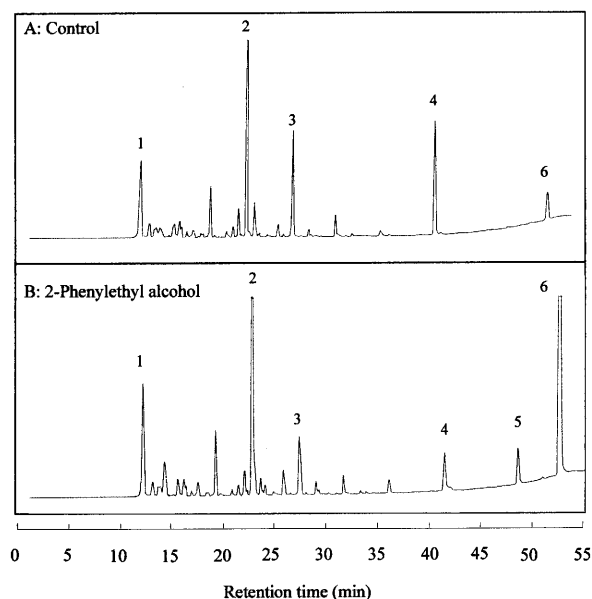
Table 1 shows the effects of chemical compounds on the emission of the other major volatiles. There was a marked increase in ethanol and a slight increase in ocimene when the inflorescences treated with ethanol or abietic acid.

In the cut inflorescences treated with 2-phenylethyl alcohol (Fig. 2), a large peak of 2-phenylethyl alcohol [retention time (RT) at 53.5 min] and a relatively small peak of 2-phenylethyl acetate (RT at 49.3 min) were detected (Fig. 2). Similarly, in the cut inflorescences treated with

**Table 1. Composition of major volatile compounds emitted from *gypsophila* inflorescences after continuous treatment with chemicals for 4 days.**

Chemicals (concentration)	Volatile compound emission (nmol·g <sup>-1</sup> FW·h <sup>-1</sup> )		
	Ethanol	Ocimene	Methylbutyric acids
Control (-)	2.87	2.70	0.54
Ethanol (500 µl·l <sup>-1</sup> )	9.90	4.15	0.59
Benzyl alcohol (500 µl·l <sup>-1</sup> )	3.13	3.19	0.33
2-Phenylethyl alcohol (500 µl·l <sup>-1</sup> )	3.21	3.03	0.14
Titanium tetrachloride (19 mg·l <sup>-1</sup> )	1.83	1.71	0.32
Abietic acid* (30 mg·l <sup>-1</sup> )	6.58	3.92	0.27

\* Ethanol (500 µl·l<sup>-1</sup>) was used as a solvent for abietic acid.



**Fig. 2.** GC profiles of volatiles from gypsophila inflorescences treated without (A) or with 2-phenylethyl alcohol (B).

Peak 1: ethanol, peak 2: ocimene, peak 3: unidentified, peak 4: methylbutyric acid, peak 5: 2-phenylethyl acetate and peak 6: 2-phenylethyl alcohol.

benzyl alcohol, we detected benzyl alcohol (RT at 51.4 min) and its acetate ester (RT at 44.9 min)(data not shown).

#### Sensory evaluation

The odor of the gypsophila inflorescences treated with the chemical solutions other than ethanol, was rated weaker than the control (Fig. 3). Especially 2-phenylethyl alcohol and abietic acid markedly reduced the unpleasant odor.

#### Discussion

Four of the five chemicals tested in this study, reduced the emission of methylbutyric acids from cut gypsophila inflorescences (Table 1), and the amount of emission coincided well with the results of sensory evaluation (Table 1, Fig. 3). This clearly indicates that the unpleasant odor of gypsophila inflorescences could be weakened by the application of these chemicals to the vase solution through reducing methylbutyric acid emission.

When aromatic alcohols such as benzyl alcohol and 2-phenylethyl alcohol were added to the vase solution, peaks of their acetate ester, in addition to those of the applied alcohols themselves, appeared on the gas chromatogram. These esters were considered to be formed from exogenous alcohols and endogenous acyl-CoA by alcohol acyltransferase (AAT). AAT is the key enzyme in volatile ester formation in some scented flowers such as *Clarkia breweri* (Dudareva et al., 1998), *Antirrhinum majus* (Dudareva et al., 2000) and *Rosa hybrida* (Shalit et al., 2003). Esterification of the aromatic alcohols by AAT in the presence of isovaleryl-CoA is supposed to be a potential factor for reducing methylbutyric acid emission, although the peaks of these isovalerate esters have not been identified on the chromatograms. The aromatic alcohols may have caused a masking effect in addition to reducing the methylbutyric acid emission, because these alcohols and

Scent level to control	Ethanol	Benzyl alcohol	2-phenylethyl alcohol	Titanium tetrachloride	Abietic acid
Stronger	●●●●●●	●●		●	
Same	●●	●	●●●	●	●●
Weaker	●●●	●●●●●●	●●●●●●	●●●●●●	●●●●●●

**Fig. 3.** Sensory sniffing test of gypsophila inflorescences treated with ethanol, benzyl alcohol, 2-phenylethyl alcohol, titanium tetrachloride or abietic acid. Dots indicate number of panelists who evaluated at each level.

their acetate esters have a floral note. 2-Phenylethyl alcohol is generally involved in the aroma of many flowers such as *Rosa* (Shalit et al., 2003), *Camellia*, *Fragaria* and *Lilium* (Knudsen et al., 1993). Another aromatic alcohol, benzyl alcohol, is also found in the volatiles emitted from many flowers with a pleasant scent such as *Jasminum* and *Cymbidium* (Knudsen et al., 1993).

The deodorizing mechanism of titanium chloride is uncertain. Titanium is known as an inorganic esterification catalyst. It may enhance the esterification of endogenous alcohols and methylbutyric acid and consequently reduce the level of methylbutyric acid emission. Furthermore, it is possible that titanium works as oxidation catalyst and it decomposes methylbutyric acid and/or its precursor. However, titanium presents as a cation in aqueous solution and is supposed to have low mobility in xylem vessels.

Abietic acid, a kind of diterpenoid, is used as deodorants. Many compounds with an unpleasant odor, such as pyridine, caproic acid, formaldehyde and hydrogen sulfide, can combine with this compound (Emori, 1985). Similarly, methylbutyric acid may have combined with abietic acid resulting in a large unknown peak on the chromatogram (data not shown). The deodorizing mechanism of this chemical as well as titanium seems to be different from that of aromatic alcohols and titanium tetrachloride, so that the combined use of these chemicals is expected to have more synergic effect.

Application of aromatic alcohols, titanium tetrachloride and abietic acid decreased the emission of methylbutyric acid without any damage to flowers and stems. Hence, addition of these chemicals to vase water may be a practical means of inhibiting and quenching the unpleasant odor of cut gypsophila flowers on a commercial scale.

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