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Volatile Floral Chemistry of *Heliotropium arborescens* L. 'Marine'

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Abstract. **Selection emphasis on cyme size and flower color of *Heliotropium arborescens* L. has led to cultivars with diminished floral fragrance. As a preliminary inquiry into the fragrance chemistry of the species, we identified 41 volatile compounds emanating from the flowers of 'Marine' via isolation (Tenax trapping) and gas chromatography–mass spectrometry. The majority of the volatile compounds emanating from the flowers were terpenes (camphene, *p*-cymene, δ-3-carene, α-humulene, δ-1-limonene, linalool, (E)-β-ocimene, α-pinene, and β-thujone), benzenoids of which benzaldehyde was the most abundant, aldehydes (decanal, heptanal, nonanal and octanal), and hydrocarbons (decane, heneicosane, heptadecane, hexadecane, nonadecane, nonane, octadecane, tetradecane, tridecane and undecane) along with a cross-section of other compounds. Subsequent identification and quantification of critical ordorants will facilitate selecting new cultivars with quantitative and qualitative improvements in fragrance.**

Heliotropium is a genus of about 250 species of flowering herbs and shrubs in the Boraginaceae family, members of which are grown in borders and greenhouses of warm regions for their fragrant flowers (Bailey, 1977). *H. arborescens* (synonymous with *H. peruvianum* L. and *H. corymbosum* Ruiz & Pav.) is the common heliotrope and the most widely cultivated, predominately for it's violet to white fragrant floral cymes. Though technically a shrub, the plant is typically cultivated as an annual and is available from a number of seed companies. In the 1950s heliotrope was a popular cut flower in the United States due to it's fragrance as much as to the striking appearance of the flowers. More recently, however, breeding emphasis has focused upon larger, more brightly colored flowers and much of the fragrance has been lost (Armitage, 2001). The common heliotrope remains a relatively obscure floricultural crop on which there has been little published research. An initial attempt to assess the volatiles produced by *H. aborescens* flowers using solid phase microextraction identified three major compounds (benzaldehyde, benzyl acetate and *p*-anisaldehyde) (Hisano et al., 1995). The cultivar Marine is day neutral with flower initiation being advanced by exposure to cool temperatures (i.e., 10 °C) before forcing (Park and Simons, 2000). It also has minimal fragrance.

Biologically active volatile compounds such as fragrances and pheromones originate from a myriad of sources and exert a diverse range of effects on living organisms (Kays and Paull, 2004). Some act as semiochemicals, functioning in the communication and interaction within or among organisms (Dunsenbery, 1992). Plants represent a major source of volatiles (Charron et al., 1995), releasing an estimated 1.4×10^9 t·year^{-1} into the biosphere (Went, 1974). A number of volatiles are known to have critical biological roles; for example, certain floral volatiles act as synomones, attracting pollinators or as kairomones or allomones (Dunsenbery, 1992). Often the interaction involves a mixture of compounds in relatively specific proportions and the relationship among organisms can be relatively complex. In some instances, three trophic levels may respond to air borne chemical information. For example, *Nicotiana attenuata* Torr. ex Wats plants under attack by several herbivores release volatile phytochemicals that attract natural predators of the insects, greatly reducing (i.e., up to 90%) the herbivore population (Kessler and Baldwin, 2001). Floral volatiles are also key elements in our enjoyment of certain species (e.g., *Gardenia jasminoides* Ellis).

Due to the past importance of the fragrance of *H. arborescens* and the potential for breeding new cultivars with improved aroma, as a preliminary inquiry we isolated and identified the major volatile compounds emanating from the flowers.

Materials and Methods

Plant material. Plants of the cultivar Marine (Ball FloraPlant, West Chicago, Ill.) growing in the Department of Horticulture ornamental display garden at the University of Georgia were used for volatile collection. Individual cymes (7 to 9 cm in diameter) comprised typically of 250 to 300 individual flowers (4 to 6 mm diameter) supported by a many branched floral stem were used; thus some tissue other than floral may have contributed to the volatiles collected. Cymes, composed of closely arranged individual flowers of differing maturities, were selected in which most flowers had reached anthesis and relatively few had senesenced. The fragrance of 'Marine' was perceptible but diminutive.

Collection of headspace volatiles. Individual cymes attached to the parent plant were placed within cylindrical glass containers (497 mL) closed at one end and having a lid with an aperture slightly larger than the diameter of the stem. The flower stem attached to the parent plant was placed through the aperture which was sealed with closed pore neoprene. Volatile samples were collected during the day using a Tenax trapping system developed for the collection of headspace volatiles (Wang and Kays, 2002). Air flow through the glass container holding the cyme was generated using a small, portable vacuum pump (model 224-44XR; AirChek Sampler, SKC Inc., Eighty Four, Pa.) with the flow rate monitored and regulated using a flow meter with value. An air flow rate of about 20 mL·min^{-1} was used throughout the experimental period. Volatiles were collected for a 4-h period which gave about 10 container volume changes. Inlet air was passed through a charcoal filter (Alltech Assoc. Inc. Deerfield, Ill.) (1 cm i.d., 5-cm-long Pyrex glass tube with a 3-cm bed of charcoal). Before use, the charcoal filter was cleaned and activated at 200 °C for 24 h by passing purified N_2 (2 mL·min^{-1}) through the trap. All connections in the volatile collection system were ground glass joints or Teflon connectors. A second collection method was employed by placing several decapitated floral cymes in the container and the volatiles were collected in a similar manner.

Volatiles were collected using a Tenax trap (Tenax-TA, 60/80) (Alltech) prepared by packing 50 mg of the adsorbent in a Pyrex glass tube (3.5 mm i.d. × 5 cm), giving a bed length of 3 cm. Silylated glass wool was used to contain the adsorbent at each end. After collection, the trapped volatiles were eluted from the Tenax with 500 μL of purified hexane containing 1 μL·L^{-1} 1-octadecene (Sigma, St. Louis, Mo.) as an internal standard. The eluted solvent was concentrated to 30 μL by slowly flushing with purified N_2.

GC and GC–MS of volitiles. Gas chromatographic analyses were performed using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Wilmington, Del.) equipped with a flame ionization detector (FID). The GC column was a 30 m × 0.32 mm (i.d.) fused silica, DB-5, 0.25 μm film thickness capillary column (J&W Scientific, Folsom, Calif.). Samples were injected in the splitless mode. Injector and detector temperatures were 180 and 250 °C, respectively. Oven temperature was initially held at 35 °C for 3 min and then programmed from 35 to 240 °C at 5 °C·min^{-1} and held 5 min. Helium was used for the carrier gas at a flow rate of 53.1 mL·min^{-1}. Air and hydrogen flow rates to the detector were 370 to 380 mL·min^{-1} and 28 mL·min^{-1}, respectively. Nitrogen was used as the makeup gas at a flow rate of 30 mL·min^{-1}. An HP3392A integrator was connected to the GC for peak area measurement.

Mass spectral analysis was performed using a Hewlett Packard mass spectrometer (MS 5970) interfaced with a HP 5890 GC. The GC column used was a 30 m × 0.32 mm i.d. fused silica column coated with DB-5. GC and temperature program conditions were as previously mentioned. The GC–MS was programmed with a 3 min solvent delay. Electron ionization was

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used and the MS conditions were ion source 200 °C; electron energy 70 eV; multiplier voltage 220 V; GC–MS interface zone 300 °C. Ions from 20 to 550 atomic mass units were monitored. The identities of compounds were based on the Wiley Spectral Library (6th ed.) and the National Institute of Standards 75 K library and, when possible, confirmed using authentic standards.

Results and Discussion

In total, 41 compounds were identified emanating from the flowers of *H. arborescens* cv. Maine (Table 1). These included a cross-section of terpenes, which have been shown to contribute to the aroma of flowers and other plant parts of a number of species (Giamakis et al., 2001; Kilic et al., 2004; Nickavar et al., 2004). Alpha-pinene is a major constituent in the volatiles from *Encyclia vespa* (Vell.) Dressler & G. E. Pollard and *E. fragrans* (Sw.) Lemee flowers (Zoghbi et al., 2002). The other terpenes identified have been reported as emanating from a variety of flowers, e.g., camphene in *Curcuma pierreana* Gagnep. (Dung et al., 1998), δ-3-carene in *Perovskia atriplicifolia* Benth. (Dabiri et al., 2001), δ-1-limonene, and *p*-cymene in *Tilia cordata* Mill. (Buchbauer et al., 1995), linalool in *Lonicera japonica* Thunb. (Miyake et al., 1998), α-humulene in *Alpinia chinensis* Rosc. (Dung et al., 1994), and β-thujone in *Lippia turbinata* Griseb. and *L. polystachya* Griseb. (Zygadlo et al., 1995). Eight benzenoid compounds were identified, one of which was previously reported from heliotrope flowers (Hisano et al., 1995). Quantitatively, benzaldehyde was the most abundant component. Each of the aldehydes (i.e., heptanal, octanal, nonanal and decanal) and a number of the hydrocarbons (i.e., nonane, decane, tridecane, hexadecane, heptadecane, octadecane and heneicosane) are known to emanate from honeysuckle flowers (*Lonicera japonica* Thunb.) (Ikeda et al., 1994).

Table 1. Volatile compounds emanating from the floral cymes of *Heliotrope arborescens* 'Marine' identified by GC–MS.

Compound
2-Hexanone
2-Hexanol
Ethyl benzene
1,2-Dimethyl benzene
p-Xylene
ethenyl benzene
Nonane
Heptanal
Methoxybenzene
α-Pinene
Camphene
Benzaldehyde
6-Methy-5-heptene-2-one
Decane
Octanal
1,4-Dichlorobenzene
δ-3-Carene
δ-1-Limonene
2-Ethyl-1-hexanol
(E)-β-Ocimene
Undecane
Linalool*
p-Cymene
β-Thujone
Nonanal
Decanal
Benzothiazole
4-Methoxy benzaldehyde
Octacosane
Eicosane
Tricosane
Tridecane
α-Humulene
Tetradecane
Hexadecane
Heptadecane
Octadecane
6,10,14-Trimethyl-2-pentadecanone
2,6,10,14-Tetramethyl pentadecane
Nonadecane
Heneicosane

*Tentatively identified.

The data presented in Table 1 represent compounds identified using both intact and decapitated cymes. Decapitation allowed substantially increasing the amount of plant material from which volatiles could be trapped, thus facilitating the identification of components that were found in relatively small amounts. While we have not quantified differences, decapitation altered the volatile profiles obtained. For example, *p*-cymene was not found in the headspace of flowers that were severed from the parent plant. A similar repression was reported for *p*-cymene in lime tree flowers (*Tilia cordata* Mill.) (Buchbauer et al., 1995). Decapitation, likewise, can result in the production of wound volatiles also altering the profile.

Although not investigated, the time of collection may also quantitatively and qualitatively alter the volatile profile (Matile and Altenburger, 1988). For example, certain species of Nicotiana exhibit nocturnal rhythms in fragrance chemistry (Raguso et al., 2003).

In the current study, volatiles present in the headspace around the flowers were sampled rather than using solvent extraction of the flowers. The latter can significantly increase the number of compounds identified and the quantity of higher molecular weight molecules (Matich et al., 2003), however, many components isolated in this manner have relatively low volatilities and are often insignificant contributors to the fragrance. In contrast, the Tenex trapping technique used in the study has a higher collection efficiency for the more volatile, lower molecular weight compounds.

The volatiles produced from plants, whether from the flowers or other organs (Wang and Kays, 2003), can vary significantly among cultivars, contributing to the diverse range in fragrances and aromas found in the plant kingdom. For example, the volatile constituents of rose flowers varied markedly among cultivars (Brunke et al., 1992). The cultivars Othello, Duchesse de Montebello and Lichtkönigin Lucia differed markedly from Queen Elizabeth indicating that substantial genetic variation exists which could be readily exploited via plant breeding to create new cultivars with distinctive fragrances. Chemically characterizing the volatiles emanating from a floral crop and identifying and quantifying the critical compounds allows developing an analytical means of making progeny selection decisions in flower breeding programs.

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