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A Survey of the Volatile Profiles of Daylily Species and Hybrids

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Abstract

Daylilies (*Hemerocallis*) lost their fragrance as a result of many years of hybridization that singularly focused on flower color and form. Using a field collection system and gas chromatography-mass spectrometry, this study assessed the fragrance profiles of 147 daylilies. Major volatile constituents and their variations in the daylily study populations were determined and suggest that fragrance could be a trait pursued in a breeding program to enhance the sensory phenotypes of new daylily varieties.

INTRODUCTION

Daylilies belong to the genus *Hemerocallis* and are monocotyledonous herbaceous perennial plants. The genus and common name reflect the blooming habit of daylilies: their flowers only last for one day. Daylilies are native to sub-tropical and temperate Asia, arising mainly from China, Korea, and Japan (Rodriguez-Enriquez and Grant-Downton, 2013). Approximately 20 species are recognized, the colorations of which are limited to yellow, orange, and fulvous red (Gulia et al., 2009). Daylily hybridization began in earnest in the early 20th century, mostly by amateur breeders that focused on increasing the diversity of flower colors, shapes, and forms (Gulia et al., 2009).

Nowadays, daylilies come in a dazzling array of colors, patterns, shapes, and sizes, and many of these "hybrids" bear little to no resemblance to the modest species from which they were derived. The American

Hemerocallis Society (AHS) is the official daylily registrar, and currently maintains an online database of more than 87,000 registered cultivars. Over the years, however, such a singular focus on flower color, pattern, and form in daylily breeding resulted in an unfortunate unintended consequence: the loss of fragrance. While a number of the original daylily species possess noticeable, distinct fragrances, those fragrances are greatly reduced or largely absent in many modern hybrids (Jiao et al., 2016). As daylily breeders seek to create novel hybrids, they have turned their attention to the long-ignored trait of fragrance (P. Genho and J. Gossard, personal communication).

Floral fragrance is composed of mixtures of volatile organic compounds (VOCs or volatiles), mostly lipophilic liquids with high vapor pressures at ambient temperatures that typically fit in families of terpenes, phenylpropanoids, or benzenoids, as well as derivatives of amino acids and fatty acids (Dudareva et al., 2013). Plant volatiles serve a number of biological functions, including attracting pollinators or seed dispersers, acting as defense compounds, protecting the plant during certain abiotic stresses, and acting as signaling molecules (Dudareva et al., 2006). Floral volatiles also serve as a sensory attractant to people. A study of consumer preference for floral attributes found that a flower that does not make fragrance at all had the largest negative effect on consumer interest, indicating that consumers prefer fragrant flowers (Levin et al., 2012).

Two studies have been conducted on daylily aroma, both in China (Jiao et al., 2016; Lin et al., 2003). Lin et al. (2003) evaluated the essential oil of a single daylily species; however, depending on the extraction process, the aroma of an essential oil can differ from the aroma experienced by a person smelling the flower from which the oil came (Tholl et al., 2006). Jiao et al. (2016) evaluated 46 daylilies and identified 37 volatiles; however, the authors used authentic compound standards to verify only three of the compounds, thus casting some doubt on the veracity of the identity of the remaining compounds (Tholl et al., 2006). These studies provide a point of reference for further investigation of daylily scent, but ultimately only reflect the volatiles emitted by a tiny fraction of the expansive modern daylily germplasm.

The objective of this research was to evaluate the volatile profiles of a larger number of daylily hybrids and a small number of daylily species in three locations across the U.S., to identify volatile compounds in those profiles, assess the variation of volatile emissions among different daylily hybrids, and finally to determine which species or hybrids may be genetic resources for different volatiles. Daylily hybridizers could use this information to selectively breed for daylilies with enhanced fragrance.

MATERIALS AND METHODS

Volatile Collection. Volatiles were collected *in situ* from three privately owned populations of daylilies in Florida, Ohio, and Utah (Table 1).

Table 1. Study population and collection details.

	Florida	Ohio	Utah
Collection Dates	5/13/16 – 7/11/16	7/10/17 – 7/12/17	7/14/17 – 7/19/17
Avg. High Temperature (°F)	91	84	96
Avg. Relative Humidity (%)	70	87	42
Number Daylilies Sampled	64	33	50
Collection Owner	J. and E. Salter, Rollingwood Gardens	J. and D. Gossard, Heavenly Gardens	P. Genho, Private Collection

The presence of two fully open flowers was the main selection criterion for volatile sampling. Beyond that, daylilies of as many colors, color patterns, and flower forms as possible were selected at random from the populations. Volatiles were sampled via headspace sorption for 1-2 hours between 1000 and 1400 hours (Huber et al., 2005). Inflorescences were inserted into a nylon resin cooking bag and the bag was gathered around the scape beneath the flower and cinched with a twist tie (Stewart-Jones and Poppy, 2006). A glass column containing approximately 50 mg HaySep Q 80-100 porous polymer adsorbent (Hayes Separations Inc., Bandera, TX) was inserted into a slit at the top of the bag above the flower and secured with a twist tie. The glass column was fastened to a wooden stake to prevent the collection apparatus from collapsing on the flower (Fig.1).

Following volatile enrichment, a single-setting vacuum pump (Barnant Company, Barrington, IL, USA) was used to pull the air out of the bag through the adsorbent trap for three minutes. On each collection date, volatiles were sampled from empty nylon resin bags to account for background contaminants. Volatiles were collected in duplicate from each daylily. Volatiles were eluted from the adsorbent polymer within 12 hours with 150 µL of methylene chloride spiked with 2 µL of nonyl acetate as an elution standard. Samples were stored at -80° C until analysis by gas Chromatography-mass spectrometry (GC-MS).

Figure 1. Schematic diagram of the collection system. The column containing the adsorbent polymer is attached to the upper part of the stake, and an empty bag, from which volatiles were sampled to account for background contaminants, is attached to the lower portion of the stake.

Volatile Analysis. Volatile samples were analyzed on an Agilent 7890A gas chromatograph fitted with a DB-5 column (5% phenyl, 95% dimethylpolysiloxane, 29 m length x 0.25 mm internal diameter x 1 µm film thickness) and coupled to an Agilent 5973A mass spectrometer (Agilent Technologies, Santa Clara, CA). Compounds were tentatively identified by comparing their mass spectra to the National Institute of

Standards and Technology (NIST) mass spectral library.

Volatile identification was achieved by comparing the retention times and mass spectra of peaks in the samples to those of authentic standards. Analysis of volatile data was performed using MassHunter Qualitative and Quantitative software programs (Agilent Technologies; Santa Clara, CA, USA). Calculation of relative amount of volatile emission was based on individual peak area relative to the peak area of the elution standard within each sample. Calibration curves for authentic standards were run in duplicate on the GC-MS under the same conditions described above.

RESULTS

The volatile profiles of 147 daylilies, six species, 98 registered cultivars, and 43 unregistered "seedlings" were evaluated, and 18 volatile organic compounds were identified. Table 2 provides information about these compounds, including their prevalence and variation within the total study population, as well as the daylily cultivar or species that emitted the greatest amount of each compound.

Table 2. Summary statistics of volatile compounds emitted by daylily study population (N = 147).

Compound	n[1]	Freq.	Mean[2] ± SE	Median	Max	Daylily Emitting Max
Acetoin	98	66%	0.39 ± 0.03	0.31	2.39	'Bright Blaze of Magic'
2-Methyl-1-butanol	26	17%	0.26 ± 0.03	0.19	0.69	'Celtic Witch'
(*E*)-2-Methyl-2-butenal	111	75%	0.35 ± 0.04	0.21	2.31	'Bright Blaze of Magic'
3-Methyl-2-butenal	123	83%	1.88 ± 0.19	1.24	11.24	'Cheddar Explosion'
Hexanal	49	33%	0.22 ± 0.03	0.13	0.70	'Midnight Crossroads'
Benzaldehyde	34	23%	0.15 ± 0.02	0.11	0.46	'Cheddar Explosion'
6-Methyl-5-hepten-2-one	6	4%	0.03 ± 0.01	0.03	0.05	'Jalapeno Crunch'
β-Myrcene	136	92%	0.77 ± 0.04	0.64	3.00	'Blue Vibrations'
2-Ethyl-1-hexanol	139	94%	0.24 ± 0.01	0.21	0.67	'Wind Rider'
(*E*)-β-Ocimene	147	100%	9.22 ± 0.59	8.00	40.52	*Hemerocallis citrina*
(*Z*)-β-Ocimene	147	100%	33.67 ± 1.81	32.47	94.33	*Hemerocallis thunbergii*
β-Linalool	92	62%	0.30 ± 0.03	0.21	1.60	*Hemerocallis thunbergii*
Phenylethyl alcohol	102	69%	0.33 ± 0.01	0.28	0.66	'Winter Halo'
allo-Ocimene	115	78%	0.04 ± 0.003	0.03	0.18	*Hemerocallis citrina*
Indole	61	41%	0.49 ± 0.07	0.25	2.74	'Oh Great One'
β-Caryophyllene	20	13%	0.22 ± 0.03	0.17	0.50	'Micro Magic'
α-Farnesene	97	65%	4.27 ± 0.58	2.31	41.35	*Hemerocallis thunbergii*
(*E*)-Nerolidol	71	48%	0.34 ± 0.05	0.20	1.90	'Wind Rider'

[1]n denotes the number of daylilies that emitted a given compound, and Freq. denotes the frequency of that compound's occurrence in the total study population (n/147).
[2]Amount of volatiles emitted is given in µg/inflorescence.

Average emissions of each volatile compound are shown by study location in Figs. 2 and 3. Table 3 provides information about the total volatile emissions in each study location. Finally, Table 4 lists the top ten most fragrant daylilies, as determined by total volatile emissions, for each study location.

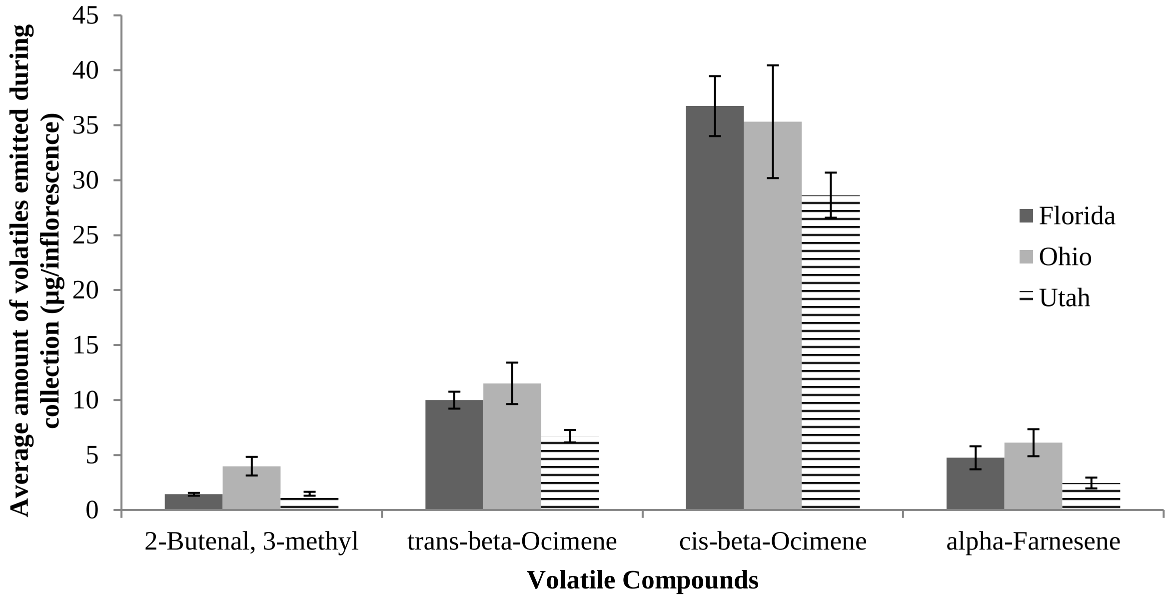

Figure 2. Average amount of high abundance volatile compounds emitted by daylilies in each study location. Standard error bars are shown for each volatile mean.

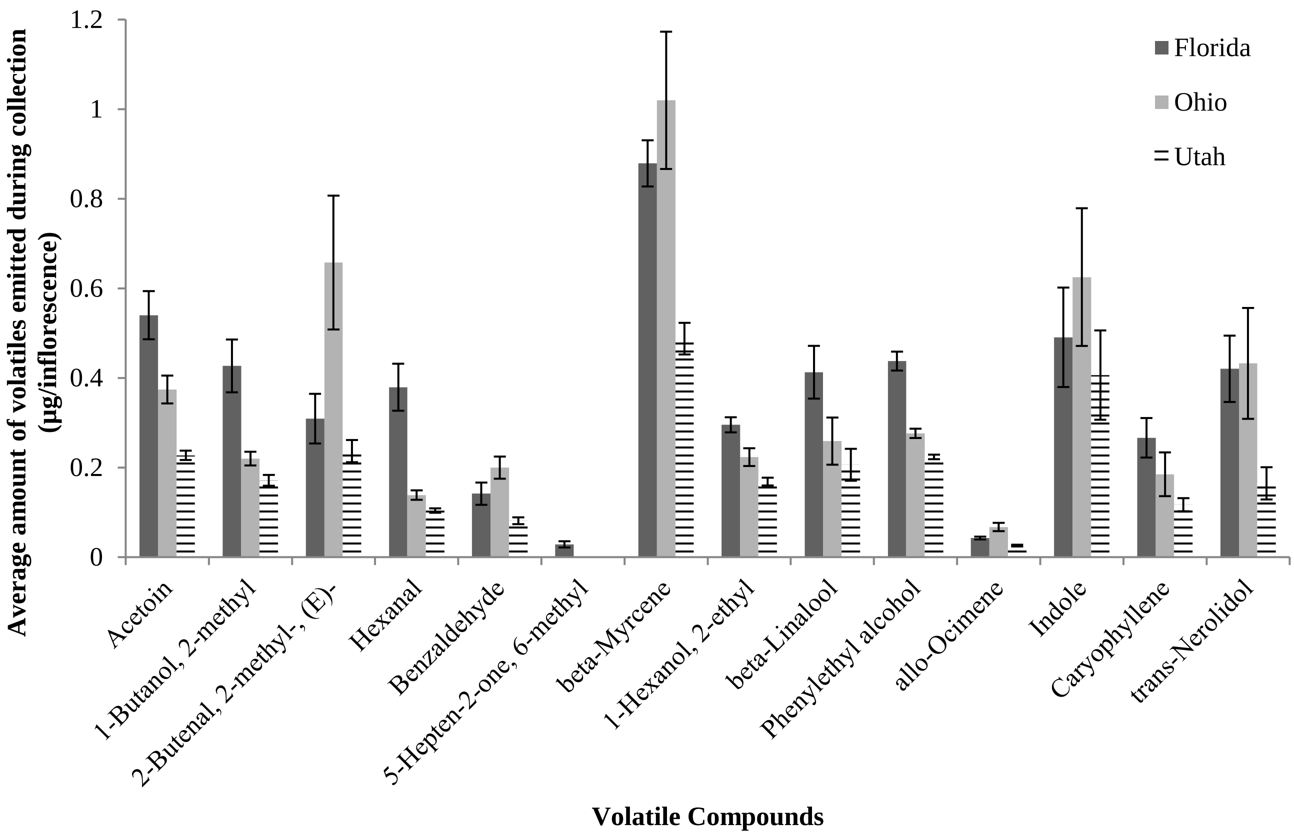

Figure 3. Average amount of low abundance volatile compounds emitted by daylilies in each study location. Standard error bars are shown for each volatile mean.

Table 3. Summary statistics for total relative volatile emissions, in µg/inflorescence, for each study location.

	Florida (n = 64)		Ohio (n = 33)		Utah (n = 50)	
	Emission	Daylily	Emission	Daylily	Emission	Daylily
Minimum	2.04	'Rim of Fire'	0.92	*H. fulva* 'Korean'	1.48	'Cheers for Now'
Mean	54.13		56.01		39.98	
Median	51.77		49.42		41.88	
Maximum	159.54	*H. thunbergii*	152.45	*H. citrina*	84.06	'Cranberry Daiquiri'

Table 4. The ten daylilies emitting the greatest total amount of volatiles, in µg/inflorescence, by location.

	Florida		Ohio		Utah	
Rank	Daylily	Total Emission	Daylily	Total Emission	Daylily	Total Emission
1st	*H. thunbergii*	159.54	*H. citrina*	152.45	'Cranberry Daiquiri'	84.06
2nd	'Spacecoast Blue Eyed Majesty'	129.59	'Out of Balance'	126.46	'Glistening Accent'	80.16
3rd	'Bridge of Dreams'	112.56	'Popcorn at the Movies'	116.30	'William Seaman'	70.63
4th	'Jalapeno Crunch'	101.29	'Blue Vibrations'	116.27	'Samurai Jack'	68.77
5th	'Spacecoast White Chocolate'	98.66	'Mystical Elf'	113.56	'Love and Marriage'	61.86
6th	'The Fantastic Barbara Watts'	82.56	'Spacecoast Devil's Eye'	113.48	'Viva Piñata'	60.65
7th	'Breakfast with Santa'	81.15	'Blackwater Captain Jack'	103.94	'Sailing'	58.71
8th	'Winter Halo'	78.90	'Double Yellow Thunder'	97.33	'Born to Run'	58.25
9th	'Heavenly Bengal Tiger'	64.58	'Cheddar Explosion'	87.20	'Ultimate Design'	58.16
10th	'Midnight Crossroads'	58.66	'Double Rays of Sunshine'	86.13	'Lover's Lemonade'	56.55

DISCUSSION

The majority of the 18 volatiles identified in this study were terpenoid compounds, including monoterpene hydrocarbons and alcohols, sesquiterpenes, and terpenoid derivatives. Similarly, Jiao et al. (2016) found that terpenoids represented over 80% of the total volatiles released by the daylilies they evaluated. While some geographic variation in the average emission amounts of the volatiles was observed, the differences between the three study locations in terms of climate, management practices like fertilization and irrigation regimens, soil type, and other factors, makes direct comparisons between the daylily populations unfeasible. Rather, each population was assessed individually.

Of the daylilies sampled in Ohio, four of the most fragrant cultivars exhibited "double" flower forms, which the AHS defines as a form with extra whorls of petals or petaloid tissue inside the normal petal whorl. As floral volatiles are emitted from petal tissue, these double daylilies may be more fragrant in part because they have more petal tissue. However, since the daylilies were part of an active breeding program, destructive sampling methods could not be employed so emissions per gram of fresh weight were not determined.

In Florida, *H. thunbergii* had a total volatile emission of almost 80 times that of the least fragrant daylily, while in Ohio, *H. citrina* had a total volatile emission of more than 150 times that of the least fragrant daylily. In comparison, over 80% of the total study population emitted less than half the amount of volatiles emitted by *H. citrina*. Jiao et al. (2016) obtained comparable results: all 38 daylily hybrids they evaluated were classified as having low or no floral aroma, with only five species exhibiting "intense or medium" floral aroma. The stark contrast between the fragrance of the species and hybrids illustrates the effect of hybridizers' traditional breeding objectives: the focus on flower form and coloration has indeed resulted in daylilies with drastically reduced fragrance. Nonetheless, out of only 147 daylilies, this study identified hybrids that do have heightened aromas, some of which are nearly as fragrant as the species already. Given the vast number of registered cultivars, many other fragrant hybrids certainly exist. As genetic resources of certain volatiles, these daylilies could be used by hybridizers in a breeding program to selectively breed for enhanced fragrance. In a practical sense, hybridizers do not need fancy analytical equipment to screen for fragrance. All they need is a decent sense of smell.

Because this research was conducted in the field on daylilies managed by different people, there were several uncontrolled variables, including soil type and fertilization regimens, among others. While the collection system was economical and practical for a field setting, it may not have been sensitive enough to detect volatiles at very low levels. Moreover, volatiles were collected at a single time point. Despite these limitations, this study yielded useful qualitative and relative quantitative data about the volatile profiles of almost 150 daylilies, highlighted the aromatic variation that exists in a slice of the germplasm, and identified daylilies that are potential genetic resources of volatiles. Hybridizers could use this information to potentially create "novel," highly fragrant daylilies that stand out from the 87,000+ existing cultivars. Daylily hybridizers are an especially avid community of plant breeders that have wrought incredible transformations in the visual characteristics of daylilies. If they increase their focus on aromatic characteristics, they will undoubtedly transform and enhance the fragrance of daylilies, too.

Figure 4. The two most fragrant daylilies, in terms of total volatile emissions, *sampled in Florida*. The species *Hemerocallis thunbergii* is shown on the left, and the hybrid 'Spacecoast Blue Eyed Majesty' is shown on the right.

Figure 5. The two most fragrant daylilies, in terms of total volatile emissions, *sampled in Ohio*. The species *Hemerocallis citrina* is shown on the left, and the hybrid 'Out of Balance' is shown on the right.

Figure 6. The two most fragrant daylilies, in terms of total volatile emissions, *sampled in Utah*. The hybrid 'Cranberry Daiquiri' is shown on the left and the hybrid 'Glistening Accent' is shown on the right.

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