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Emission of Volatile Chemicals from Flowering Dogwood (*Cornus florida* L.) Flowers

XIAOFENG ZHUANG, WILLIAM E. KLINGEMAN, JUN HU, AND FENG CHEN*

Department of Plant Sciences, 2431 Joe Johnson Drive, University of Tennessee, Knoxville, Tennessee 37996-4561

Reproduction of flowering dogwood trees occurs via obligate out-crossing, and U.S. native bees have been suggested to be primary pollinators of this ecologically and economically important deciduous tree. Whether floral volatiles play a role in reproduction of the dogwood remains unclear. Objectives of this study were to identify principal volatile chemicals emitted from dogwood flowers and to assess a temporal volatile emission profile and volatile consistency across four cultivars. Inflorescences with intact bracts and 5 cm flower pedicel were removed from dogwood trees and subjected to headspace volatile collection. Six principal volatile compounds were detected from the flowers of the cultivar 'World's Fair' with 3-formylpyridine as the most abundant constituent. Subsequent headspace analyses performed using inflorescences without bracts or floral pedicels alone indicated that 3-formylpyridine, *E*-β-ocimene, *S*-linalool, and ketoisophorone were mainly emitted from inflorescences. Experiments were also conducted to determine whether volatile emissions differed across time and between different cultivars of flowering dogwood. When volatile emission was analyzed for 48 h using 12 h light/dark cycles, the emission of several volatile compounds displayed diurnal patterns. Finally, whereas florets in inflorescences of four different dogwood cultivars emitted similar levels of the six principal floral volatile chemicals, 'Cherokee Brave' flowers alone yielded 4-methoxybenzaldehyde and germacrene-D. The implications of the findings of this study to dogwood breeding programs are discussed.

KEYWORDS: ***Cornus florida*; flowering dogwood; gas chromatography−mass spectrometry; headspace; native plant; pollination; scent**

INTRODUCTION

Flowering dogwood (*Cornus florida* L.) is a small, deciduous dogwood tree species native to eastern North America and popular as an ornamental plant. In spring, flowering dogwood trees become the focus of many community festivals across the southeastern United States (*1*). A striking feature of flowering dogwoods is that the inflorescence is surrounded by four large, petaloid, white, pink, or reddish bracts that provide a spectacular springtime display before or at the very early stage of leaf emergence. Each flowering dogwood inflorescence is composed of over 20 florets, each of which is relatively inconspicuous and contains four greenish yellow, 1 mm long petals.

Dogwood populations have declined dramatically since the late 1970s, attributed mainly to mortality from dogwood anthracnose (*2*) and dogwood powdery mildew, fungal plant diseases caused by *Discula destructiva* and *Erysiphe pulchra* (*3*), respectively. Although research has revealed commercial flowering dogwood cultivars and novel plant germplasm with resistance to either one plant pathogen or the other (see, e.g., ref *4*), a key plant breeding objective remains for developing a flowering dogwood with resistance to both plant pathogens. Understanding the reproductive biology of dogwood is important for such breeding programs.

Dogwood is an obligate out-crossing tree species (*5*). Because the flowers of dogwood produce only a sparse amount of pollen, wind pollination has been suggested to be unimportant, or at most a minor factor, in the pollination of dogwood (*5*). Hand pollination, which has been used as the primary means of pollination in dogwood breeding, is labor intensive and inefficient. Further advances in classical breeding of the species may depend on the development of a more effective pollination model. Researchers have realized that this process could be optimized by enhancing the activity of potential insect pollinators. Indeed, many insects species visit blooming flowers of dogwood including bees, beetles, flies, and butterflies (*6*). Of these, the most common insect visitors to the dogwood inflorescences include *Andrena* sp. miner bees (Hymenoptera: Andrenidae) and *Lasioglossum* (=*Dialictus*) sp. sweat bees (Hymenoptera: Halictidae) that also carry large loads of dogwood pollen (*7*). The cues for attracting natural pollinators to dogwood flowers, however, have not been extensively studied.

Plants pollinated mainly by bees typically have showy flowers, and some bee-pollinated flowers also have strong scent.

* Corresponding author [telephone (865) 974-8521; fax (865) 974-1947; e-mail fengc@utk.edu].

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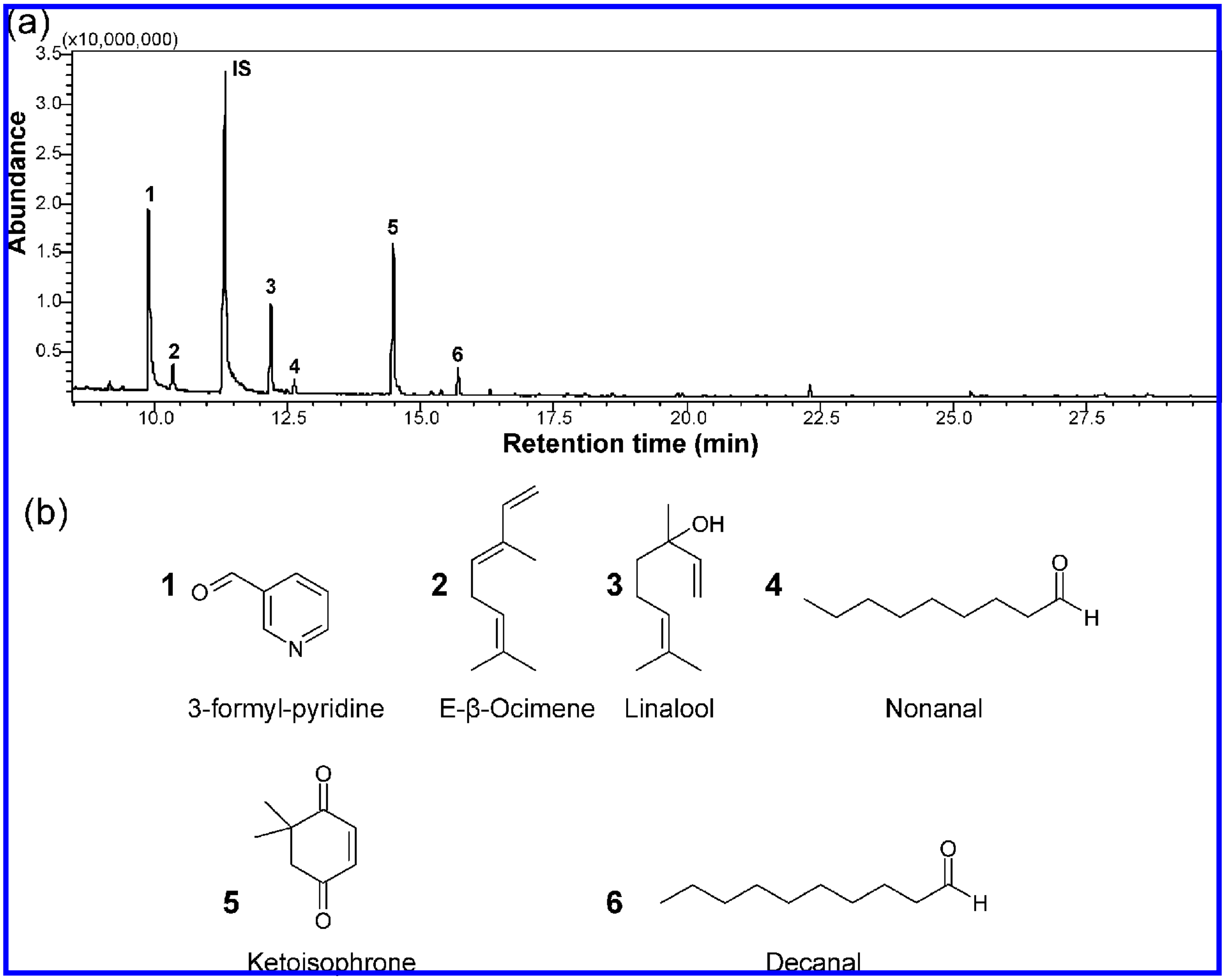

Figure 1. Volatile chemicals emitted into the headspace surrounding flowering dogwood cultivar 'World's Fair' inflorescences with bracts and flower stems retained: (**a**) gas chromatogram of volatile chemicals collected from the headspace surrounding dogwood tissues. IS represents internal standard. (**b**) structures of the chemical compounds that were identified numerically in (**a**).

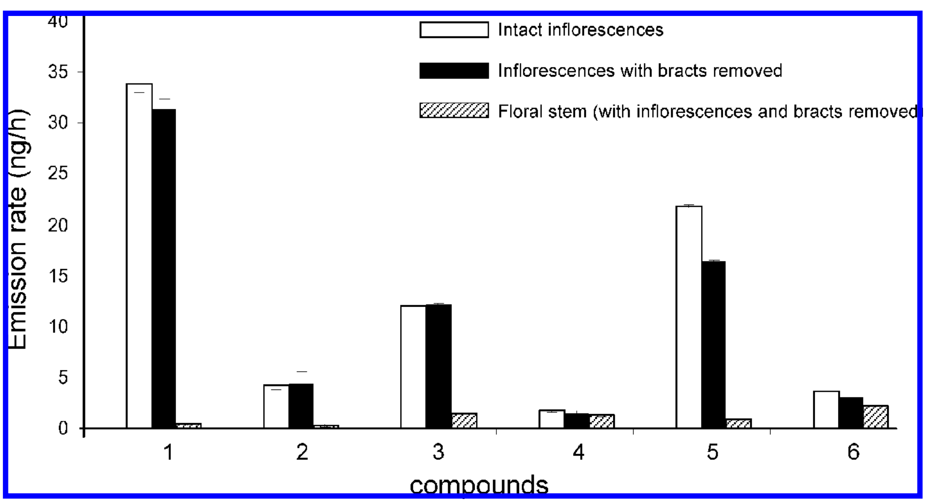

Figure 2. Release rates of six major volatile compounds from dogwood flowers and parts of the flower structures from six floral units. Bars 1−6 correspond to 3-formylpyridine, *E*-*β*-ocimene, *S*-linalool, nonanal, ketoisophorone, and decanal, respectively.

For example, bee-pollinated snapdragon flowers emit a large number of sweet-smelling scent volatiles (*8*). Because floral scent volatiles are both chemically complex and variable across species, floral odors can be expected to affect pollinator choice and subsequently affect plant reproductive outcome (*9*). Floral scent chemistry can also benefit plants by deterring herbivores and other floral visitors (*10*). For example, nicotine recovered in the headspace surrounding *Nicotiana attenuata* Torrey ex. S. Watson flowers was suggested to have a defensive role against herbivores (*11*).

Arabidopsis thaliana (L.) Heynh, which is the most widely studied model species for plant biology research, is capable of a high level of self-pollination under growth-room conditions (*12*). Yet, a recent study showed that *A. thaliana* flowers, which are perceived to be aesthetically scentless to humans, emit a large number of volatile chemicals (*13*) that were suggested to

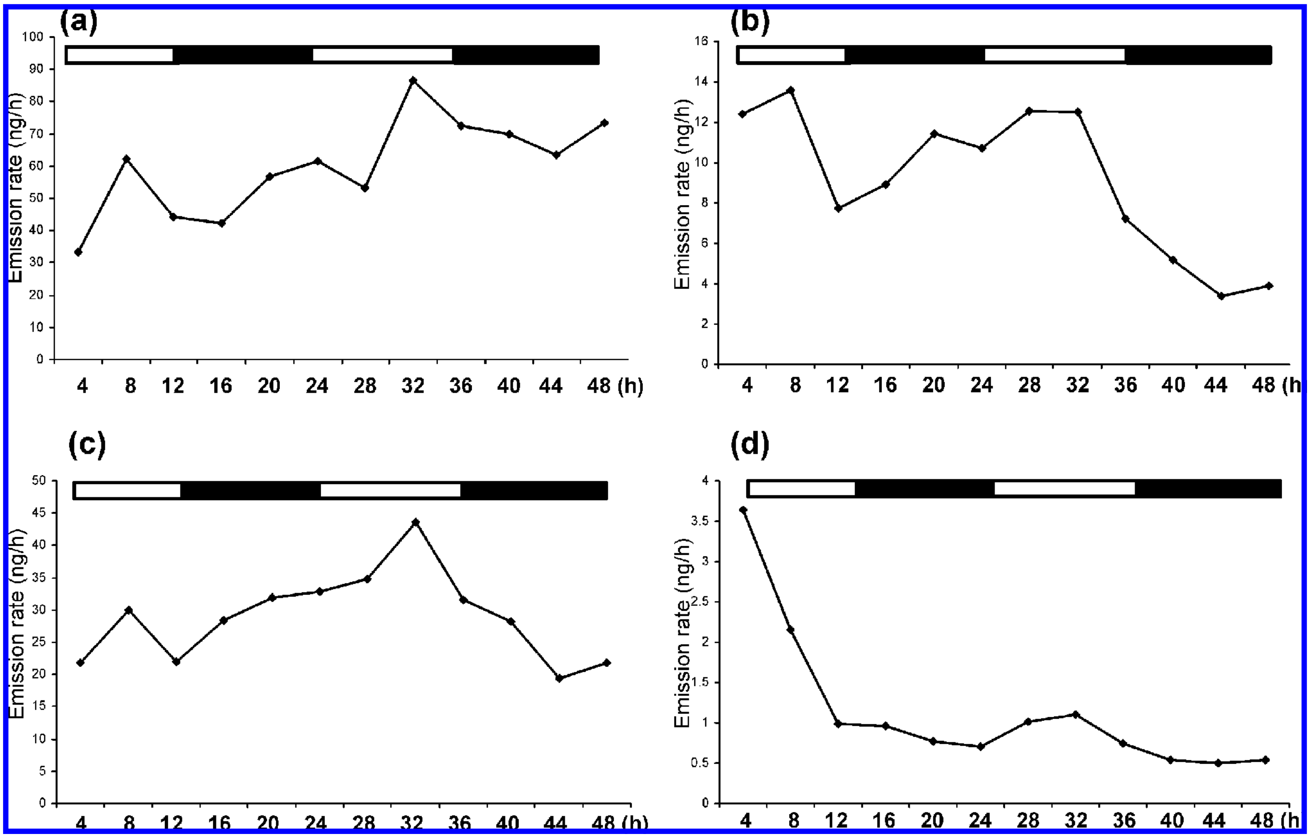

Figure 3. Emission rates of four major compounds, 3-formylpyridine (**a**), *S*-linalool (**b**), ketoisophorone (**c**), and decanal (**d**), during a 48 h period from six floral units. White and black bars depict daytime and nighttime, respectively. Two replicates yielded similar results. Only emission rate from one replicate is presented.

be involved in attracting pollinators in the wild for cross-pollination (*14*). This finding implies that the role of floral volatiles in the reproduction of certain plant species may have been underestimated. Like *A. thaliana*, dogwood flowers are generally perceived to lack distinguishing scents. Whether *C. florida* flowers emit volatiles and, if so, whether the floral volatiles play a role in reproduction of dogwood remain unknown.

As with *A. thaliana*, dogwoods have a well-established phylogeny (*15*) and have been an important genus for basic plant biology research, including studies of floral evolution (*16*). Whereas dogwood flowers are generally believed to be scentless, dogwood fruits have been shown to emit volatile compounds (*17*), in turn suggesting that certain biochemical pathways for production of volatile chemicals in flowering dogwood are functional. The objective of this study was to assess whether dogwood flowers emit volatile chemicals and if so, to determine whether volatile emission profiles differ across time and between different horticultural cultivars.

MATERIALS AND METHODS

Plant Material. Inflorescences each with about 20 flowers clustered above subtending bracts and stem tissues were analyzed from four *C. florida* cultivars, including 'World's Fair', growing on the campus of the University of Tennessee, and 'Cherokee Brave', 'Cherokee Princess', and 'Appalachian Spring' trees growing in 3 gal (11.4 L) nursery containers. 'Cherokee Princess', 'World's Fair', and Appalachian Spring' are flowering dogwood cultivars with white bracts, and 'Cherokee Brave', which is unrelated in parentage to 'Cherokee Princess', possesses pink bracts. 'World's Fair' is also reported to be drought resistant, flowers at a young age, and is only distinguishable from 'Cloud 9' [= 'Barton' (*18*)] because it flowers about 1 week earlier (*19, 20*). 'Appalachian Spring' is resistant to the fungal pathogen causing dogwood anthracnose (*21*), and pink-bracted 'Cherokee Brave' has moderate resistance to dogwood powdery mildew (*3, 20*).

'World's Fair' reproductive tissues of approximately 25 bracts with subtending inflorescences that included floral pedicles were removed and analyzed on April 11, 2008. Flowering tissues of 'Cherokee Brave' were collected and analyzed on April 15, 'Cherokee Princess' on April 16, and 'Appalachian Spring' on April 18, 2008. In each case, inflorescences attached to an approximately 5 cm long floral pedicle were cut from the tree, placed in a flask filled with distilled water, and transported to the laboratory for volatile analysis. At the time of tissue collection, approximately half of the florets in individual inflorescences were open. Two samples for each cultivar were analyzed.

Headspace Volatile Collection. Volatiles emitted from dogwood tissues were collected in an open headspace sampling system (Analytical Research Systems, Gainesville, FL). Dogwood reproductive structures were collected at 8:30 a.m. and immediately placed in a flask filled with water. Six floral units, which included inflorescences with up to 20 individual florets, subtending bracts, and stem tissues, were placed in single flasks with about 150 mL of distilled water and held in 10 cm (3.9 in) diameter by 30 cm (11.8 in) tall glass chambers that included a removable O-ring snap lid with an air outlet port. Charcoal-purified air entered the chamber at a flow rate of 0.8 L/min from the top through a Teflon hose. To more precisely determine which portions of the dogwood tissues emitted volatiles, headspace collection was performed with inflorescences from which bracts were removed as well as tissues from which both bracts and florets were removed (i.e., only stem tissues remaining). Volatiles were collected for 4 h by pumping air from the chamber through a SuperQ volatile collection trap (Analytical Research Systems).

Determination of Emission Dynamics of Individual Floral Volatiles of Dogwood. To better understand temporal emission dynamics of floral volatile compounds from dogwood, headspace collections were performed continuously with one elution every 4 h through two complete 12:12 h (L/D) photoperiods. Volatiles collections were initiated at 9:00 a.m., which marked the beginning of the light period.

At each collection time, volatiles were eluted with 100 μL of methylene chloride, and 1-octanol was added as an internal standard for quantification. Analyses of both time course and cultivar of volatile profiles were based on two data set replicates.

GC-MS Analysis. Plant volatiles were analyzed on a Shimadzu 17A gas chromatograph coupled to a Shimadzu QP5050A quadrupole mass selective detector. Separation was performed on an SHR5XLB column (30 m × 0.25 mm i.d. × 0.25 μm thickness, Shimadzu, Columbia, MD). Helium was the carrier gas (flow rate of 5 mL min^{-1}), a splitless injection (injector temperature = 250 °C) was used, and a temperature gradient of 6 °C/min from 60 °C (3 min hold) to 300 °C was applied. The chirality of linalool was determined using an RT-GAMMA-DEXsa column (Restek, Bellefonte, PA) using hydrogen as the carrier gas. Products were identified using the National Institute of Standards and Technology (NIST) mass spectral database and by comparison of retention times and mass spectra with authentic reference compounds obtained from Sigma (St. Louis, MO).

RESULTS

Dogwood Flowers Emit Floral Volatiles. Six major volatile compounds were detected from six 'World's Fair' flowering dogwood inflorescence clusters of florets, bracts, and pedicles following collection for 4 h: 3-formylpyridine, *E*-β-ocimene, *S*-linalool, nonanal, ketoisophorone, and decanal (**Figure 1**). 3-Formylpyridine and ketoisophorone were tentatively identified. All others were determined on the basis of comparisons to authentic reference compounds.

Analysis of portioned dogwood tissues revealed that of the six major volatile constituents identified, 3-formylpyridine, *E*-β-ocimene, *S*-linalool, and ketoisophorone were primarily emitted from florets. In contrast, nonanal and decanal were mainly emitted from pedicles (**Figure 2**).

Emission Dynamics of Individual Floral Volatiles of Dogwood. Three floral volatile compounds, 3-formylpyridine, *S*-linalool, and ketoisophorone, showed similar emission patterns across two light/dark photoperiods. Emission of these compounds escalated during the day and then declined during the night. Decanal, however, showed a different emission pattern and was detected at its highest level during the first 4 h following excision of the inflorescences and bracts (**Figure 3**).

Emission of Floral Volatiles from Different Dogwood Cultivars. Floral volatile profiles of four commercially important dogwood cultivars revealed that the scent chemistries of tested pink-bracted ('Cherokee Brave') and white-bracted forms ('World's Fair', 'Cherokee Princess', and 'Appalachian Spring') were very similar, although with minor differences. For example, whereas the six major floral scent compounds were consistent between cultivars and elution rates were highly similar to those of 'World's Fair', 'Cherokee Brave' emitted two additional compounds not detected in 'World's Fair': 4-methoxybenzaldehyde and germacrene-D. The former is an aromatic compound and the latter a sesquiterpenoid. Floral volatile profiles of 'Cherokee Princess' and 'Appalachian Spring' were essentially identical to that of 'World's Fair' (data not shown).

DISCUSSION

The role of floral volatiles in the reproduction of scented outcrossing plant species is easy to understand. Their role in reproduction of scentless plants, however, has been elusive. *A. thaliana* is a self-compatible plant and naturally yields approximately 2% cross-pollination-derived seeds (*22*). Yet, small insects including solitary bees have been observed visiting *A. thaliana* flowers (*23*). The identification of volatile compounds from *A. thaliana* flowers strongly suggests that these floral volatiles, which could not be detected by humans, likely play a role in attracting pollinators in the wild (*11*). The identification of volatiles from flowers of dogwood, which are also generally considered esthetically "scentless" to humans, suggests that these chemicals may have a role in the plant's reproduction.

We showed in this paper that flowering dogwood flowers emit a number of volatile chemicals (**Figure 1**). Some of these floral volatiles, such as the monoterpene linalool, are constituents of floral scents common to many plant species (*24*). Linalool has been shown to be attractive to a broad spectrum of pollinators (*25*). Other compounds, such as 3-formylpyridine and cyclic ketone ketoisophorone, have not been reported as floral scent compounds. It is interesting that volatiles from flowers are different from volatiles produced by dogwood fruits. Fruits of dogwood emit a number of volatile compounds, with ethyl acetate and 3-methylbutan-1-ol predominating (*17*), which are not constituents of floral volatiles (**Figure 1**). The difference in volatile composition suggests that these volatile compounds may have specific biological or ecological roles. For example, fruit volatiles may be involved in attracting seed dispersers, whereas floral volatiles may serve as cues to attract pollinators. For many plant species, floral scent emission displays rhythmic patterns (*8*), with peak emission often coinciding with the most active visitation of the pollinators. The emission of floral volatiles from dogwood flowers also exhibits a diurnal pattern (**Figure 3**). Because the observed pollinators of dogwood, including bees and butterflies, have a diurnal lifestyle, it will be interesting to determine whether the timing of the peak emission of volatiles from dogwood flowers coincides with the timing of active visitation of native pollinators. Such information will be useful for understanding the role of dogwood floral volatiles in pollinator attraction.

Additional research will be necessary to examine the responses of certain natural pollinators to pure, synthetic volatile compounds individually or in combination using electroantennography, which will help determine the key constituent for pollinator attraction and whether there are synergistic effects among individual compounds. Similarly, the efficacy of these compounds in pollinator attraction can be assessed both singly and as component blends in attracting the primary pollinators of flowering dogwoods in the field. A comparison of visitation frequency and the pollinator species complex attending different cultivars of dogwood, for example, 'World's Fair' and 'Cherokee Brave' flowers that differed in floral volatile composition, will also help to verify roles of these compounds in dogwood pollination and fruit set. If shown to be important, these compounds can be used as an important tool to enhance insect-mediated cross-pollination of dogwood, which will accelerate the production of novel cultivars of flowering dogwood, such as the ones with resistance to both dogwood anthracnose and dogwood powdery mildew, through breeding.

The finding presented in this paper can also be important for the nursery industry to produce dogwood with fragrant flowers. As mentioned in the Introduction, most native flowering dogwood trees, as well as commercially available ornamental cultivars, are esthetically scentless. Interestingly, Santamour and McArdle (*19*) list 'Fragrant Cloud' and 'Pink Sachet' as white- and pink-bracted flowering dogwoods, respectively, that have fragrance resembling a blend of gardenia, honeysuckle, and *Calycanthus* scents. Unfortunately, it has not been possible to locate these cultivars either in the commercial trade or arboreta, and they may have been lost (R. N. Trigiano, personal communication). Nonetheless, their inclusion in the checklist does suggest it is possible to create new cultivars of dogwood trees with fragrant flowers. Additional chemical analyses of floral volatiles from a greater variety of *C. florida* cultivars may provide useful information for guiding such breeding efforts.

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