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Floral scent is different between sexual phases within individuals in a synchronously dichogamous shrub (*Canella winterana*) but there is no distinct female or male scent profile across individuals

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ABSTRACT

Floral scent is a highly complex and variable floral characteristic that is involved in pollinator attraction. One possible cause for variation in floral scent can be sexual identity of the flower. Here, we examine the floral scent bouquet of a synchronously dichogamous shrub, *Canella winterana* (L.) Gaertn.We used dynamic headspace extraction and gas chromatography – mass spectroscopy, followed by statistical analysis using non-metric multidimensional scaling, SIMPER, MANOVA, and PERMANOVA to identify and compare the scent profiles of *Canella winterana* in its female and male phase for multiple individuals as well as multiple inflorescences of a single tree over one cycle of its entire sexual phase transition. The scent profile of *C. winterana* is composed of 49 volatile organic compounds and dominated by five compounds. We found no evidence for distinct male or female scent profiles; however, there were significant differences in scent emission between different sexes within some individuals. Two compounds explained over half of the variation between sexual stages within individuals. Our exploration of a single tree's sexual phase transition, including neuter phase, found that five compounds dominated the female phase scent bouquet and that female phase was distinct from male and neuter phase. This study offers new insight into the role that variability in floral scent between sexual phases might play in variable pollinator behavioral responses. These results suggest partial support for two distinct hypotheses regarding the differences between the sex phases (1) honest signaling and (2) sexual mimicry.

1. Introduction

Floral scent is a complex blend of volatile compounds produced by floral tissues that is highly diverse in terms of ecological function. Perhaps most notable is its importance to the reproductive success of many plant species. A number of studies find that increased floral scent emission is associated with higher fitness (Miyake and Yafuso, 2003; Ashman et al., 2005; Raguso, 2008; Majetic et al., 2009; Wright and Schiestl, 2009; Scheistl, 2010; Parachnowitsch et al., 2012; Rosensteil et al., 2012). Furthermore, a plant's floral scent bouquet can vary temporally and is often correlated with peak pollinator activity – tightly linking floral scent and pollinator attraction (Huber et al., 2005; Burdon et al., 2015; Majetic et al., 2015). While the last 20 years have shown marked improvements in our understanding of how floral scent signaling to mutualists can affect the reproductive success of flowering plants, there is still much progress to be made in this field (Schiestl, 2015; Haber et al., 2018). Of particular interest, given limited previous study, are questions regarding how mating systems and scent production interact, and in turn how these effects influence pollinators in natural environments.

Floral scent can show patterns of variability that are linked to key aspects of reproductive biology (Ashman et al., 2005; Dötterl and Jürgens, 2005; Füssel et al., 2006; Waelti et al., 2009). Studies in dioecious species show that there can be substantial intraspecific variation of floral scent between sexes in terms of both quantitative and qualitative characteristics. For example, in *Magnolia kobus* and *Silene latifolia*, certain compounds dominate the scent of individuals of one sex while present in relatively small amounts in the other sex (Azuma et al., 2001; Dötterl

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and Jürgens, 2005). In extreme cases, differences of even a single compound can be responsible for changes in sex-biased pollinator behavior, as seen for *Fragaria virginiana* (Ashman et al., 2005). Studies also suggest an association between floral scent components and types of pollinators, with specific compounds associated with attraction of specific groups of pollinators (Moya and Ackerman, 1993; Dobson et al., 1997; Knudsen et al., 2001). These prior studies suggest a role of sex specific floral scent identity and its role in differential pollinator attractions. However, those that do explore mating systems focus mainly on dioecious species, and little work has been conducted to explore these possibilities in other mating systems (as reviewed in Ashman, 2009).

One mating system that has been overlooked in these scent profile studies is dichogamy, which especially surprising given that it is a taxonomically widespread plant breeding system (Bertin and Newman, 1993; Centibas, 2014). In dichogamous systems, the maturation of sexual organs is temporally separated, so that there is limited overlap between the release of pollen from anthers and the acceptance of pollen on stigmas within the hermaphroditic flower (Endress, 2010). It is hypothesized that dichogamy, by preventing overlap between the staminate and pistillate function within a flower, evolved in response to selection pressures associated with negative impacts of self-pollination (Bhardwaj and Eckert, 2001). Although dichogamy is sufficient in preventing self-fertilization in single flowered plants (Narbona et al., 2011), this system often does not prevent self-pollination in species bearing multiple sexually mature flowers on an inflorescence (Jong et al., 1993). In these cases, geitonogamy, i.e. self-pollination between flowers on the same plant, often occurs as pollinators forage for resources from multiple flowers found on the same inflorescence (Nattero et al., 2011). However, dichogamy coupled with gender coordination between all flowers on an inflorescence should reduce or completely prevent geitonogamous self-pollination (Bhardwaj and Eckert, 2001; Galloway et al., 2001).

Canella winterana (L.) Gaertn is a plant that exhibits a highly synchronous form of gender coordination within its flowers, known as synchronous dichogamy (Wilson, 1985). Flowers of *C. winterana* specifically exhibit synchronous protogynous sexual development, wherein flowers initially express pistillate function and transition to staminate function after 24 h (Garrett, personal observation). We sought to explore the possibility that scent differs between sex phases in a dichogamous system by characterizing the overall scent of *C. winterana*, examining scent bouquet differences between female (pistillate) and male (staminate) sexual phases, as well as investigating the transition period between sexual phases (i.e., a neuter stage where the flower is not fully expressing male or female function). One possible explanation for distinct sex specific profiles is the honest signaling of rewards – the scent of the nectar in the female phase and pollen in the male phase is honestly signaling the available floral rewards. Alternatively, if the scent of the female and male phase is similar, there may be sexual mimicry occurring, suggesting the scent profile is under selective pressure to remain similar between the sexes to minimize sex-biased pollinator discrimination.

2. Materials and methods

2.1. Study species –

Canella winterana (L.) Gaertn. is a monotypic genus in the family Canellaceae. In the United States, flowering occurs from late May to early August but the length and intensity of the flowering season is dependent on the amount of precipitation. Inflorescences are borne at the ends of branches as terminal cymes that contain anywhere from 1 to 60 crimson red aromatic flowers and each plant can contain anywhere from one to more than a hundred cymes (Garrett, personal observation). Typically, 2-3 flowers will bloom in a cyme at a time but there can occasionally be up to 5 flowers blooming at once if the environmental conditions are favorable. Each flower is subtended by 3 green fleshy sepals, has 5 fleshy crimson red petals, and a single bicarpellate pistil with five ovules (Wilson, 1960). The pistil is surrounded by stamens, which have fused at the filament to form a staminal column around the style. When the bright yellow stigmatic surface becomes receptive to pollen, it protrudes out of the top of the staminal column making it easy to identify when flowers are in the female phase of sexual expression (Fig. 1). Similarly, the anthers produce bright yellow pollen which provides a striking contrast to the crimson petals, which indicates the flowers are in the male sexual phase (Wilson, 1966).

Both the number of cymes on a plant and the number of flowers within each cyme can be highly variable. In general, plants found in habitats with high exposure to the sun, such as those found on edge habitats and in canopy gaps, will have more cymes and more flowers per cyme relative to plants growing in the subcanopy where light is less available (Vermeulen, 2014).

2.2. Synchronous dichogamy in Canella winterana –

Wilson (1985) originally discovered that *Canella* exhibits a highly regulated form of dichogamy with a specific schedule of protogynous floral development that is synchronized between all flowers within a single plant. Wilson's original description of flowering phenology in *Canella* is now recognized formally as synchronous dichogamy. More detailed recent research shows that *Canella* exhibits a very specific 3-phased schedule of synchronously dichogamous sexual expression (Garrett, personal observation). In **phase one** of this cycle, all flowers are female first and open expressing pistillate function, which persists for 12-24 h. **Phase two** is a transition phase where flowers are neuter, i.e. pistillate function ceases and staminate function has not yet begun. Specifically, pistillate function ceases due to the stigma becoming dry and/or clogged with pollen so that it can no longer effectively receive pollen. The length of phase 2 typically lasts for approximately 12 h but can be variable; it can be as short as 1 h but is generally no longer than a full day. In **phase three,** flowers exhibit male (staminate) function, which is marked by the release of pollen into the environment from the anthers. The pollen then either persists in the flower for another 12-48 h or is dispersed by pollinators to another plant.

2.3. Sampling location –

Canella has a subtropical-tropical distribution and is found commonly throughout the West Indies (Smith and Vankat, 1992). However, *Canella* is considered rare in the northern-most part of the species range; it is a state-listed endangered species in Florida (Ward et al., 2003). These trees inhabit inland coastal hammocks, and are most commonly found growing directly out of limestone substrates. We utilized ten reproductively mature plants found in three distinct populations in protected areas of southern Florida: six plants from John Pennekamp Coral Reef State Park, three plants from the Key West Tropical Forest and Botanical Garden, and one plant from the Native Tree Nursery property in Homestead, FL (which grows *C. winterana* from wild-collected seed).

2.4. Scent collection –

The scent of ten unique *C. winterana* individuals with up to 3 inflorescences per individual were sampled in both female and male phase by dynamic headspace collection (as described in Majetic et al., 2017) during June 2017 and 2018. Dynamic headspace collection involved first enclosing the inflorescence in a Reynolds oven bag (Reynolds, Richmond, Virginia.) and puncturing the bag with a scent trap. The scent trap, a Pasteur pipette filter containing 10 mg 80/100 mesh Super Q chromatography packing material (Alltech Associates, Deerfield, Illinois) between plugs of silanized quartz wool, was then attached to a PAS-500 personal air sampler (Spectrex Corp., Redwood City, California) and air was pulled through the trap at a rate of ~200 mL/min for an

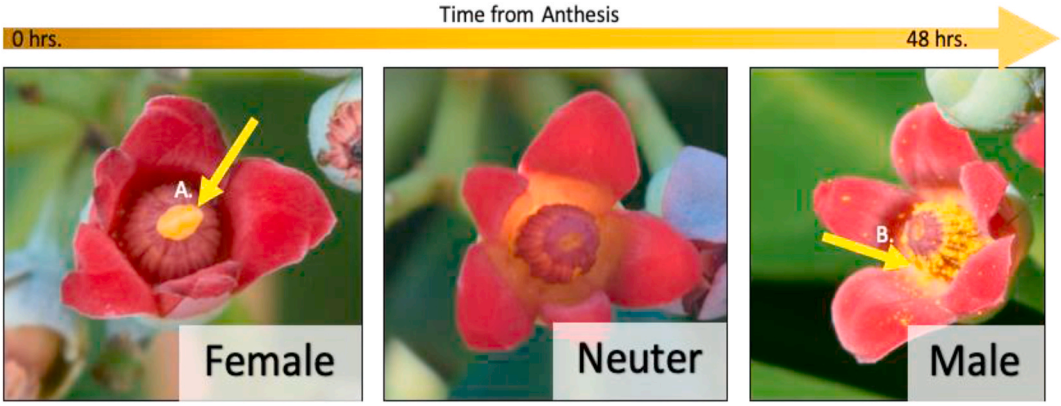

Fig. 1. Stereotypical pattern of sexual expression transitions in *Canella winterana* flowers. Each panel shows the phenotypic differences used to define the sexual identity of flowers in the field at certain time points during flowering phenology. Left panel: Female flowers are characterized by the opening of the bright yellow stigmatic surface (A) out of the opening formed by the staminal column. Middle panel: The neuter phase is defined as when the stigmatic surface is no longer wet, open, or accepting pollen and anthers have not yet dehisced. Typically, this manifests at the end of the first day of flowering, marking the transition between the female and male sexual phase. Right panel: Male phase is marked by the anthers dehiscing to release bright yellow pollen into the environment (B).

hour (as in Majetic et al., 2019). Traps were then eluted with 300 μL hexane into glass vials with Teflon-lined lids following each collection period; scent elutions were stored on ice in the field and then in a −20 °C freezer until chemical analysis was completed. After collection from female phase flowers, the inflorescence was bagged with mesh to prevent pollination, and thus post-pollination odor changes (Schiestl et al., 1997), and marked for identification purposes. Twenty-four hours later, when the flowers were in male phase, the collection protocol was repeated. After collection of volatiles from both sexual stages, the inflorescences were harvested; number of opened flowers, unopened buds and mass of inflorescence tissue sampled was recorded. Ambient air and vegetative samples were taken at all field sites to control for background noise in the data (as in Majetic et al., 2019).

To capture the scent during the transition between sexes, including the neuter phase, in *C. witnterana,* more intensive sampling across multiple time intervals within an inflorescence was conducted. Scent from six inflorescences on a single *C. winterana* plant was sampled at seven different time points at John Pennekamp Coral Reef State Park. Scent collection began when the tree was presenting fully female flowers (time 0), then continued until the flowers were fully male (time 6). Scent collection periods were 1 h with a subsequent 2-h equilibration period. Due to the use of a State Park, access to the tree was limited to the park's hours of operation. Therefore, scent sampling did not occur between dusk and dawn (approximately 21:30 to 8:00). Collection start times were 10:20 (time 0), 13:17 (time 1), 16:15 (time 2), and 18:55 (time 3) on June 18, 2018, and 8:29 (time 4), 11:18 (time 5), and 16:07 (time 6) on June 19, 2018.

2.5. Chemical analysis –

Prior to chemical analysis, all samples were blown down to a volume of 100 μL with a gentle stream of N2 gas. This technique standardizes volumes across samples before GC-MS. An internal standard of 5 μL 0.03% toluene (16 ng) was added to each sample following blow-down to assist in quantifying scent emission rates. One μL aliquots of sample were then injected with an autosampler (model AI/AS 3000, ThermoFisher) into a Thermo-Scientific trace gas chromatograph using an SSL injector in splitless mode with constant septum purge (inlet temp was 240C). We used an EC-wax GC capillary column (TR-Wax MS, 30m length, I.D. of 0.25 mm, column film thickness was 0.25um) and a DSQ II mass spectrometer in electron-impact mode at Saint Mary's College (Notre Dame, IN, USA) for analysis. The GC-MS settings consisted of an initial 40 °C hold for 3 min, followed by an increase from 40° C to 260° C at intervals of 10° C per minute and a final hold at 260° C for 5 min. Ion source temp was 200C, and MS transfer line temp was 260C. Following GC-MS, biologically relevant compounds were identified by comparing mass spectra and calculated Kovats index to published spectra and Kovats indexes in Pherobase (www.pherobase.org), and NIST V.2 library spectra (release year 2005, as in Majetic et al., 2019).

2.6. Statistical analysis –

Emission rate of each identified compound (ng scent/g fresh floral mass/hour) was calculated using TIC for peak areas of each compound and the toluene standard: (peak area of compound * 16 ng toluene)/ peak area toluene (Majetic and Sinka, 2013). To adjust for background noise, we subtracted the volatiles present in the ambient air and vegetative sample controls taken at each collection. PERMANOVA with *post-hoc* pairwise comparisons were conducted on the data from the 10 individuals' males and females and, separately, the single tree intensive time sampling data, to assess differences in whole scent profiles between individuals, between sexes nested within individuals, and across multiple timepoints using PRIMER+ (Version 6.1.16, 2013; Clarke and Gorley, 2006). Non-metric multidimensional scaling (NMDS) analyses were also performed to visualize any differences. Individual samples with similar scents were expected to be grouped together in the NMDS space (following Majetic et al., 2019). For the male and female phases only, we also performed a similarity percentage analysis (SIMPER) to compare the average abundance of each compound and the relative percent contribution to the overall differences between sexual stages within individuals.

3. Results

We detected 49 volatile organic compounds (VOCs) in the scent emission profile of *Canella winterana,* of which 19 could be fully identified using chemical library information. Over all individual plants and inflorescences examined, five VOCs made up, on average, 91.8% of the total scent emission, with four identified as terpenes: *E*-β -ocimene, beta-pinene, *Z*- β -ocimene, and linalool. The fifth was identified as the benzenoid p-anisole (Fig. 2). The remaining 44 minor compounds contributed less than 10% to the total scent emission. The known minor compounds included 21 benzenoid/phenylpropanoids, 3 terpenes, 1 fatty acid derivative, and 1 nitrogen-carbon ring compound. Twenty-nine compounds could not be fully identified using chemical libraries and were therefore categorized as unknowns or unknowns with a chemical category designation (i.e., unknown benzenoid) if category-

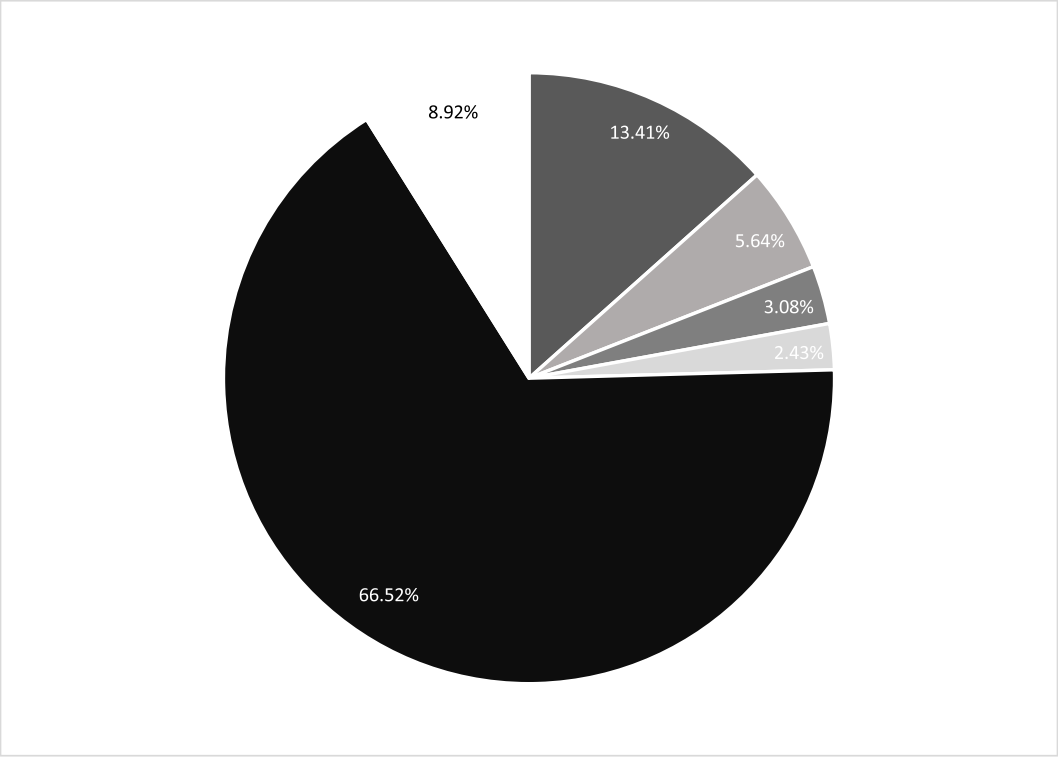

Fig. 2. Average percentage contribution of each compound to the scent profile of *Canella winterana* across all sexual phases. Scent of this species is dominated (an average of 91.8% of emission) by four common terpenes; *E*-β -ocimene, beta-pinene, *Z*-β-ocimene, and linalool, (pictured in shades of gray and listed in descending order) and p-anisole (black), a benzenoid. The remaining 44 minor compounds (white) are listed in S1.

stereotypical mass spectra were clearly present (for full list of compounds, mass spectra, and retention times, see Supplemental Table 1).

PERMANOVA results indicated a significant difference between individuals for the total data set (Pseudo-F = 1.9165, Permutation P = 0.035), and for sex nested within individual (Pseudo-F = 1.5976, Permutation P = 0.023). Six samples were found to have substantially larger emissions compared to all others, and so were removed for further analysis. After this removal, PERMANOVA results indicate no significant difference between individuals (Pseudo-F = 1.8262, Permutation P = 0.103), but the significant differences between sex nested within

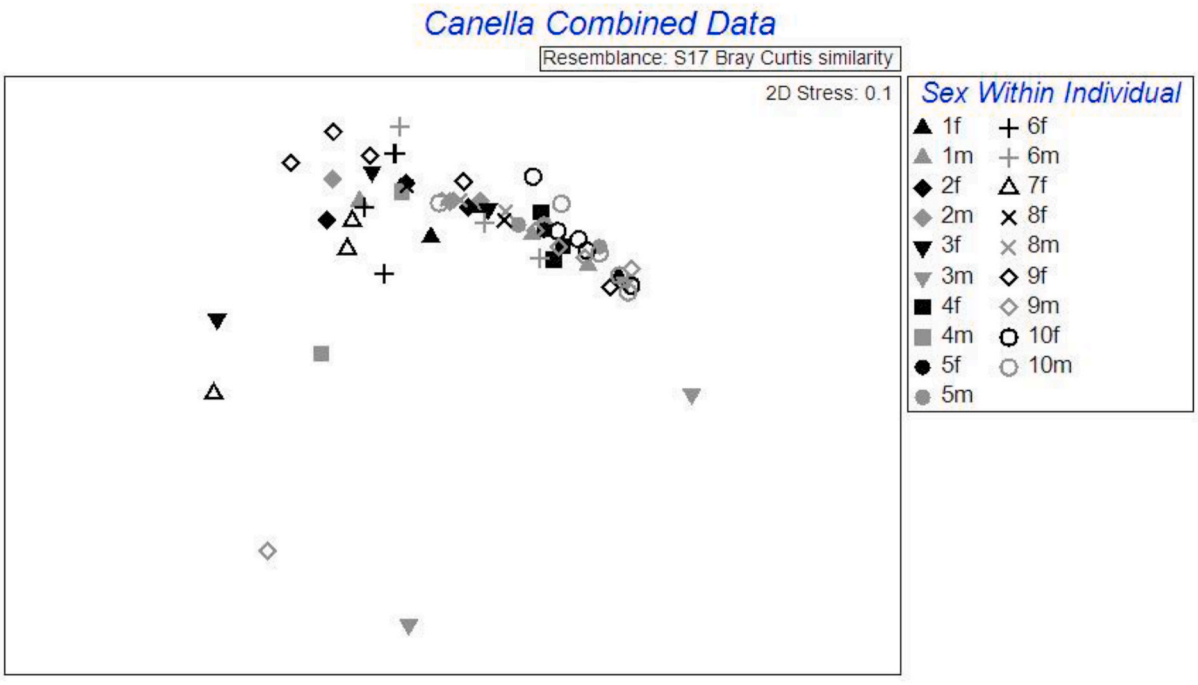

Fig. 3. Non-metric multidimensional scaling of floral scent similarity including all samples. Different colors represent different genders: black is female and gray is male. Different symbols represent different individuals (see key in figure).

individual persisted (Pseudo-$F = 1.6301$, Permutation $P = 0.04$).

A NMDS analysis containing all data points showed no apparent patterning and highlighted the six outliers described above (Figs. 3 and 2D Stress = 0.1). After removal of outliers, individuals 9 and 4 showed a clear pattern of separation between sex stages (Figs. 4 and 2D Stress = 0.06). It is important to note, however, we were only able to obtain a single male stage inflorescence sample from individual 4. Other individual plants showed less distinct separation between sexual stages.

We performed a similarity percentage analysis (SIMPER) to compare the average abundance of each compound during female and male sexual phases within individuals and the relative percent contribution of each compound to overall differences between phases. Two of the five major compounds emerged as contributing more than 50% to scent differences between sex phases within individuals (Table 1). P-anisole made contributions ranging from 40.73% to 81.87% to the differences between phases for individuals; emission of this compound was greater in females for three individuals (1, 4, and 8) and greater in males for 6 individuals (2, 3, 5, 6, 9, and 10; Table 1). *E*-β -ocimene was the next most important compound for determining differences between phases within individuals. This compound's percent contribution to differences between sexes ranged from 9.8% for some individuals to 20.25% for others (Table 1). *E*-β -ocimene emission was greater in females for 2 individuals (1 and 10) and greater in males for 7 individuals (2, 3, 4, 5, 6, 8, and 9; Table 1). For individual 3, 92.71% of the difference between its female and male phase flowers was explained by these two compounds (Table 1).

While p-anisole and *E*-β -ocimene explained over half of the variation between sex phases within individuals, there were other compounds with greater than 5% contribution to differences between sex phases within certain individuals. For Individual 5, linalool contributed 18.5% (male average abundance = 0.62, female average abundance = 0.15) and *Z*-β-ocimene contributed 6.32% (male average abundance = 0.62, female average abundance = 0.15) to its observed differences between sex phases. B-pinene contributed 12.3% (male average abundance = 0.42, female average abundance = 0.75) to sex differences in Individual 6 and unknown benzenoid 11 contributed an additional 10% (male average abundance = 0.13, female average abundance = 0.66). Individual 10 had 8.09% of its sex differences explained by Unknown N (male average abundance = 0, female average abundance = 1.25).

For our time series data, NMDS indicated a clear clustering and separation of samples taken at time-point zero, the female phase, from all other samples (Figs. 5 and 2D Stress = 0.05). Out of a total of 43 compounds found for the scent profile of this specific *Canella winterana* individual, five were found in greater abundance at time point zero than at any other time point: p-anisole, *E*-β -ocimene, *Z*-β-ocimene, methyl benzoate, and unknown N (Fig. 6). These compounds were present in all of the neuter and male phase in lesser amounts. Later time points showed less distinct clustering and more spread overall; the male phase (time point 6) showed some loose clustering but overlapped with many time points representing the neuter phase. PERMANOVA confirmed that there was a significant effect of time on scent profile similarity (Pseudo-$F = 2.83$; $P = 0.002$). Pairwise comparisons between time zero and all other time points (six comparisons) indicated, as visualized in NMDS, that the female sex phase time point was significantly different from all other points (all $t > 2.2$; all $P < 0.007$). Pairwise comparisons of all other time points (15 comparisons) were generally found to be non-significant (all $t<1.7$; $p>0.09$), with the exception of two time point comparisons: times two and three ($P = 0.041$) and times two and six ($P = 0.046$).

4. Discussion

In this study we characterized the scent of *Canella winterana*'s flowers in their female, neuter, and male phase in natural environments. The floral scent chemistry of *C. winterana* showed variability among individuals and between sexes within an individual; however, there was not a typical female or male phase scent profile overall and differences between sexes within individuals were more complex than predicted. These data suggest partial support of both the honest signaling and mimicry hypotheses.

The overall scent of *C. winterana* was dominated by 5 compounds, suggesting a clear cut "Canella" odor. Two of these five major compounds, P-anisole and E-β-ocimene, contributed to more than 50% to scent differences between sex phases within individuals, supporting our hypothesis of differences between the sex phases. However, we found no consistent sex-specific trends between individuals; in other words, there was no characteristic female or male scent for the species as a whole (Table 1). It can therefore be inferred that the nectar and pollen are not affecting the scent of the sexes, as has been previously found in other species (Dobson and Bergstrom, 2000; Raguso, 2004; Ashman, 2009), refuting the possibility of honest signaling of rewards. Instead, some

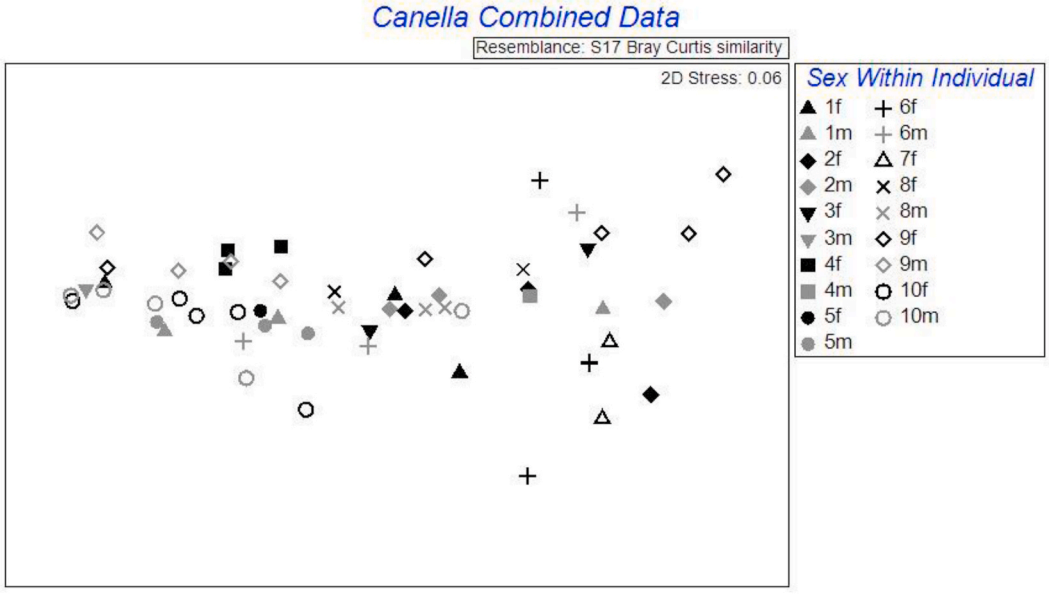

Fig. 4. Non-metric multidimensional scaling of floral scent similarity excluding 6 outliers. Different colors represent different genders: black is female and gray is male. Different symbols represent different individuals (see key in figure).

Table 1
SIMPER results. Note that there is no individual 7 due to a loss of samples of the male phases during chemical analysis.

Tree	P- anisole			*E*-β -ocimene		
	Female average abundance (ng scent/g fresh floral mass/hour)	Male average abundance (ng scent/g fresh floral mass/hour)	Contribution to difference (%)	Female average abundance (ng scent/g fresh floral mass/hour)	Male average abundance (ng scent/g fresh floral mass/hour)	Contribution to difference (%)
1	11.35	8.06	63.11	1. 97	2.30	11.93
2	2.11	2.81	62.09	0.88	0.92	20.25
3	3.19	29.97	81.87	0.65	4.18	10.84
4	11.69	1.85	80.06	0.79	0.74	3.82
5	8.98	9.74	40.73	3.21	3.94	18.50
6	1.33	5.45	43.22	0.56	1.16	12.71
8	4.35	4.18	57.69	0.75	1.20	13.53
9	6.53	16.32	80.95	0.69	0.93	5.54
10	14.73	16.76	62.12	3.80	3.76	9.80

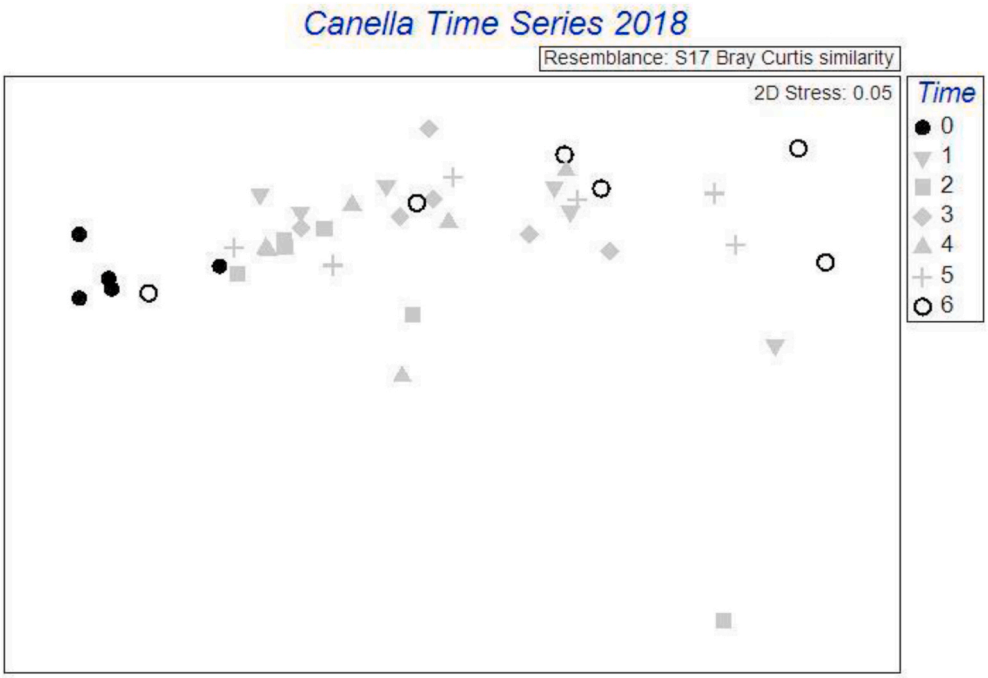

Fig. 5. NMDS for overall scent composition of *Canella winterana* at sequential scent collection time points. Time point zero (closed black dots) represents fully female sex phase of *Canella winterana*. Time point six (open black dots) represents fully male. Time points one through five (various shapes in gray) represent various times during the transitional neuter sex phase of *Canella winterana.*

plants had females with higher production of p-anisole or E-β-ocimene while the males of other individuals showed higher abundance of the same compound. If the honest signaling of floral rewards was occurring, we would expect a distinct female scent, driven by the presence of nectar and a distinct male scent caused by the presence of pollen. While we see a distinct female scent profile in our intensive time period sampling (Fig. 5), there is no difference between the neuter and male phase, further suggesting that there is not a pollen specific scent that is driving the difference between the female and male phase scent profiles within an individual. Future assessment of the volatiles of pollen and nectar for *C. winterana* will help to verify this inference.

One possible explanation for the profile overlap seen here may be that the sexes of different individuals emit the same compounds to minimize pollinator discrimination. This phenomenon is often termed the intersexual mimicry hypothesis (Ashman, 2009). Pollinator discrimination can negatively affect pollinator ability to cross-pollinate (Charlesworth, 1993). In many cases, this occurs when the pollinator favors the rewards and/or attractive traits of one sex over the other, causing the favored sex to be visited more frequently than the other and limiting effective pollen transfer. This can result in a decline in the reproductive success of the species. The potential for such sexual dimorphism in scent leading to pollinator discrimination has been demonstrated for flowers of *Fragaria virginiana*; females are deficient in a significant pollinator attractant volatile compared to hermaphrodites and pollinators respond behaviorally to these differences (Ashman et al., 2005). In our study we found differences in the floral scent between males and females. However, there was no compound that was consistently lacking between all the male or female individuals. This suggests that individuals of *C. winterana* may exhibit similar compound profiles (albeit not always in the same amounts) at different sexual phases as a consequence of selective pressure to ensure visits to both the female and male stage flowers, often termed the intersexual mimicry hypothesis (Ashman, 2009).

One key assumption of the intersexual mimicry hypothesis, however, is problematic for the current study. This hypothesis generally assumes that the sex engaged in mimicry does not produce any beneficial award; pollinators are therefore "accidently" cross-pollinating, then leaving quickly to find rewards (Baker, 1976). This assumption cannot be supported in *C. winterana* because both sexes provide a beneficial reward to pollinators: nectar in the female phase and pollen in the male phase. It is

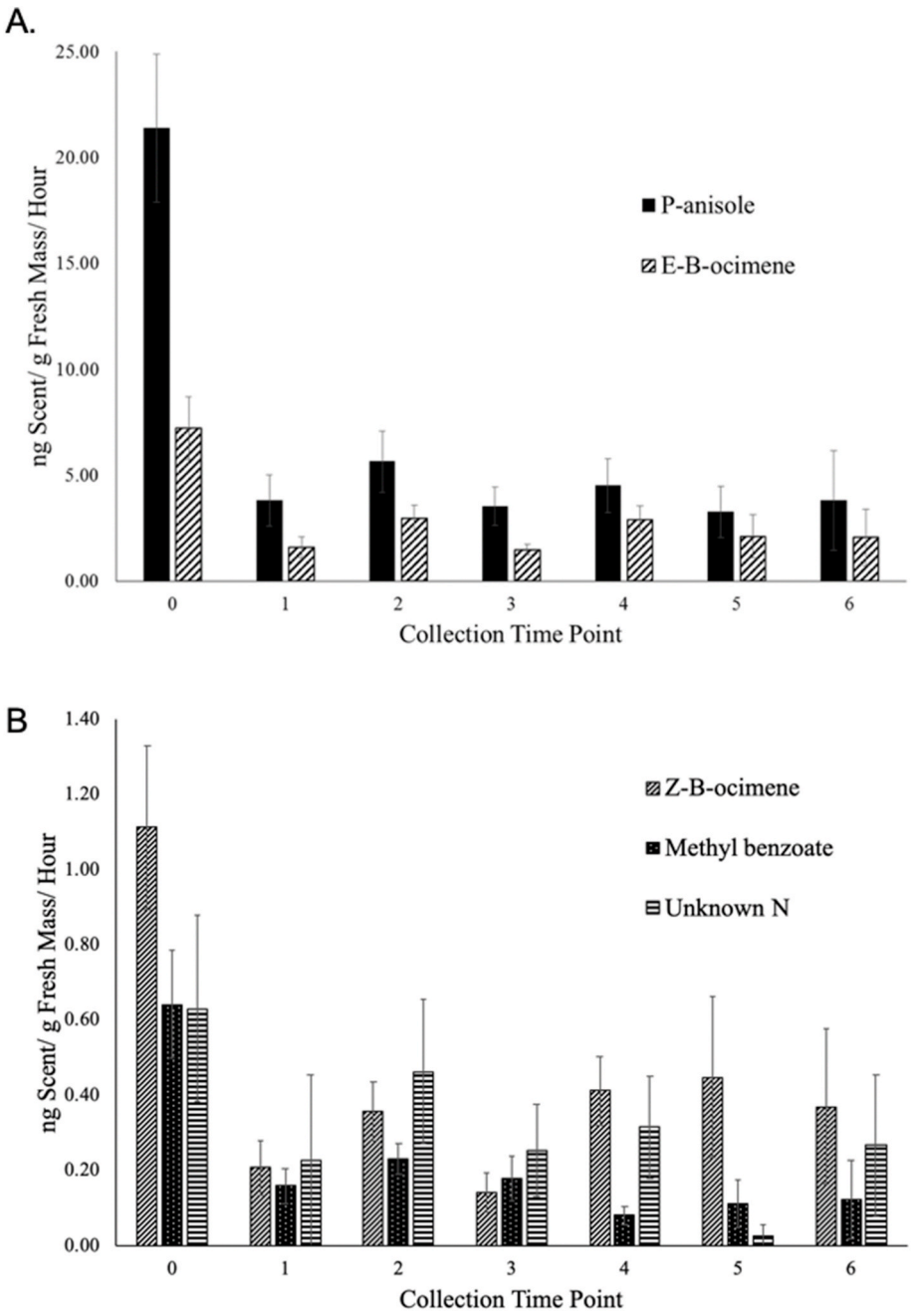

Fig. 6. Emission rates and standard errors of (A) p-anisole and *E*-β -ocimene and (B) *Z*-β-ocimene, methyl benzoate, and unknown N across time points for *C. winterana*. These five compounds were found by similarity percentage analysis to be the major contributors to scent differences over time. Time point zero represents fully female sex phase while time point six represents fully male sex phase.

thus likely that *C. winterana* is displaying elements of both honest signaling and intersexual mimicry. The similarity in scent between males and females across individuals would cause increased outcrossing, since pollinators may be unable to distinguish which phase they are visiting if relying on scent alone as a cue. This would provide explanation for the minor pollinator fauna overlap between the sexes. However, the mimicry does not serve to "trick" the pollinator since both female and males provide a reward, generating some discrimination once pollinators access other cues. The end result is therefore visitation to males that maximizes their pollen export and reward the pollinator with pollen, while visits to females can maximize pollen deposition and in turn provide a nectar reward for the pollinator. Different reward preferences by pollinators could then explain the larger and more obvious patterns of discrimination seen between sexes following arrival at the flower.

These results are the first to our knowledge to explore floral scent profiles between sexual phases of a synchronously dichogamous plant. We found that there was no distinct female or male scent across individuals, as has been found in sex morphs in dioecious systems (Azuma et al., 2001; Dötterl and Jürgens, 2005), suggesting partial support of the intersexual mimicry hypothesis. There were, however, differences between sexual phases within an individual – suggesting partial support for honest signaling of rewards and complicating the possibility of intersexual mimicry as the sole explanation of the patterns. More intensive studies of dichogamous systems from a scent perspective would further improve our understanding of how mating systems and scent production interact, and in turn how these effects influence pollinators in natural environments.

Author contributions

Hanna Makowski: Conceptualization, Methodology, Field work, Chemical and data analysis, Writing – led original draft preparation. **Cassie Majetic**: Conceptualization, Methodology, Field work, Supervision of Chemical and data analysis, Writing – reviewing and editing. **Patrick Garrett:** Conceptualization, Methodology, Field work, Writing – original draft preparation. **Sophia Johnson:** Methodology, Field work, Chemical and data analysis, Writing – original draft preparation, **Paige Schurr:** Methodology, Field work, Chemical and data analysis, Writing – original draft preparation. **Rich Moore:** Writing – reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2021.104270.

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