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Title: Floral scent of eastern skunk cabbage (*Symplocarpus foetidus*: Araceae)

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ABSTRACT:

Symplocarpus foetidus (L.) Salisb. (eastern skunk cabbage) occurs across a broad geographic range of northeastern North America, blooming in winter between December and March. The inflorescences are well-known for their thermogenic and thermoregulatory metabolic capabilities. The perceptual qualities of their fetid floral aroma have been described widely in the literature, but to date the floral volatile composition remained largely unknown. Here we present a detailed study of the floral scent produced by *S. foetidus* collected from intact female- and male-stage inflorescences and from dissected floral parts. Our results show a large range of biosynthetically diverse volatiles including nitrogen- and sulfur-containing compounds, monoterpenes, benzenoids, and aliphatic esters and alcohols. We document high inter-individual variation with some organ-specific volatile trends but no clear strong variation based on sexual stage. Multivariate data analysis revealed two distinct chemotypes from our study populations that are not defined by sexual stage or population origin. The chemotype differences may explain the bimodal perceptual descriptions in earlier work which vary between highly unpleasant/fetid and pleasant/apple-like. We discuss the results in ecological contexts including potential for floral mimicry, taking into account existing pollination studies for the species. We also discuss the results in evolutionary contexts, comparing our scent data to published scent data from the close sister species *Symplocarpus renifolius*. Future work should more closely examine the chemotype occurrence and frequency within these and other populations, and the impact these chemotypes may have on pollinator attraction and reproductive success.

KEY WORDS:

GC-MS; volatile organic compounds; chemotype variation; ocimene; dimethyl disulfide; acetoin; isoamyl isovalerate; sabinene; floral mimicry

1. INTRODUCTION

Eastern skunk cabbage, *Symplocarpus foetidus* (L.) Salisb. is an herbaceous plant native to northeastern North America. This species belongs to the predominantly tropical family Araceae, and it employs the floral thermogenic properties found in a number of genera across the family (Seymour, 2010) to shift its blooming time to mid-winter or very early spring (**Figure 1**). The floral thermogenic and thermoregulatory capacities are the most studied aspect of this odd, early-blooming plant (Ito, 1999; Ito et al., 2003; Ito et al., 2004; Ito and Ito, 2005; Knutson, 1979; Onda and Ito, 2005; Seymour and Blaylock, 1999; Seymour, 2004; Takahashi et al., 2007). The above-ground vegetation for this species dies back in late summer, leaving very little physical presence of the plant above ground, aside from the maturing infructescence of the spadix. As flowering begins in mid-winter, a leathery spathe emerges directly from the soil surface from the underground root system. Spathe color ranges from mottled deep purple-maroon to highly variegated patterns of maroon and yellow-green (**Figure 1**). The coloration of the spathe makes the emerging inflorescence quite difficult to discern visually from the surrounding leaf litter, at least for human observers (**Figure 1**). As flowers on the spadix become sexually mature, the spadix begins its thermoregulatory metabolic processes (Barriault et al., 2021; Knutson, 1979). The flowers are protogynous, with female-stage indicated by a spadix covered with single pistils (**Figure 1**), and male-stage indicated by a spadix covered by clumps of mature stamens shedding copious pollen (**Figure 1**). As flowers become sexually mature, they emit a foul floral odor similar to carrion, and odor emission persists throughout both female and male stages.

For centuries botanists have been fascinated, if perhaps a bit repulsed, by the floral and vegetative odors of *Symplocarpus foetidus*. In an early account *New England's Rarities Discovered* (1672), Josselyn describes the plant as being scented "as strong as a fox." Delpino named *S. foetidus* as an exemplar species for the floral odor type of "mephitic or viverine" in the class of "nauseous odors" (Delpino, 1874). Other

authors have described its floral scent as combining “suspicion of a skunk, putrid meat, and garlic” (Blanchan, 1900), imitating “decaying flesh” and reminiscent of “the smell of a mustard plaster and raw onions” (Matthews, 1902). Although most descriptions of the floral scent are in the genre of malodorous or fetid, authors frequently note the variability of the odor between individual plants. Some descriptions are more pleasant, describing it as “not especially repulsive” (Williams, 1919) or likening some inflorescences to the scent of apples (Kevan, 1989). In most accounts, the foul floral aroma is suspected of being a key attractant for carrion-seeking insects active in winter and very early spring that are fooled into acting as pollinators for this plant (Barriault et al., 2021; Cady, 1936; Gibson, 1901; Williams, 1919).

The pollination ecology of the plant, especially the potential for floral mimicry of carrion, is still a bit unclear. Observations from across the 19th and 20th century report flies and bees as floral visitors (Doolittle 1900; Gibson, 1901; Kevan, 1989; Knutson, 1979; Lovell, 1920; Shufeldt, 1918; Trelease, 1879). Floral characteristics (purple-mottled coloration, fetid floral scent, terrestrial presentation) led to expectations of carrion mimicry and pollination by carrion-seeking insects – thus the observation of honey bees visiting and collecting pollen were somewhat surprising. However, the bees observed were non-native (and not adapted to local North American flora), and the copious pollen presented by *S. foetidus* represents one of the only pollen resources at the time of year when blooming occurs (Gibson, 1901; Kevan, 1989). The most recent work on pollination for this obligatory cross-pollinated species (Barriault et al., 2021) does not report bees, but does report diverse fly taxa, a number of which were observed to be carrying pollen. These authors (Barriault et al., 2021) suggest that the pollination strategy may be more generalized than narrowly-defined “carrion mimicry,” involving a large diversity of Coleoptera and Diptera (including Chironomidae, Sphaeroceridae, Mycetophilidae, Phoridae, Chloropidae). Furthermore, it remains unclear whether this pollination system is deceptive – as suggested by the characteristic unpleasant floral scent – and/or a breeding site mutualism as emerging insects from the infructescences have been observed (Barriault et al., 2021; Grimaldi and Jaenike, 1983).

In this paper, we present a detailed floral scent analysis for *Symplocarpus foetidus*, across floral developmental stages and dissected inflorescence parts. This work follows the earlier work of Elizabeth Kozen, whose Master's thesis (2013) showed dimethyl disulfide to be a major floral scent compound for the species, but could not confirm additional chemical composition for the floral scent. Better understanding the composition of the floral scent for this species will allow us to compare its chemical similarity to carrion and other substrates to examine potential floral mimicry. We also aim to document potential variation of floral scent between individuals or between inflorescence parts; and finally, to compare its floral volatile blend to the published floral scent composition of close sister species *Symplocarpus renifolius* (Oguri et al., 2019) to explore aspects of shared evolutionary history.

2. RESULTS & DISCUSSION

2.1 Overall chemical composition

The floral scent of *Symplocarpus foetidus* is comprised of a wide range of compounds from a diverse array of underlying biosynthetic pathways, with high variation among individuals (e.g within populations) as has been shown in the deceptive pollination strategies of European *Arum* species (Chartier et al., 2016; Gferer et al., 2021; Szenteczki et al., 2021). The combined floral scent analysis for whole inflorescences and all dissected parts identified 106 total compounds, a majority of which were identified with a high degree of certainty (**Supplemental Table 1**). The major biosynthetic classes identified include nitrogenous compounds, sulfur-containing compounds, oxygenated short-chain aliphatics, monoterpenoids, and benzenoid compounds. Major volatile compounds, found in more than 80% of individuals (at least 14 out of the 17 individuals sampled) and typically representing >5% of total peak area per sample include dimethyl disulfide, 3-methyl-1-butanol, acetoin, isoamyl isovalerate, sabinene, cis-beta-ocimene, trans-beta-ocimene, and two unknowns identified by their calculated retention index (RI): 877 and 973 (**Figure 2; Supplemental Table 1**). The two unknowns listed here have very consistent mass

spectra (**Supplemental Figure 1**) that did not yield a reliable library match. One of these unknown compounds (with retention index 973 and likely mass ion of 115) might be tentatively identified as 2-methyl butyraldoxime-O-methyl ether (Kaiser, 1995; S. Dötterl pers comm) but as a precaution until its certain identification, it will be referenced as RI 973 throughout the manuscript.

The elucidated chemical composition matches well to the existing varied scent descriptions in the literature. Dimethyl disulfide (DMDS) is a major compound found in the floral scent of all individuals sampled, and would account for the floral scent descriptions in existing literature characterizing it as fetid and similar to carrion, cabbage, or garlic. Other compounds that likely contribute to the “fetid” floral scent descriptions include dimethyl trisulfide (DMTS; described perceptually as similar to fish and cabbage), trimethylamine (perceived as rotting fish), and methanethiol (described as similar to sulfur, gasoline, or garlic). While these particular compounds all suggest floral mimicry of protein decomposition (mimicry of carrion or feces; Stavert et al., 2014), only DMDS is found in large proportions consistently across samples. A number of other compounds such as sabinene and isoamyl isovalerate are found in high proportions across samples and are not associated with odors of carrion or feces. The monoterpene sabinene is perceptually described as similar to pepper, turpentine, or wood; the aliphatic ester isoamyl isovalerate is described as fruity, sweet, or similar to green apple. It is of course not possible to determine the scent characteristics of the two major unknown compounds (RI 877 and RI 973) without further determination of their structure and identity.

Human perceptions of individual compounds or the overall odor blends from these inflorescences are not particularly ecologically-informative, and we must be cautious in using human perceptions to predict insect perceptions or mimetic overlap with a substrate. However, the varied range of human depictions of the floral scent for this species is informative and consistent with the compositional variation we documented between individuals (**Figure 2**). Furthermore, the somewhat bimodal descriptions in the literature, ranging from similar to a skunk or putrid meat, causing one to “flea the area” (Blanchan, 1900)

to “not particularly repulsive” (Williams, 1919) or even similar to “apple” (Kevan, 1989) may reflect the type of compositional variation we describe between chemotypes 1 and 2, detailed below.

2.2 Whole-inflorescence scent variation and chemotypes

The proportions of major compounds and biosynthetic classes for whole inflorescence samples are shown in **Figure 2**, illustrating the high level of variation between individuals although this variation appears to be due more to proportional composition than the presence/absence of compounds. Initially we grouped samples by sexual stage (female vs male stage inflorescences) to determine whether floral scent varied ontogenetically during anthesis. PERMANOVA revealed a significant difference (pseudo-F value $_{1,12} = 3.65$; $p = 0.029$) between female and male inflorescence scent, with the groupings overlapping somewhat in the 2-D NMDS representation of the dataset (**Figure 3a**). Hierarchical cluster analysis revealed two discrete clusters within the dataset that were not defined by sexual stage, but that better explained the overall variation in the data. These two clusters are hereafter referred to as chemotype 1 and chemotype 2. PERMANOVA run based on these chemotype groupings of samples revealed a more strongly significant difference between groups (pseudo-F value $_{1,12} = 9.17$; $p = 0.001$) coupled with a more obvious visual separation of the sample data points in the 2-D NMDS representation (**Figure 3b**). Patterns of relative percentage composition for female vs. male stage inflorescences and chemotype 1 vs chemotype 2 inflorescences can be seen in **Figure 2**. Furthermore, although hierarchical cluster analysis sorted individual 7F and 16M (Figure 2) into chemotype 1 and these samples were scored as such in Figure 3b, they both have the highest proportion of ocimene compounds for samples scored as chemotype 1 and their positions highlighted in Figure 3b indicate that they could be intermediate between chemotypes 1 and 2. More data are needed to discuss further this hypothesis.

Compounds that contribute to the highest percentage of overall variance between chemotypes 1 and 2 are listed in descending order in **Figure 4** and include dimethyl disulfide, cis- and trans-beta-

ocimene, sabinene, and the two unknowns RI 973 and RI 877. While the listed percentages represent the contribution of each compound to the overall variance between chemotypes, the percentages shown in the pie graphs reflect the average peak area of each compound for whole inflorescence samples in chemotype 1 and chemotype 2. Of particular interest are the high average percentage peak areas of cis- and trans-beta-ocimene (and allo-ocimene) for samples in chemotype 2 relative to chemotype 1. The constant-sum constraint inherent in compositional data such as these might explain the lower average percentages of dimethyl disulfide and unknowns 973 and 877 in chemotype 2 relative to chemotype 1. However, for the same reason, it is interesting that the average percentages of sabinene and isoamyl isovalerate (and other monoterpenes and aliphatic esters) do not drop in a similar way as might be expected if their absolute amounts remained constant. In other words, chemotype 2 appears to not only be defined by the addition of high amounts of ocimenes, but also by potentially higher amounts of other monoterpenes and aliphatic esters.

2.3 Dissected floral parts scent variation and chemotypes

While examining the scent data for dissected parts we noted a clear “signal strength” difference between floral organ types. While the SPME methodology used here does not allow precise quantification, we feel that it is worth noting the large difference in overall sample quantity (“strength”) by comparing the total peak areas of samples from different sample types. The average total peak area for dissected spadix samples was not significantly different from the average total peak area for whole inflorescence samples. Meanwhile, the average total peak area varied significantly among whole and dissected inflorescence samples (ANOVA $df = 2, 23$; $F = 11.394$; $p < 0.001$). The average total peak area for dissected spathe samples was significantly lower than both spadix and whole inflorescence samples (post-hoc Tukey-Kramer test; $Q = 3.542$; $p < 0.05$). Pollen samples ($n = 2$) were comparable in total peak area to spathe samples even if not statistically tested (**Figure 5**). This suggests that most of the floral scent emitted from whole inflorescences is generated by the spadix. In Araceae, it is common to have a majority of

inflorescence odor generated by part or all of the spadix (Gonçalves-Souza et al., 2017; Hadacek and Weber, 2002; Marotz-Clausen et al., 2024), with the spathe generating no detectable odor or a weak compositionally different odor (Buzgo, 1998; Hoe and Wong, 2016; Kumano and Yamaoka, 2006). However, *Lysichiton americanus*, the closest North American relative of *S. foetidus* (Lee et al., 2019) is a notable exception, with odor generated by the spathe as a major attractant for the rove beetle pollinator *Pelecomalium testaceum* (Brodie et al., 2018; Pellmyr and Patt, 1986).

Importantly, we note that patterns of scent composition variation between dissected individuals suggests that these individuals might fall within the two chemotype groupings described for the whole inflorescence samples. As shown in **Figure 6**, dissected individuals 2 and 4 match patterns seen in chemotype 2, including high percentage of ocimenes and relatively low dimethyl disulfide and unknowns RI 973 and RI 877. Meanwhile, dissected individuals 1, 3, 5, and 6 more closely match volatile patterns seen in chemotype 1. This same subset of dissected individuals (1, 3, 5, 6) also show high percentages of ethanol, 3-methyl-1-butanol, acetoin, and 2,3-butanediol relative to individuals 2 and 4; however, these four “fermentation-associated” compounds are not noticeably higher in chemotype 1 whole inflorescence samples. The dissected individuals with high relative percentages of ethanol, 3-methyl-1-butanol, acetoin, and 2,3-butanediol represent both female and male stage inflorescences (**Figure 6**), and thus a range of floral ages. While it is possible that these compounds are due to tissue damage (wounding) from the dissection process, dissected individuals were all healthy inflorescences that were freshly collected from the field site an hour or so before dissection and scent collection. Thus, while these volatiles are frequently associated with fermentation processes, they cannot be attributed to decay or fermentation of the inflorescence tissue any more than would similarly be possible from the inflorescences sampled whole.

Since overall scent emission (evidenced by total peak area comparisons) suggests that spadix samples are similar to whole inflorescence samples, we re-ran PERMANOVA analysis for whole inflorescence plus spadix samples to test how well the chemotype groupings explained overall variance

within the expanded dataset. When spadix samples were included in the analysis, we found a stronger significant difference between chemotypes (pseudo-F value $_{1,18} = 11.52$; $p = 0.0001$), with individuals 2 and 4 scored as chemotype 2 and individuals 1, 3, 5, and 6 scored as chemotype 1. In the combined dataset (whole inflorescence + spadices), chemotype 1 is represented by 14 samples composed of 7 female-stage and 7 male-stage inflorescences, while chemotype 2 is represented by 6 samples, 1 of which is female-stage and 5 male-stage inflorescences. When PERMANOVA was re-run for the expanded dataset (whole inflorescence + spadix samples) using sexual stage as a factor, the variation between sexual stages was not significant (pseudo-F value $_{1,18} = 2.32$; $p = 0.072$).

Similar to whole-inflorescence samples, we noted high variation between dissected individuals, with different dissected parts within an individual showing more similarity than the same dissected parts across individuals (**Figure 6**). However, there were some organ-specific trends that we outline here. In all cases, the proportion of DMDS emitted from the spadix was much higher than for the spathe, and DMTS was only identified from the spadix. In fact, most of the nitrogenous and sulfur-containing volatiles were either only identified in spadix samples, or in much higher proportions in spadix samples than in spathe samples. Similarly, most aliphatic esters and benzenoid compounds were identified only in spadix (and pollen) samples. In contrast, short-chain aliphatic alcohols and ketones typically associated with fermentation (especially acetoin) were in higher proportions in the spathe than they were in spadix samples of the same inflorescence for individuals 1, 3, 5, and 6 (chemotype 1), whereas the proportion of these same volatiles were small in both spadix and spathe samples for individuals 2 and 4 (chemotype 2). In the dissected chemotype 2 individuals (with high ocimene and overall monoterpene proportions), the ocimene and other monoterpene proportions were consistently high in both spathe and spadix (and pollen for individual 4).

Although pollen was only sampled from two individuals and representative of a very small tissue mass relative to the spadix or spathe samples, there are a few potential differences between pollen and

other parts that may indicate pollen-specific odors (Dobson and Bergström, 2000). Several nitrogenous compounds including trimethylamine, isobutylaloxime, and 1-nitro-2-phenylethane represent higher proportions of pollen scent than they do other floral parts. Most other compound identities and ratios for the pollen are quite close to the spadix compositions for the same individual inflorescence which is no doubt in part because the spadices retained a good amount of pollen on the intact anthers, and the close positioning of the anthers/pollen to the rest of the spadix tissues may allow the pollenkit to adsorb any spadix-generated odors (Dobson and Bergström, 2000). Our volatile collection from pollen didn't result in identification of any VOCs not identified from other floral parts or whole inflorescences, but additional sampling of larger quantities of pollen is needed for better comparison.

2.4 Implications of underlying biosynthesis and evolutionary history

A large subset of the esters and nitrogenous compounds from our data point to a discrete set of underlying biosynthetic pathways involving the essential amino acids valine, isoleucine, leucine, phenylalanine, and methionine. Bizzio et al. (2022) nicely summarize groupings of branched-chain volatiles that are most likely synthesized through catabolism of branched-chain amino acids valine, isoleucine, and leucine, or from excess branched-chain keto acids in the process of branched-chain amino acid biosynthesis. This would account for at least 18 different volatile compounds: 2-methylpropyl, 3-methylbutyl, and 2-methylbut(en)yl alcohols, and alkyl or acyl esters (e.g. 3-methylbutyl ester of 2-methylbutanoic acid, isoamyl isovalerate, and isobutyl tiglate) and some corresponding nitrogenous compounds (e.g. isobutylaloxime and 2-methylbutanenitrile) found in *S. foetidus*. Similar biosynthetic steps involving phenylalanine would yield compounds identified from our dataset such as ethyl phenylacetate, and benzyl cyanide (Maoz et al., 2022). Furthermore, the pathways catabolizing methionine into methanethiol, dimethyl disulfide, dimethyl trisulfide, and thioethers such as 2-(methylthio) ethanol have been documented in plants (Maoz et al., 2022), and it is possible that similar pathways produce these compounds in *S. foetidus*. The presence of amino acid derived volatiles in flowers and fruits has been

suggested as important advertisements for animals (pollinators or seed dispersers) seeking these essential amino acids for their diets (Maoz et al., 2022). These volatiles may be particularly important for deception of saprophagous insects that feed as either adults or larvae on decomposing substrates high in protein (carrion or omnivore/carnivore feces).

Nitrogenous and sulfur-containing compounds are unusual in floral scent blends (Dötterl and Gershenzon, 2023; Knudsen et al., 2006), and specific compounds such as DMDS, DMTS, trimethylamine, putrescine (1,4-diaminobutane), and pyrazines are commonly associated with vertebrate decomposition (Kasper et al., 2012; Paczkowski and Schütz, 2011), and with flowers mimicking carrion (Jürgens et al., 2013). However, a number of the branched-chain nitriles, aldoximes, and nitroalkanes identified in our dataset are not commonly reported volatiles from vertebrate decomposition – although the associated branched-chain alcohols, aldehydes, and esters putatively derived from valine, leucine, and isoleucine are more commonly reported (Kasper et al., 2012; Paczkowski and Schütz, 2011). Alternatively, these branched-chain aldoximes, nitriles, and nitro alkanes have been documented in a number of night-blooming hawkmoth-pollinated flowers including multiple species of *Oenothera* and *Nicotiana* (Raguso et al., 2003; Raguso et al., 2007). Such pollinators were not registered among the visiting entomofauna in a skunk cabbage Canadian population (Barriault et al. 2021) but their attractive role can't be ruled out and needs further study.

Another interesting detail is the occurrence of volatiles such as ethanol, 2,3-butanediol, and acetoin. The biosynthesis of these volatiles is well-studied in fermentation pathways for yeasts and some bacteria, but their biosynthesis is less understood in plants (but see Forlani et al., 1999). However, these volatiles sometimes co-occur in floral scent blends from species mimicking fermenting substrates in order to attract fermentation-seeking insects for pollination (e.g. *Asimina triloba* or *Calycanthus occidentalis*; Goodrich et al., 2023 and Gottsberger et al., 2021, respectively). Their role in the floral scent of *Symplocarpus foetidus* is less clear, since they represent a small proportion of most whole-inflorescence

floral scent and are in combination with DMDS and other volatiles associated with protein-rich decomposing substrates. If they play a role in attraction of saprophagous insects as pollinators (e.g. floral mimicry of decomposing substrates), they may help create the illusion of carrion under particular conditions or a particular stage of decay – as these compounds sometimes co-occur in carrion scent blends along with more “typical” sulfur or nitrogenous compounds (Kasper et al., 2012; Stavert et al., 2014). Alternatively, addition of these volatiles typically associated with fermenting substrates (e.g. Goodrich et al., 2023) might blur the line between fermenting substrates and carrion, attracting a wider range of saprophagous insects.

A final point of interest regarding underlying biosynthesis for floral scent of *Symplocarpus foetidus* is the “cassette” of ocimene compounds which largely define chemotype 2 relative to chemotype 1. Cis-, trans-, and allo-ocimene collectively contribute 30% of the overall variation between the chemotypes, comprising between 19% and 54% of total peak area in whole inflorescence individual samples for chemotype 2, whereas in chemotype 1 they are either entirely absent or present at lower proportions. Other monoterpenes such as sabinene, beta-myrcene, limonene, and 1,8-cineole are all relatively consistent across samples and do not co-vary with the ocimenes. Furthermore, most of the monoterpenes identified in *S. foetidus* were also identified in *Symplocarpus renifolius* (Oguri et al., 2019) likely reflecting shared evolutionary history – but none of the ocimene compounds were reported in *S. renifolius*, suggesting that these compounds may be a novel evolutionarily addition to the floral blend. The presence of ocimenes might account for differential attraction of bees (Füssel et al., 2007; Gong et al., 2014; Peng et al., 2017), and prior pollinator observations for some populations of skunk cabbage have indicated high bee visitation and collection of pollen (e.g. Kevan, 1989).

Other interesting comparisons can be made between the floral scent composition of *Symplocarpus foetidus* and *S. renifolius*, although it should be noted that different collection methods were used (thermal desorption with Tenax adsorbent vs. SPME with DVB/PDMS coated fibers) which may

contribute artificial differences when comparing datasets. Both species overlap in a number of high-proportion compounds, including DMDS, isoamyl isovalerate, sabinene, 1,8-cineole, and limonene. Both species appear to lack sesquiterpene volatiles, although Oguri et al. (2019) report up to 21 “other terpenoid” compounds representing small proportions of samples. For nitrogenous compounds, Oguri et al. name only one pyrazine compound (we identified no pyrazine compounds) and none of the nitrile, aldoxime, or nitroalkane compounds we have identified; however, they do report up to 12 “other N-containing compounds” representing small proportions of samples. Three sulfur-containing volatiles were identified in both species: DMDS, DMTS, and methanethiol. While DMDS represents a high proportion of floral scent for both species, it represents approximately 10% of the relative percentage floral scent for *S. renifolius*, with isoamyl isovalerate having a higher relative percentage (approx. 29%). In *S. foetidus*, DMDS represents approximately 30% of the relative peak area for whole-inflorescence samples, followed by sabinene (17.2% average) then isoamyl isovalerate (5.2% average). Another notable chemical difference is that *S. renifolius* has a number of aliphatic aldehydes all lacking in *S. foetidus* while the floral scent of *S. foetidus* has a relatively high number of aliphatic esters, most all of which are lacking in *S. renifolius* (except isoamy isovalerate – a key compound in both species).

3. CONCLUDING REMARKS:

The floral scent of *S. foetidus* is chemically diverse and variable. The high variability between individuals within a population may contribute to a deceptive pollination strategy, making it more challenging for duped pollinators to learn to avoid disappointing mimics. Despite this inter-individual variation, the most striking result was the occurrence of two main chemotypes representing potentially different scent types. Chemotype 1 is dominated by DMDS, sabinene, isoamyl isovalerate, and the unknowns RI 877 and RI 973. Chemotype 2 shares these compounds with the addition of a high proportion of cis- and trans-beta-ocimene, and correspondingly lower proportions of DMDS and unknowns RI 877

and RI 973. The proportions of sabinene, isoamyl isovalerate, and several other monoterpenes and aliphatic esters remain relatively constant between the two chemotypes, suggesting potentially that they represent early intermediates of a biosynthetic pathway that is modified further downstream, or simply that their independent biosynthesis is not affected by chemotype differences. It will be interesting in future work to sample more broadly both within the current study populations and across geographically distant populations to see whether these chemotypes are consistent. It will also be interesting to test for differences in pollinator attraction between the two chemotypes.

The pollination strategy for *S. foetidus* remains something of a mystery. None of the data reported here rules out the potential for carrion mimicry as has been proposed in previous works. However, the range of volatiles reported, along with the range of pollen-carrying visitors identified by Barriault et al. (2021) may indicate mimicry of a broader range of decomposing substrates, and/or a true brood-site mutualism. Finally, the high proportion of ocimenes only present in some individuals (chemotype 2) poses interesting questions about the underlying biosynthetic control of these compounds relative to other monoterpenes emitted more consistently across all individuals, and what impact this might have on pollinator attraction. Similarly, the distinctive set of amino acid derived alcohols, esters, and nitrogenous compounds point to interesting underlying biosynthesis – yet their functional significance, if any, is unclear.

4. EXPERIMENTAL

General Experimental Procedures. Floral volatile collections were done using static headspace methods and solid-phase microextraction fibers (SPME field collection, Supelco) coated with DVB/PDMS. Samples were analyzed by GC-MS.

Study sites and floral scent collections. We studied two populations of *Symplocarpus foetidus*: one large population of over 100 plants at Houston Park (“HP”; Wallingford Twp., Delaware County, PA,

39.875491, -75.363881), and the other a smaller population of approximately 20 plants at Taylor Arboretum ("TA"; Wallingford Twp., Delaware County, PA; 39.8719845, -75.3709336). Populations at both field sites were on river floodplains of Ridley Creek. Field work and scent collection was carried out in January and February 2022 and 2023. Since the spathes are challenging to visually locate in the surrounding leaf litter in the absence of snow (snow was only briefly present during this study, in February 2022), inflorescences were marked with flagging tape tied to a stick or bamboo skewer stuck into the ground close to the base of the inflorescence. We only sampled floral scent from plants with a single inflorescence, although a small number of plants at both field sites had two inflorescences per plant. Eight intact inflorescences were sampled at field sites (TA $n = 2$; HP $n = 6$); of these, three inflorescences at Houston Park were sampled twice, 4 days apart (all female at first sampling, two transitioned to male phase by second sampling, one remained in female stage). All sampled inflorescences were assessed in the field to have floral odor detectable to the human nose. This seemed the best available method to rule out inflorescences which were still immature or inflorescences which were past the viable male phase. Inflorescences were scored as female if the spadix was uniformly covered in individual pistils. Inflorescences were scored as male when most or all individual flowers on the spadix were presenting mature (pollen bearing) stamens. Inflorescences which were clearly intermediate (half female-stage, half male-stage flowers) were avoided for sampling.

Floral scent was collected from intact inflorescences at the field sites, and from cut inflorescences brought into the lab. For scent collection from intact inflorescences at the field site, a 30 cm bamboo skewer (GoodCook everyday 12" bamboo skewers) was bent in half, remaining connected at the break point, to form an inverted "V". A 6 cm X 6 cm polyacetate oven bag (Toppits®, Germany) was then pulled over the "V" such that the skewer acted something like a tent frame to hold the bag open. The ends of the inverted "V" were then stuck into the soil on either side of a sexually mature inflorescence with the open oven bag covering the entire inflorescence structure down to ground level. Care was taken not to

injure the spathe tissue while covering the inflorescence, to avoid any wounding volatiles. Site/environmental controls were collected during each round of field scent collection by creating a similar bag/skewer “tent” over leaf litter and soil lacking an inflorescence.

For in-lab collection of inflorescence scent and scent from dissected floral parts, stems of healthy, sexually mature inflorescences were cut several centimeters below the base of the spathe (usually also several centimeters below the soil line). The cut end of the stem was immediately placed in a cup with water and brought to the lab for sampling. A total of 9 inflorescences were cut and brought to the lab. In the lab, 6 cm X 6 cm polyacetate oven bags were used for scent collection of cut whole inflorescences (n = 3) and for dissected parts (n = 6 inflorescences for dissection; 2 female and 4 male). For whole inflorescences, a bag was placed over the entire structure and secured at the stem just below the spathe. For dissected parts, a sterile razor was used to carefully slice the spiral base of the spathe away from the stem. The removed spathe was placed into an oven bag, and the remaining spadix with a short segment of stem was similarly secured within an oven bag. Two of the male-stage dissected individuals had copious pollen being shed from anthers. Prior to securing the spadix into an oven bag, the pollen was carefully shaken onto a flat piece of paper and transferred into a clean 7mL glass vial with Teflon screw cap (Supelco, Sigma-Aldrich, 595 N Harrison Rd, Bellefonte, PA 16823).

Once oven bags were secured around the sample at the field site or in the lab, the scent inside the bag was allowed to equilibrate for 15 minutes. Then a Supelco SPME portable field sampler with 65 μm DVB/PDMS coating (Supelco, Sigma-Aldrich, 595 N Harrison Rd, Bellefonte, PA 16823) was inserted into the bag for an additional 15 minutes for sample collection. For the pollen samples collected into clean glass vials, the screw caps were removed and replaced with a small piece of oven bag. Scent was allowed to equilibrate for 15 minutes, then the SPME fiber was inserted through the bag into the vial airspace for an additional 15 minutes.

All samples were analyzed on a Shimadzu Nexis GC-2030 gas chromatograph with a Supelcowax 10 fused silica 30 m capillary column, 0.2 mm diam, 0.2 μm film thickness (Supelco, Sigma-Aldrich, 595 N Harrison Rd, Bellefonte, PA 16823), and Shimadzu QP-2020 quadrupole mass spectrometer (electronic ionization, 70eV) detector. Injection temperature was 260°C with an initial oven temperature of 60°C and a two-minute hold, followed by a ramp of 4°C/minute to a final temperature of 200°C. We used splitless injection with a solvent delay of 0.5 min (He carrier gas) at a column flow rate of 0.66 mL/min. Mass spectrum ion source and interface temperature was 250°C, with m/z range 35.00 to 500.00. Chromatogram peaks were manually integrated using Shimadzu GC-MS LabSolutions software.

Mass spectra for sample peaks were identified using the reference libraries Adams, NIST20, Perkin FF2013 and Wiley Registry™ 8th Edition, and standardized retention indices were calculated using an alkane ladder (C6-C20 Millipore Sigma, Darmstadt, Germany). Compound identities were confirmed with known standards whenever possible, using the same equipment and method. When known standards were not available, tentative compound identifications were assigned with high library spectral matches ($\geq 90\%$) and calculated retention indices matching published retention indices for the compound. All other peaks were classified as “unknowns” and mass spectra were reported for each unknown compound.

Statistical analyses were performed with RStudio version 4.3.2 (R Core Team 2023). The Non-metric multidimensional scaling (NMDS) to visualize the chemical similarities between samples were done with the metaMDS function on the matrix of Bray-Curtis dissimilarity index calculated with the vegdist function (package vegan) on the compound proportions. The hierarchical cluster analysis to find chemotype groupings was visualize with a dendrogram with functions hclust (library vegan) and dendro.gp (library RVAideMemoire) using the same dissimilarity matrix as for the NMDS. Chemical group differences were tested with permutational multivariate analysis of variance using distance matrices with the function adonis2 (package pairwiseAdonis) on the Bray-Curtis dissimilarity values. A Simper (Similarity Percentage) analysis between the two chemotypes was performed on compounds relative proportions using the Bray-

Curtis dissimilarity measure (multiplied with 100) with Past 4.14 statistical software (Hammer et al., 2001). This simple analysis method allowed us to assess which volatile compounds are primarily responsible for an observed difference between groups of samples. An ANOVA was run on total peak area values (log transformed to normalize), to compare VOCs emissions between whole inflorescence (n = 14 samples), spadix (n = 6), and spathe (n = 6). Tukey-Kramer post hoc analysis was used to test for significant differences between groups (Past 4.14).

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6. FIGURES and legends, TABLES, and REFERENCES:

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Figure 1. Mature inflorescences of *Symplocarpus foetidus*. The range of typical spathe coloration and pattern is illustrated in photos a thru c. Mature inflorescences emit enough heat to melt through snow (d). Images e and f show male spadix with an unidentified Drosophilidae, and a female spadix with an unidentified Heleomyzidae, respectively. Inflorescences are visually cryptic in leaf litter (g) even when the population is in full bloom (h). Scale bars = 1 cm; Red arrows indicate position of inflorescences.



Figure 2. Relative percentage scent composition for whole inflorescence samples. Samples are separated by sexual stage (female vs. male) and by chemotype. “Chemotype 1” shown with white background; “Chemotype 2” shown with grey background. Individual plants are identified by number below each bar, with individuals 7, 8, and 9 sampled at two different time points, 4 days apart.

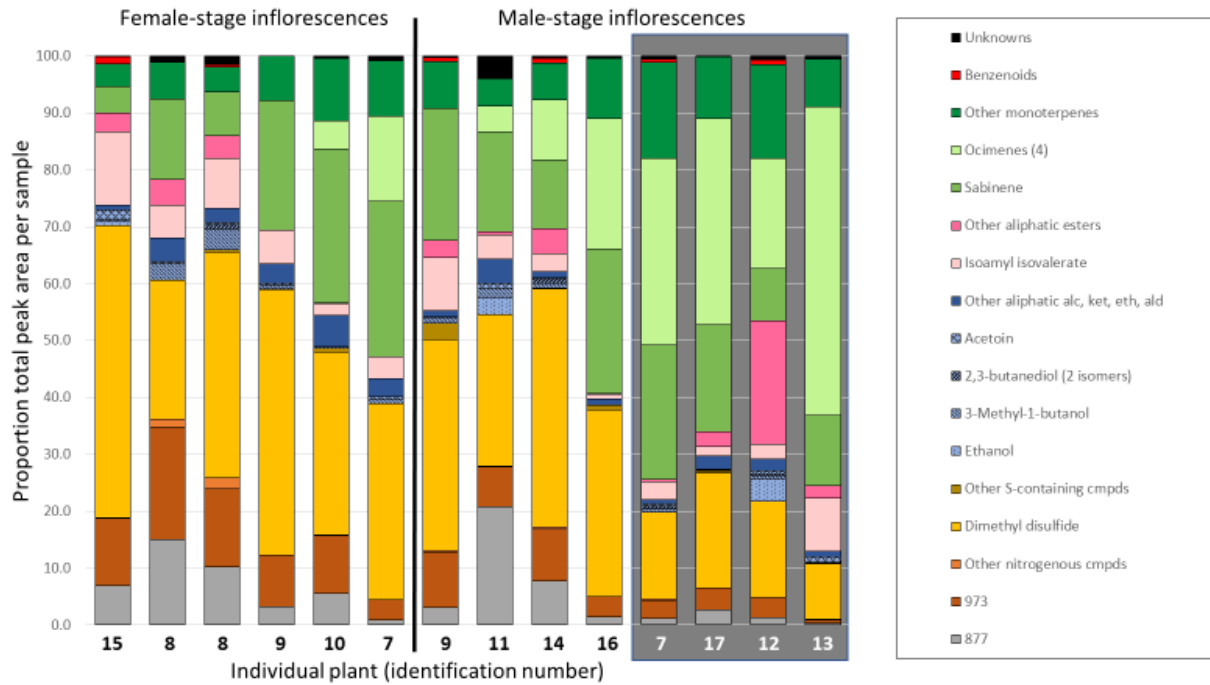


Figure 3. NMDS representation of whole inflorescence samples in two dimensions. Clusters in panel a are defined by sexual stage (female = blue circles; male = red triangles) and clusters in panel b are defined by chemotype (chemotype 1 = no shape outline; chemotype 2 = black shape outline). Samples 7F and 16M are identified in panel b to illustrate their somewhat intermediate position relative to chemotype 2 samples.

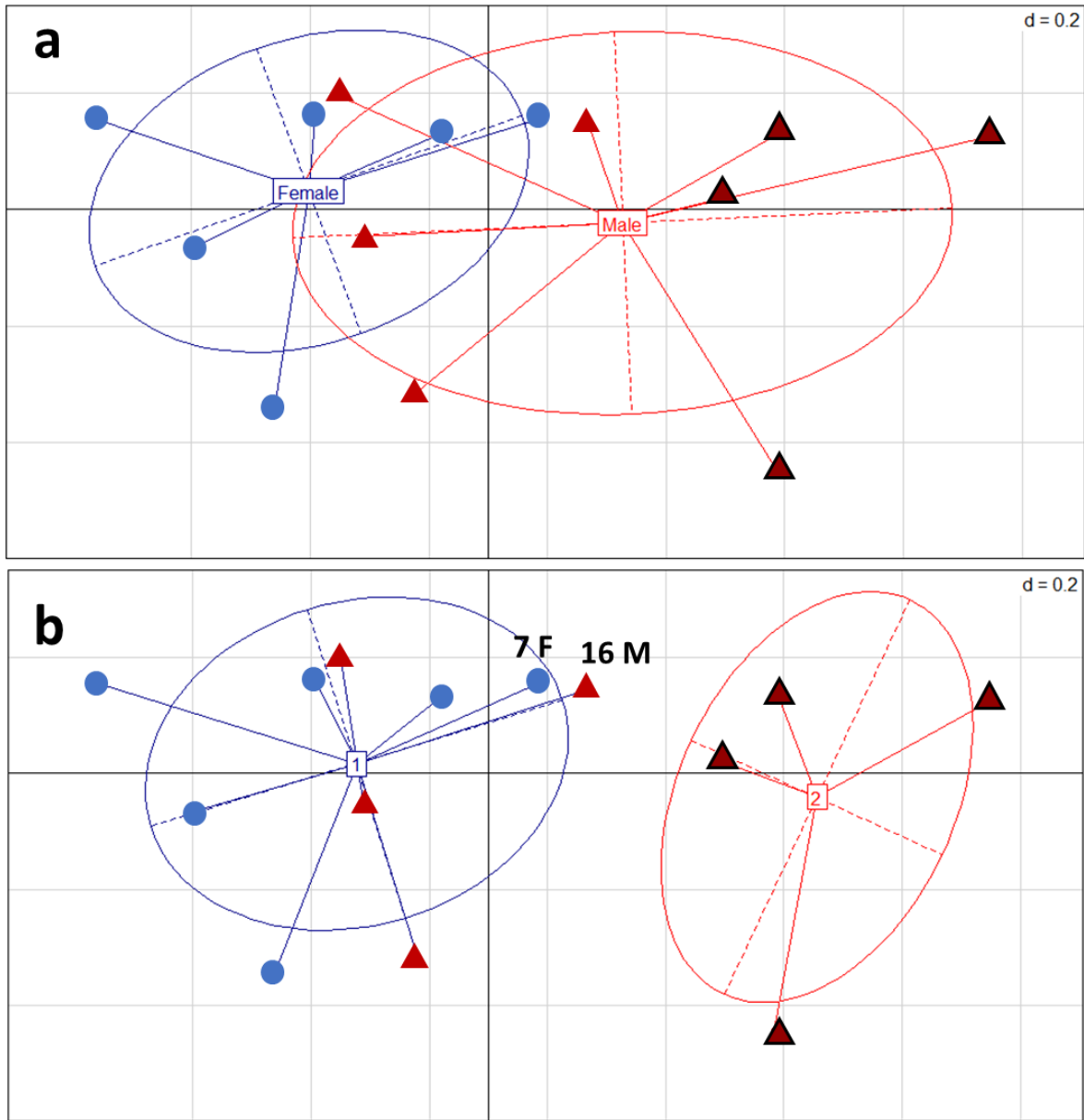


Figure 4. SIMPER analysis of compounds contributing most to the overall variance between chemotype 1 and chemotype 2. Percentage values in the table indicate the % that each compound individually contributes to the overall difference, ranking the compounds from highest to lowest. Pie graph percentage are based upon each compound's average percentage peak area for samples within each chemotype grouping.

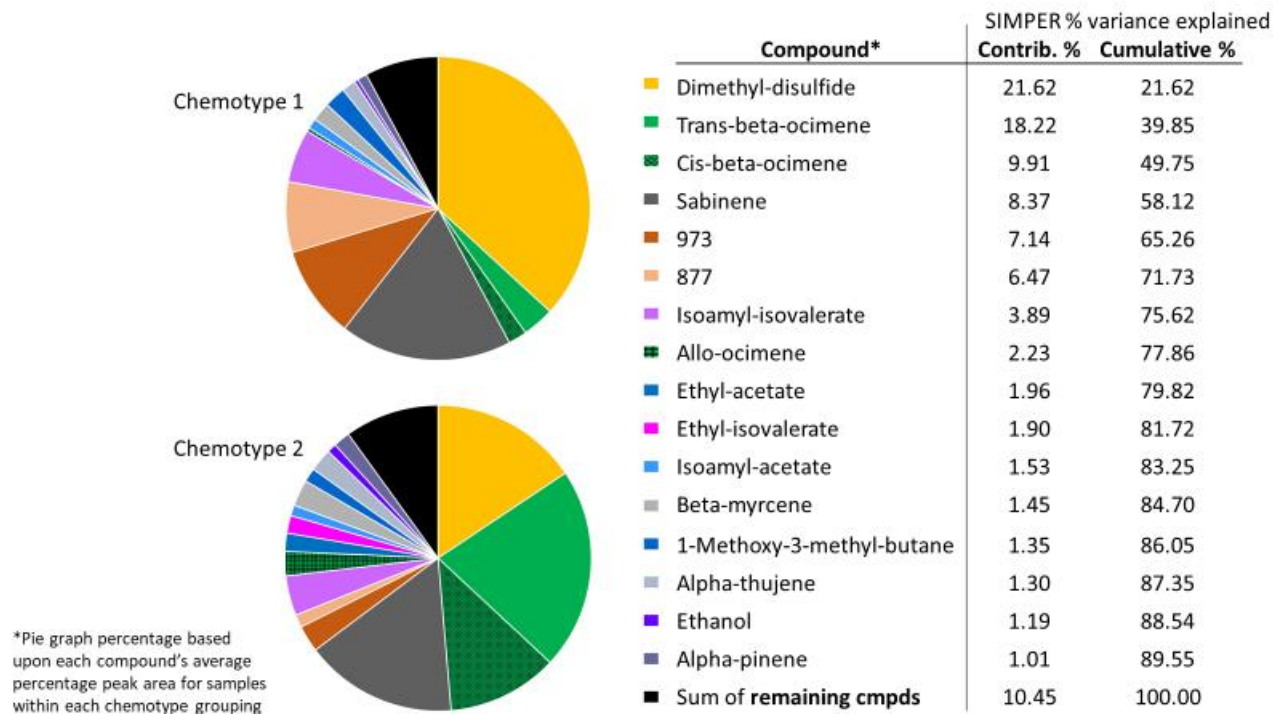


Figure 5. Box plot of average log-transformed total peak areas by sample type. Sample types include whole inflorescence (n = 14), spadix (n = 6), spathe (n = 6), and pollen (n = 2). Letters indicate the result of the Tukey's pairwise post-hoc test ($p < 0.05$). Different letters indicate a statistically significant difference between the treatments. Pollen samples are plotted for visual comparison but were not included in the statistical test due to their low sample size.

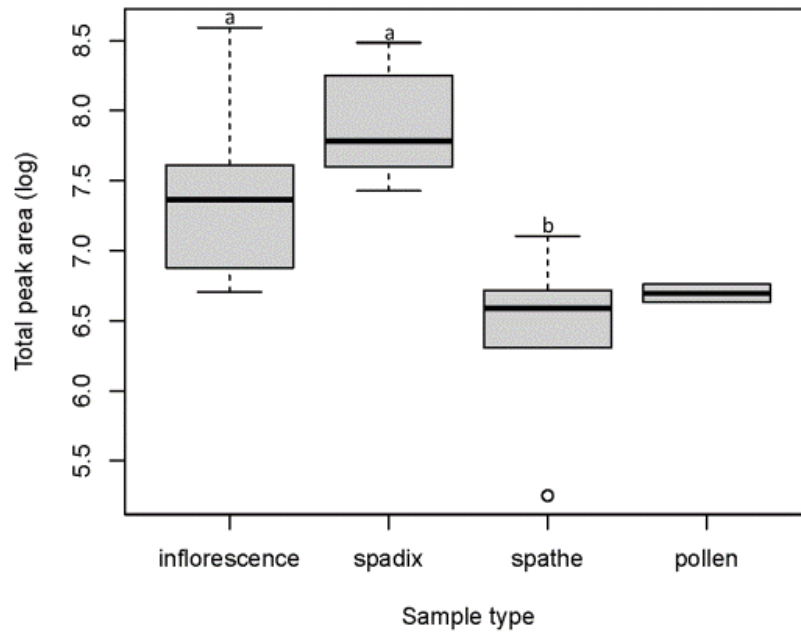
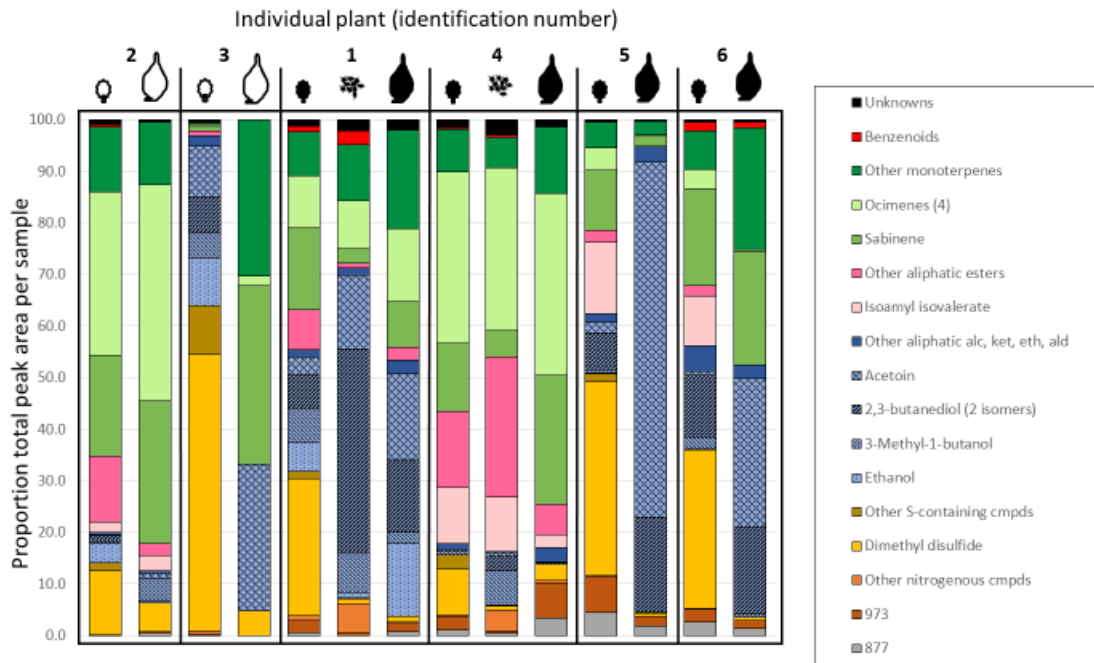
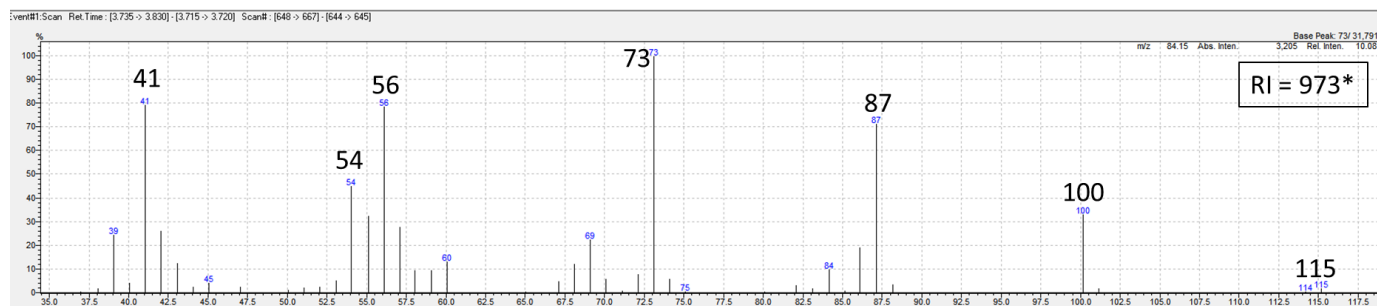
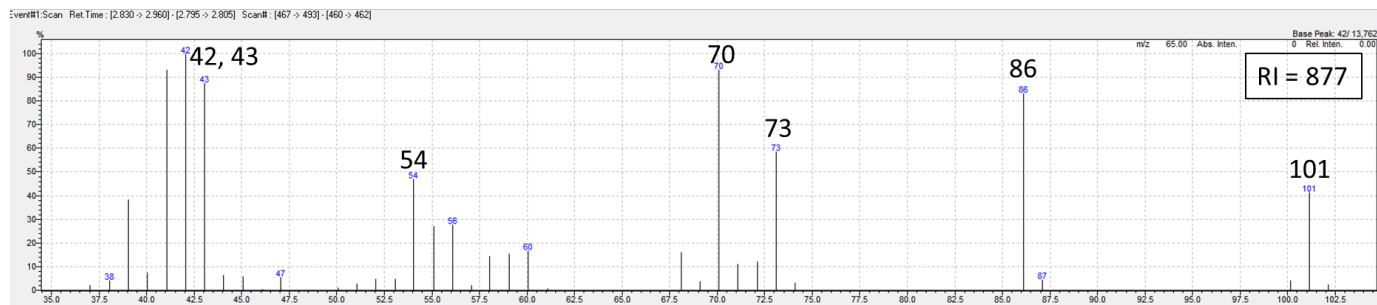


Figure 6. Relative percentage scent composition for dissected inflorescence parts. Six individual inflorescences were dissected into spathe (🍷) and spadix (🍷). Female-stage inflorescences are shown with open symbols, male-stage inflorescences are shown with black symbols. In two individual males, there was sufficient pollen to isolate and collect pollen scent (🍷).



Supplemental Figure 1. Mass spectrum for two unknown compounds comprising a large proportion of the total peak area for most samples. Top panel shows mass spectrum for unknown with calculated retention index of 877. Bottom panel shows mass spectrum for unknown with calculated retention index of 973.



***tentatively identified as 2-methyl butyraldoxime methyl ether**