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# Germplasm diversity of sunflower volatile terpenoid profiles across vegetative and reproductive organs

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**Abstract.** Cultivated sunflower (*Helianthus annuus*) is the fourth most important oilseed crop globally and is known to have experienced multiple genetic bottlenecks during domestication and improvement. Homogenization of crop germplasm may limit breeding efforts to improve pest and pathogen resistance or optimize other biotic interactions like pollinator attraction. Such interactions are often strongly influenced by plant phytochemistry, especially volatile compounds like terpenoids. Here we use solid-phase microextraction gas chromatography mass spectrometry (SPME GC-MS) to evaluate volatile phytochemistry across leaves, involucral bracts, disc florets, and ray floret petals in a collection of twelve inbred lines selected to represent a cross-section of sunflower germplasm diversity. Results indicate considerable compositional diversity of volatiles among lines, though substantial reduction in total volatile abundance relative to wild *H. annuus*. From leaves and bracts to disc florets and petals, we observe a strong increase in the proportion of monoterpenoids relative to sesquiterpenoids accompanying the transition to reproductive structures, with consistently over 85% monoterpenoids in disc florets and petals. This pattern is driven by substantially higher production of monoterpenoids (especially alpha-pinene and sabinene) in reproductive structures. Sesquiterpenoid production is roughly similar across organs, and in leaves varies among lines from 21–55% of volatiles, dominated by cadinene-type sesquiterpenoids. This work suggests that the compositional diversity of volatile terpenoids within cultivated germplasm may be sufficient for many breeding applications, though for breeding increased volatile production the use of wild *H. annuus* and other wild *Helianthus* germplasm may be necessary.

**Key words:** SPME GC-MS, *Helianthus*, monoterpenoids, phytochemistry, sesquiterpenoids.

## INTRODUCTION

Plant domestication is one of the most important events initiating human civilization (Childe, 1936). Although the Fertile Crescent (modern day regions of Iraq, Syria, Kurdistan, Lebanon, Iran, Turkey, etc.) is the oldest center of plant domestication and among the first and best-known cradles of civilization (Zeder, 2011; Haas et al., 2019), there are many other regions that have contributed crops to modern diets. Cultivated

sunflower (*Helianthus annuus* L.) is one of the few crops that was domesticated in North America (Crites, 1993; Blackman et al., 2011). Native Americans as pioneer sunflower breeders developed the first sunflower landraces that had increased seed yield and oil content and were suitable for cultivation, descendants of which gave rise to diverse extant landraces like Hopi, Havasupai, Seneca, Mandan, Hidatsa, and Arikara, among many others (Heiser, 1954; Heiser et al., 1969; Seiler, 1984; Seiler, 1985; Seiler, 1992; Snow et al., 1998; Lentz et al., 2008; Park & Burke, 2020). These landraces became the foundational genetic material for all the other sunflower landraces, varieties, and breeding lines developed everywhere else (Blackman et al., 2011; Baute et al., 2015; Palmgren et al., 2015; Park & Burke, 2020). Today, modern sunflower lines are mostly short-statured and early-flowering, with specific oil profiles and decreased hull content (Heiser et al., 1969; Blackman, 2013; Baute et al., 2015). Sunflower seeds (achenes) can contain up to 55% oil by weight (Ismail & Arafat, 2014; Harun, 2019), as well as substantial protein content alongside phenolic compounds and essential oils (Ceccarini et al., 2004; Weisz et al., 2009; Zilic et al., 2010). Beyond nutritional value for human and animal consumption, a more recent application of sunflower oil and biomass is the production of biofuels (Jasinskas et al., 2008; Kolchinskij, 2008; Cedik et al., 2018). Given that global food security is under threat by climate change and land degradation, the use of edible oils for biofuel production has been questioned (Naylor et al., 2010; Ghosh et al., 2019), and technologies have been sought for the use of precursor-rich non-edible crop byproducts like sunflower stalks (Ziebell et al., 2013; Nargotra et al., 2018; Vital Brazil et al., 2019; Manmai et al., 2021.

There are two major market types of sunflowers: oilseed varieties, and confectionary (or non-oil) varieties. Generally, oilseed types have smaller seeds with thinner hulls and higher oil content, while confectionary types have larger seeds with thicker hulls and lower oil content (Heiser et al., 1969; Adeleke & Babalola, 2020). Oilseed sunflower is a profitable annual crop, and the fourth most important source of edible oil worldwide (FAO, 2019). Sunflower varieties also can be grouped based on their membership in major breeding pools (Korell et al., 1992), the most important of which are the HA (maintainer) and RHA (restorer) pools which have experienced major genetic divergence (Mandel et al., 2013; Badouin et al., 2017; Talukder et al., 2019). In addition, marker-based, phylogenetic, and genome-wide assessments have more fully described the impacts of founder events and genetic bottlenecks on the cultivated sunflower germplasm, indicating that wild sunflower accessions have around three times the number of alleles per microsatellite locus as elite inbred lines used in breeding (Tang & Knapp, 2003), and that cultivated sunflower as a whole has lost approximately one-third of allelic diversity present in wild *H. annuus* (Mandel et al., 2011). Further, modern cultivars contain about half the expected heterozygosity genome-wide as wild *H. annuus* accessions (Hübner et al., 2019), and cultivated sunflower exhibits around a 12-fold reduction in effective population size (Park & Burke, 2020). However, approximately 27% of the 61,205 genes in cultivated sunflower are variable across the 483 cultivated genotypes in the sunflower pangenome (Hübner et al., 2019), such that both cultivated and wild *H. annuus* are both viable sources for genetic variation for use in breeding. However, only a single cytoplasmic male sterility system and very few fertility-restoring alleles are used to create commercial hybrid seed for oilseed and confectionary production, resulting in much lower genetic variability on-farm (Seiler et al., 2017; Talukder et al., 2019).

Although domestication and improvement turned the grassland wildflower *Helianthus annuus* into a modern uniform high-yielding crop, cultivated sunflower is susceptible to numerous environmental stresses including diseases (rust, powdery mildew, downy mildew, charcoal rot, Verticillium, Phomopsis, Sclerotinia, and sunflower mosaic virus), insect pests (weevils, moths, and beetles), and abiotic stresses like drought and salinity (Seiler, 1984; Seiler, 1992; Palmgren et al., 2015; Seiler et al., 2017). A long-standing hypothesis in crop evolution posits that the process of domestication and improvement has favored selection for growth and yield at the expense of resistance to stress, particularly biotic stresses like herbivory (Becerra et al., 2009; Agrawal, 2011; Carmona et al., 2011; Whitehead et al., 2016). Across global agriculture, insect pests alone destroy around 32% of potential crop yield annually (Oerke, 2006). Stress resistance is a complex phenotype that is not linked consistently to specific chemical, physiological, or morphological traits across species (Whitehead et al., 2016). Several studies of the effects of domestication on insect interactions in sunflower have identified substantial increases in herbivore oviposition, feeding preference, survival and reproduction, and even reductions in beneficial parasitoid engagement in cultivated sunflower relative to wild *H. annuus* (Rogers et al., 1987; Chen & Welter, 2002, 2003, 2005, 2007; Michaud & Grant, 2009; Mayrose et al., 2011). Studies of phytochemistry have noted a domestication-associated reduction in the production of nonvolatile sesquiterpene lactones (Rowe et al., 2012; Prasifka et al., 2015), though no known comparisons to date have been published for volatile terpenoids. Leaves and flowers of wild and cultivated sunflowers are fragrant and rich in both volatile and nonvolatile terpenes (Marechal & Rigal, 1999; Ceccarini et al., 2004; Ukiya et al., 2007; Prasifka et al., 2015; Lawson et al., 2019). It is well documented that terpenoids mediate plant-environment interactions (Pichersky & Raguso, 2018; Liu et al., 2020; Zhou & Pichersky, 2020), and in sunflower are involved in a wide range of functions including repelling or killing herbivores (Rogers et al., 1987; Charlet et al., 2008; Gopfert et al., 2009; Rowe et al., 2012; Prasifka et al., 2015), inhibiting fungal pathogen growth (Mayrose et al., 2011; Lawson et al., 2019), allelopathic effects against competing plants (Macias et al., 2002), and even free radical scavenging (Liu et al., 2020). Beside these, volatile compounds in different species are responsible for many antimicrobial, antifungal, and antioxidant activities as well (Vasinauskiene et al., 2006; Lawson et al., 2019; Liu et al., 2020; Lukosiute et al., 2020). Volatile terpenoids have also been demonstrated to influence the quality of biofuel production (Mikulova et al., 2014; Pausas et al., 2016; Vitazek et al., 2018), and common monoterpenoids and sesquiterpenoids can form the basis of the production of specialty biofuels (Peralta Yahya et al., 2011; Joyce et al., 2012; Zhang et al., 2014; Yang et al., 2016; Mewalal et al., 2017).

Further work is needed to clarify the roles of specific volatile terpenoid metabolites in cultivated sunflower, as well as to describe the diversity of phytochemical profiles present in sunflower germplasm that can be leveraged to breed cultivars with desirable terpenoid-mediated phenotypes - whether that be repelling or inhibiting harmful pests or pathogens, attracting beneficial pollinators or parasitoids, or for industrial applications. The specific composition and relative ratios of terpenoids can be as important as their abundance, due to synergistic effects that occur when multiple compounds act together in a cocktail (Richards et al., 2016), or antagonistic effects such as those that occur due to preferential oxidation of major compounds by insect detoxification enzymes resulting in enhanced effects of minor compounds (Scalerandi et al., 2018). Sparse previous work

performed in individual sunflower cultivars indicates that volatile profiles vary among organs and that monoterpenoids are dominant compounds (Ceccarini et al., 2004; Lawson et al., 2019). In wild *H. annuus*, geographic origin appears to drive large-scale variation in both abundance and proportional composition of profiles (Adams et al., 2017), such that genetic variation within the cultivated sunflower germplasm should be predicted to translate into parallel variation. Despite the value to breeding efforts, to date there has not been a comprehensive evaluation of volatile profiles across the cultivated sunflower germplasm. Here in this study, we performed analytical chemistry to describe volatile profile variation in four aerial organs - leaves, involucral bracts, disc florets, and ray floret petals - across twelve cultivated lines spanning breeding pools and market classes within the Sunflower Association Mapping (SAM) panel (Mandel et al., 2011; Mandel et al., 2013). The objectives of this study were to determine abundance and composition of volatile compounds and estimate overall quality and quantity of volatile compounds in cultivated sunflower. The results of this work inform the approach for broader targeted screening of the germplasm resources available for sunflower (Kane et al., 2013; Kantar et al., 2015; Seiler et al., 2017), germplasm selection for studies evaluating the consequences of volatile terpenoid variation on biotic interactions (e.g., Prasifka et al., 2015), and potential limits on the independence of phytochemistry among organs that may constrain the development of cultivars that optimize multiple functions like foliar pest resistance, floral pest resistance, and pollinator attraction.

## MATERIALS AND METHODS

### Germplasm selection

Twelve inbred lines were selected for evaluation in this study, the so-called 'Core 12' lines within the Sunflower Association Mapping (SAM) panel (Mandel et al., 2011; Mandel et al., 2013). The full panel contains 288 inbred lines of cultivated sunflower selected to capture approximately 87% of allelic diversity present within the sunflower germplasm repositories of the United States Department of Agriculture (USDA) National Plant Germplasm System and the French Institut National de la Recherche Agronomique (INRA), propagated by single-seed descent to remove residual heterozygosity (Mandel et al., 2011; Mandel et al., 2013). The 'Core 12' lines were selected by rarefaction to represent the most divergent genotypes, together containing just under half of the allelic diversity within the full SAM panel (pre Mandel et al., 2011), and includes three HA-Oil lines, two HA-NonOil lines, three RHA-Oil lines, one RHA-NonOil line, one INRA-HA line, and two open-pollinated varieties (Table S1). These twelve genotypes should reflect a cross-section of genetic diversity across all sunflower germplasm.

### Plant growth

In 2019, the Core 12 lines were grown alongside the full 288-line SAM panel in a randomized complete block design across two agricultural high tunnels on the University of Central Florida campus in Orlando, FL, United States. The Core 12 lines were planted in mid-March, with six replicate plants of each line grown in each agricultural high tunnel, which served as statistical blocks, totaling a target of 12 replicates for each line. Seeds were planted directly into 18.6 liter pots filled with pine-bark-based potting soil. Each pot received four tablespoons (64 grams) of slow-release fertilizer (Osmocote Plus 15-9-12; Scotts, Marysville, OH, USA) to ensure non-limiting nutrient supply. Plants

were watered daily to field capacity with automatic drip irrigation to ensure non-limiting water availability. Photoperiod, light, and temperature levels were ambient, with flowering of the Core 12 lines occurring during a two-week period in May based on genotype-derived variation in flowering phenology.

**Sampling**

At flowering (R5 stage; Schneiter & Miller, 1981), samples were taken of the four target aerial organs. The lamina of the most recently fully-expanded leaf (hereafter 'leaf') was cut with scissors down the midrib, with one side rolled and placed into a microcentrifuge tube. One or more involucral bracts on the back of the composite head (hereafter 'bract') were removed with scissors and placed into a microcentrifuge tube. Multiple ray floret petals (hereafter 'petal') were plucked from the circumference of the composite head with forceps and placed into a microcentrifuge tube. Several dozen newly open disc florets were removed from the center disc of the composite head with forceps and placed into a microcentrifuge tube. All organ samples were immediately snap-frozen in liquid nitrogen upon sampling, and kept in a -80 °C freezer until preparation for analysis. Scissors and forceps were cleaned with ethanol between samples to prevent cross-contamination. Only undamaged healthy structures were sampled, excluding any organs with visible wilting, damage, or necrosis, and excluding any replicate plants with substantial herbivory, pathogen infection, partially broken stems, or other visible factors that might influence phytochemistry. Within each genotype, between 2–9 samples were obtained for each organ type - typically more for leaves and bracts, and fewer for petals and disc florets given the narrower time window for sampling these more ephemeral organs (just a few days for each composite head) and our strict quality criteria. The total number of samples obtained was 240, or an average of $n = 5$ samples per organ per genotype.

**Phytochemical analysis**

In this experiment, solid-phase microextraction gas chromatography-mass spectrometry (SPME GC-MS) was performed using a single quadrupole GCMS-QP2020 (Shimadzu, Inc.) to identify volatile compounds in the samples. The leaf, bract, petal, and disc floret samples were ground with a mortar and pestle to a fine powder in liquid nitrogen, and 200 (± 20) mg of the tissue was put into 10 mL glass headspace vials with the total sample mass recorded. To start the phytochemical analysis, the vials were incubated at 75 °C for 15 minutes with agitation at 250 rpm. Then to extract volatiles from the headspace, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVS/CAR/PDMS) SPME fiber was introduced to the vial and incubated at 75 °C with agitation at 250 rpm for 10 minutes. The SPME fiber was then desorbed for 3 minutes into the inlet of the GC-MS at 250 °C. The fiber between samples was conditioned for 10 minutes at 270 °C. Column flow was 1.91 mL $min^{-1}$ with splitless injection using a purge flow of 3.0 mL $min^{-1}$ after 3.5 minutes sampling time. Initial GC temperature was 35 °C, then increased to 80 °C at 10 °C $min^{-1}$, held for 5.5 minutes, then increased to 140 °C at 15 °C $min^{-1}$, held for 5.5 minutes, then increased to 220 °C at 20 °C $min^{-1}$, and held for 2 minutes. The MS source and interface temperatures were kept at 200 °C and 250 °C, respectively. The mass spectra of peaks were compared against the National Institutes of Standards and Technology standards database (Lemmon et al., 2017), and minimum similarity of 75% was used to select peaks identities as naming conventions. Potential mislabeling was avoided by manually processing the raw data using retention

time and mass spectra similarity hits for each peak. For our purposes, the top NIST library hit for each peak was reported regardless of isomer identity (full differentiation of isomers can be difficult for many compounds with GC-MS); due to this our dataset contains multiple instances of some metabolites that might be isomers of the same compound. The peak area for each compound was divided by the sample mass placed into the headspace vial to generate mass-normalized peak area, our metric of compound abundance.

**Statistical analysis**

Additional summary statistics were calculated for each sample using mass-normalized peak areas. Sums of mass-normalized peak areas for compounds in particular focal classes were calculated to derive estimates of the abundance of total monoterpenoids, total sesquiterpenoids, total diterpenoids, total terpenoids (the sum of monoterpenes, sesquiterpenes, and diterpenes), total fatty acid derivatives, and an additional category of total 'other compounds' for all other miscellaneous non-terpenoid compounds (including various ketones, epoxides, benzaldehydes, alkanes, alkenes, and alcohols). By summing all mass-normalized peak areas, a semiquantitative relative estimate of the total volatile abundance in each sample was generated. The proportional contribution of each individual compound to the total volatile abundance was calculated by dividing the mass-normalized peak areas for each compound by the total volatile abundance in each sample, then expressed as a percentage. Likewise, the proportional contribution of monoterpenoids, sesquiterpenoids, diterpenoids, fatty-acid-derivatives, and other compounds were similarly calculated by dividing the total abundance of each class by the total volatile abundance in each sample.

The number of compounds detected and identified within each sample was also recorded as an estimate of volatile compound diversity. To improve focus on the dominant compounds in volatile profiles, 'major compounds' were identified in three ways. First, across the entire dataset 'major compounds' were identified as compounds that were both present in all organs of all genotypes and contributed on average > 1% of total volatile abundance across the 48 organ-by-genotype combinations. Within each organ, this process was again repeated to identify 'major compounds' that were present in all 12 genotypes and contributed on average > 1% of total volatile abundance across the 12 genotypes. Within each line, this process was again repeated to identify 'major compounds' within each line, identified as those compounds that were present in all four organ types and contributed on average > 1% of total volatile abundance across the four organs. Potential trait-trait associations within and between organs were investigated using pairwise Pearson correlations with the corr package (Makowski et al., 2020) in R version 1.4.1717 (R Core Team, 2022). Graphs were drawn using Microsoft Excel v. 2210.

## RESULTS AND DISCUSSUION

**Volatile compound diversity across organs and genotypes**

Across the 240 samples analyzed, 196 unique compounds were detected and identified, of which 69.4% were terpenoids (33.7% monoterpenoids, 34.7% sesquiterpenoids, and 1% diterpenoids), 3.6% fatty acid derivatives, and 27% other compounds (Appendix 1). Among organs, leaves had the fewest unique compounds while disc florets and bracts had the most (Table 1). Across organs the proportion of

identified compounds that were terpenoids (72.6–77.7%), fatty acid derivatives (0–4.8%), and other compounds (19.7–25.2%) were quite similar, though the proportional breakdown within the terpenoid class was more variable (Table 1). Within leaves, there were around twice as many sesquiterpenoid compounds identified as monoterpenoids, while the opposite pattern was observed in petals (Table 1). Both disc florets and bracts had roughly even proportions of both monoterpenoids and sesquiterpenoids (Table 1). Among the twelve genotypes, the total number of identified compounds varied from 71 to 107, with between 74.8–88.2% terpenoids, 0–3.7% fatty acid derivatives, and 11.7–21.6% other compounds (Table S2). Within the terpenoid class, the proportion of monoterpenoids and sesquiterpenoid compounds were roughly similar among genotypes with more of either subclass in different genotypes (Table S2).

**Table 1.** Total number of volatile compounds detected and identified via SPME-GC-MS in the four organ types assessed (pooling all twelve plant genotypes), as well as the proportional breakdown of compounds classified as terpenoids (divided into monoterpenoids, sesquiterpenoids, and diterpenoids), fatty acid derivatives, and other compounds

| Organ | Number of compounds | % terpenoids | % monoterpenoids | % sesquiterpenoids | % diterpenoids | % fatty acid derivatives | % other compounds |
|---|---|---|---|---|---|---|---|
| Petals | 83 | 73.5 | 49.4 | 24.1 | 0 | 4.8 | 21.6 |
| Disc florets | 95 | 72.6 | 38.9 | 32.6 | 1.0 | 2.1 | 25.2 |
| Bracts | 94 | 76.4 | 38.2 | 37.2 | 1.0 | 0 | 23.4 |
| Leaves | 72 | 77.7 | 25.9 | 50.6 | 1.2 | 2.4 | 19.7 |

**Volatile compound abundance and profile composition**

Across all genotype-by-organ combinations, mean total volatile abundance varied by nearly an order of magnitude, and the mean number of compounds detected ranged from 12.0 to 38.6 (Table 2, Fig. 1). Profiles were consistently terpenoid-dominated (87–99% of abundance), but with very large variation in the proportional abundance of monoterpenoids (38–98%) and sesquiterpenoids (0.5–55%) (Table 2). Regardless of organ or genotype, the diterpenoid and fatty acid derivative classes were minute fractions of total volatile abundance (< 1% in all cases, often undetected).

Considering organ-driven variation, petals contained on average the fewest detected compounds (16.8), while leaves contained the most (29.2) and disc florets and bracts were intermediate (Table S3). This cross-organ pattern holds within most of the twelve genotypes considered individually (Table 2). The mean number of compounds in each organ was unrelated to the total volatile abundance, which on average was highest in disc florets and under half as abundant in leaves, with bracts and petals intermediate (Table S3). On average, 93–98% of total volatile abundance was composed of terpenoids, but the balance of monoterpenoids and sesquiterpenoids varied widely among organs (Fig. 1, Table S3). In reproductive structures, total abundance was dominated by monoterpenoids (90–98% in petals, 85–94% in disc florets, 71–91% in bracts; Fig. 1, Table 2, Table S3). In leaves the balance between monoterpenoids and sesquiterpenoids was much more variable, with 38–69% monoterpenoids and 22–55% sesquiterpenoids among genotypes (Fig. 1, Table 2). Leaves have by far the highest proportion of sesquiterpenoids, as well as the highest proportion of non-terpenoid compounds (Table 2, Table S3).

**Table 2.** Volatile profiles for each organ within each plant genotype, as assessed by SPME-GC-MS. The average number of compounds detected and total volatile abundance (mass-normalized peak area) are reported, along with the proportional breakdown of mass-normalized peak area by compound class: terpenoids (divided into monoterpenoids, sesquiterpenoids, and diterpenoids), and other compounds. Values represent mean ± SE for each metric reported, calculated across all replicate samples. Fatty-acid derivatives are excluded as a category in this table, as only five organ-genotype combinations had detectable quantities. Entries with values representing detected compounds between 0–0.1% of volatile profile composition are rounded up to 0.1%, and percentages may not sum to 100% due to rounding

| Core 12 genotype | Organ | Number of compounds | Total volatile abundance | % terpenoids | % monoterpenoids | % sesquiterpenoids | % diterpenoids | % other compounds |
|---|---|---|---|---|---|---|---|---|
| SAM 020 | Petal | 16.8 ± 1.0 | 32,193 ± 672 | 99.3 ± 0.3 | 95.8 ± 0.8 | 3.4 ± 0.8 | 0 | 0.6 ± 0.3 |
| | Disc Floret | 21.5 ± 1.6 | 56,381 ± 16,607 | 98.4 ± 0.3 | 89.0 ± 2.7 | 9.2 ± 2.5 | 0 | 1.7 ± 0.3 |
| | Bract | 27.0 ± 2.5 | 26,831 ± 2,120 | 95.8 ± 1.2 | 73.0 ± 1.4 | 22.7 ± 1.6 | 0.1 ± 0.1 | 4.1 ± 1.2 |
| | Leaf | 28.1 ± 3.2 | 19,797 ± 4,814 | 89.4 ± 4.9 | 40.6 ± 2.9 | 48.3 ± 3.9 | 0.4 ± 0.2 | 10.5 ± 4.9 |
| SAM 022 | Petal | 18.2 ± 0.4 | 43,053 ± 18,789 | 97.6 ± 1.4 | 95.4 ± 1.3 | 2.2 ± 0.3 | 0 | 2.3 ± 1.4 |
| | Disc Floret | 24.7 ± 3.1 | 55,973 ± 25,032 | 96.9 ± 0.8 | 85.5 ± 6.0 | 11.3 ± 6.2 | 0 | 2.8 ± 0.9 |
| | Bract | 21.4 ± 2.4 | 36,174 ± 14,221 | 95.8 ± 0.9 | 79.5 ± 4.0 | 16.2 ± 4.1 | 0.1 ± 0.1 | 4.1 ± 0.9 |
| | Leaf | 25.1 ± 2.5 | 16,762 ± 2,645 | 91.9 ± 3.2 | 55.2 ± 5.7 | 36.1 ± 5.6 | 0.5 ± 0.1 | 8.0 ± 3.2 |
| SAM 027 | Petal | 17.5 ± 1.3 | 32,568 ± 3,785 | 96.4 ± 1.5 | 90.6 ± 1.6 | 5.8 ± 0.5 | 0 | 3.3 ± 1.4 |
| | Disc Floret | 22.6 ± 2.0 | 40,946 ± 5,777 | 94.3 ± 1.4 | 87.8 ± 1.4 | 6.4 ± 0.4 | 0 | 5.6 ± 1.3 |
| | Bract | 26.2 ± 1.0 | 30,837 ± 3,672 | 97.5 ± 0.7 | 81.0 ± 1.5 | 16.5 ± 1.7 | 0 | 2.4 ± 0.7 |
| | Leaf | 26.8 ± 4.9 | 21,356 ± 8,474 | 95.0 ± 1.1 | 57.5 ± 4.1 | 37.1 ± 3.8 | 0.3 ± 0.1 | 4.9 ± 1.1 |
| SAM 093 | Petal | 16.0 ± 1.8 | 20,579 ± 3,480 | 97.6 ± 1.0 | 92.1 ± 0.8 | 5.5 ± 0.5 | 0 | 2.3 ± 1.0 |
| | Disc Floret | 24.5 ± 2.8 | 43,219 ± 6,242 | 96.4 ± 1.2 | 91.4 ± 1.2 | 5.0 ± 0.8 | 0.1 ± 0.1 | 3.5 ± 1.2 |
| | Bract | 22.1 ± 2.7 | 20,855 ± 3,423 | 96.0 ± 1.3 | 85.0 ± 1.2 | 10.9 ± 0.6 | 0 | 3.9 ± 1.3 |
| | Leaf | 18.5 ± 2.6 | 7,779 ± 1,538 | 87.0 ± 4.8 | 55.3 ± 6.3 | 31.0 ± 5.2 | 0.6 ± 0.5 | 12.9 ± 4.8 |
| SAM 094 | Petal | 17.0 ± 1.7 | 31,427 ± 5,742 | 98.0 ± 0.8 | 95.2 ± 1.0 | 2.7 ± 0.4 | 0 | 1.9 ± 0.8 |
| | Disc Floret | 23.7 ± 3.4 | 55,033 ± 14,133 | 98.0 ± 0.5 | 93.4 ± 0.4 | 4.5 ± 0.6 | 0 | 1.9 ± 0.5 |
| | Bract | 25.6 ± 1.7 | 23,948 ± 1,887 | 91.8 ± 3.3 | 73.8 ± 3.4 | 18.0 ± 1.7 | 0 | 8.1 ± 3.3 |
| | Leaf | 34.0 ± 2.8 | 36,717 ± 9,236 | 97.0 ± 0.7 | 58.6 ± 3.7 | 38.3 ± 3.3 | 0.1 ± 0.1 | 2.9 ± 0.7 |
| SAM 176 | Petal | 12.0 ± 0.4 | 33,519 ± 8,194 | 99.1 ± 0.2 | 96.8 ± 0.4 | 2.3 ± 0.4 | 0 | 0.8 ± 0.2 |
| | Disc Floret | 24.2 ± 1.5 | 46,579 ± 2,097 | 98.6 ± 0.3 | 92.5 ± 1.0 | 6.0 ± 0.9 | 0.1 ± 0.1 | 1.3 ± 0.3 |
| | Bract | 21.2 ± 1.7 | 27,179 ± 7,499 | 96.1 ± 1.4 | 84.2 ± 2.3 | 11.8 ± 1.5 | 0 | 3.8 ± 1.4 |
| | Leaf | 23.1 ± 1.5 | 14,518 ± 1,384 | 94.1 ± 1.3 | 61.6 ± 5.2 | 31.7 ± 4.2 | 0.7 ± 0.2 | 5.8 ± 1.3 |

*Table 2 (continued)*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SAM 185 | Petal | 16.6 ± 4.0 | 21,906 ± 6,279 | 97.7 ± 1.7 | 92.8 ± 2.3 | 4.8 ± 2.0 | 0 | 2.2 ± 1.7 |
| | Disc Floret | 22.8 ± 2.3 | 30,926 ± 2,861 | 93.2 ± 3.9 | 86.3 ± 4.4 | 6.9 ± 0.6 | 0 | 6.7 ± 3.9 |
| | Bract | 25.7 ± 2.1 | 25,477 ± 3,319 | 98.3 ± 0.6 | 78.0 ± 1.5 | 20.3 ± 1.4 | 0 | 1.6 ± 0.6 |
| | Leaf | 29.5 ± 3.8 | 20,733 ± 4,974 | 94.1 ± 2.3 | 51.7 ± 3.4 | 41.9 ± 4.2 | 0.4 ± 0.2 | 5.8 ± 2.3 |
| SAM 191 | Petal | 17.7 ± 1.6 | 35,969 ± 5,178 | 98.9 ± 0.4 | 95.0 ± 1.4 | 3.8 ± 1.3 | 0 | 1.0 ± 0.4 |
| | Disc Floret | 30.7 ± 1.4 | 53,286 ± 2,961 | 98.5 ± 0.2 | 89.8 ± 1.1 | 8.6 ± 0.9 | 0 | 1.4 ± 0.2 |
| | Bract | 22.0 ± 1.7 | 22,987 ± 2,157 | 93.2 ± 2.2 | 71.3 ± 4.8 | 21.8 ± 4.6 | 0 | 6.7 ± 2.2 |
| | Leaf | 30.5 ± 3.4 | 22,195 ± 4,838 | 94.1 ± 1.3 | 38.1 ± 6.1 | 55.4 ± 5.3 | 0.5 ± 0.2 | 5.8 ± 1.3 |
| SAM 203 | Petal | 18.0 ± 0.9 | 43,979 ± 4,321 | 98.5 ± 0.3 | 97.9 ± 0.4 | 0.5 ± 0.1 | 0 | 1.4 ± 0.3 |
| | Disc Floret | 20.5 ± 2.1 | 50,213 ± 10,528 | 96.6 ± 1.0 | 94.0 ± 0.7 | 2.6 ± 0.7 | 0 | 3.3 ± 1.0 |
| | Bract | 17.3 ± 1.2 | 28,527 ± 13,367 | 96.9 ± 1.2 | 91.2 ± 2.1 | 5.3 ± 1.1 | 0.3 ± 0.2 | 3.0 ± 1.2 |
| | Leaf | 26.8 ± 2.2 | 18,996 ± 3,275 | 91.8 ± 2.4 | 62.5 ± 3.1 | 28.4 ± 1.5 | 0.8 ± 0.2 | 8.1 ± 2.4 |
| SAM 237 | Petal | 14.3 ± 0.6 | 19,245 ± 1,703 | 99.1 ± 0.6 | 93.9 ± 1.3 | 5.1 ± 0.7 | 0 | 0.6 ± 0.6 |
| | Disc Floret | 24.6 ± 0.3 | 32,925 ± 3,077 | 94.8 ± 0.8 | 85.0 ± 0.1 | 9.7 ± 0.9 | 0 | 5.1 ± 0.8 |
| | Bract | 25.0 ± 1.5 | 22,806 ± 2,152 | 94.9 ± 2.3 | 75.3 ± 3.9 | 19.5 ± 2.2 | 0 | 5.0 ± 2.3 |
| | Leaf | 38.6 ± 2.6 | 28,727 ± 5,449 | 95.2 ± 0.1 | 44.7 ± 1.9 | 50.1 ± 2.0 | 0.3 ± 0.1 | 4.7 ± 0.1 |
| SAM 240 | Petal | 18.3 ± 2.7 | 42,294 ± 7,998 | 97.8 ± 1.0 | 94.1 ± 2.9 | 3.7 ± 1.8 | 0 | 2.1 ± 1.0 |
| | Disc Floret | 26.5 ± 3.5 | 66,735 ± 9,890 | 98.4 ± 0.2 | 93.7 ± 1.1 | 4.7 ± 0.8 | 0 | 1.5 ± 0.2 |
| | Bract | 32.0 ± 2.0 | 59,356 ± 10,996 | 98.5 ± 0.2 | 84.4 ± 2.4 | 14.1 ± 2.6 | 0 | 1.4 ± 0.2 |
| | Leaf | 37.8 ± 1.3 | 46,592 ± 2,361 | 97.2 ± 0.5 | 59.0 ± 2.2 | 38.1 ± 2.3 | 0.1 ± 0.1 | 2.7 ± 0.5 |
| SAM 262 | Petal | 19.5 ± 0.5 | 40,037 ± 3,575 | 96.3 ± 1.6 | 93.8 ± 2.1 | 2.4 ± 0.4 | 0 | 3.6 ± 1.6 |
| | Disc Floret | 25.0 ± 1.0 | 72,697 ± 5,727 | 97.0 ± 0.1 | 94.3 ± 0.1 | 2.7 ± 0.1 | 0 | 2.9 ± 0.1 |
| | Bract | 14.6 ± 1.4 | 15,832 ± 1,116 | 97.7 ± 0.9 | 88.9 ± 2.5 | 8.6 ± 1.4 | 0.2 ± 0.1 | 2.2 ± 0.9 |
| | Leaf | 31.6 ± 3.4 | 27,771 ± 4,980 | 91.1 ± 4.2 | 68.8 ± 5.9 | 21.9 ± 3.5 | 0.3 ± 0.1 | 8.6 ± 4.2 |

While organ-driven variation was large, several substantial genotype-driven patterns are evident. First, overall volatile abundance varies substantially among genotypes no matter which organ is considered (Fig. 1, Table 2). Genotype-level means averaged across organs show two-fold variation in total volatile abundance (Table S4), while in comparison across all 48 genotype-by-organ combinations the variation in total volatile abundance was over nine-fold (Table S5). Taking each organ individually, variation among genotypes in total volatile abundance was a bit over two-fold in petals and discs, over three-fold in bracts, and nearly six-fold in leaves (Table S6).

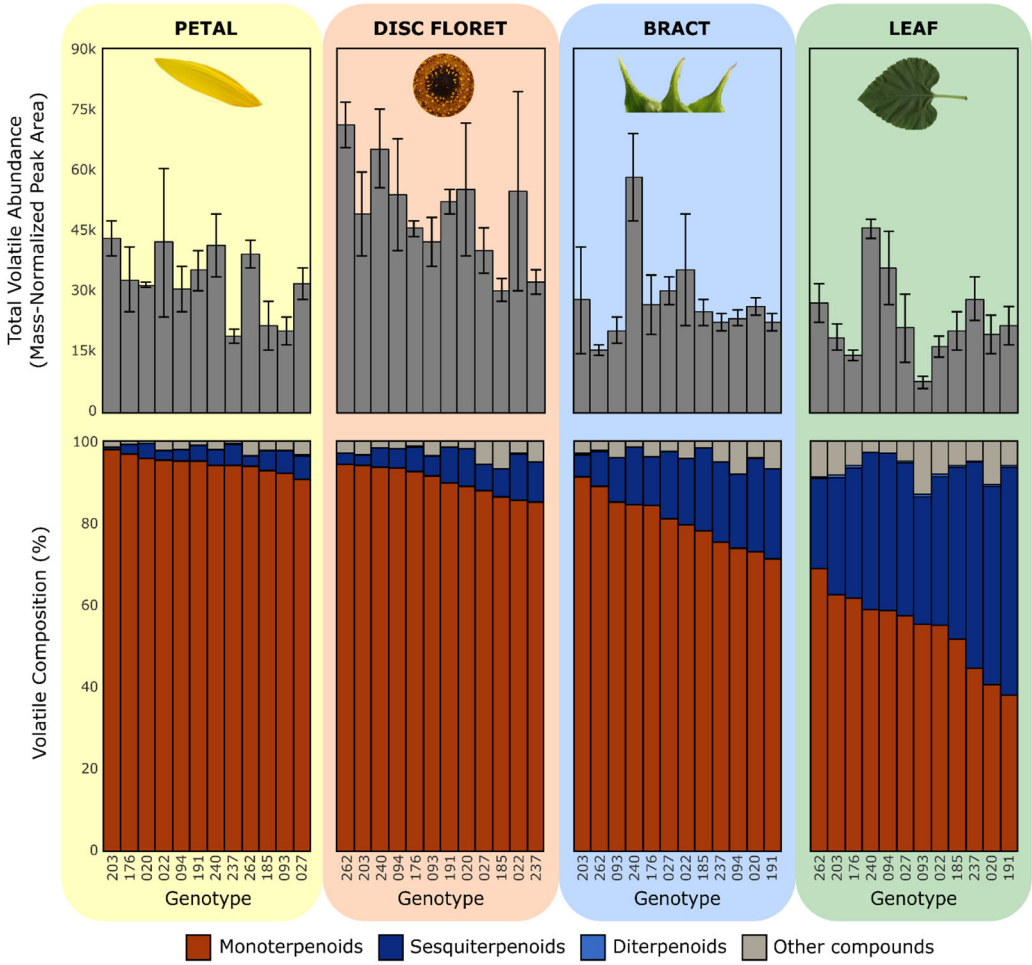


**Figure 1.** Total volatile abundance (top panels) and volatile profile composition (bottom panels) for the four focal organs (petal, disc floret, bract, and leaf) across the Core 12 genotypes (SAM 020, SAM 022, SAM 027, SAM 093, SAM 094, SAM 176, SAM 185, SAM 191, SAM 203, SAM 237, SAM 240, SAM 262). Total volatile abundance is expressed as the total mass-normalized peak area of all detected compounds averaged across replicate samples of a given organ within a genotype, and error bars represent standard error of the mean. Volatile profile composition is the relative proportion of total mass-normalized peak area comprised of monoterpenoids, sesquiterpenoids, diterpenoids, or other non-terpenoid compounds.

This variation among genotypes within each organ is similar in magnitude to the variation among organs within each genotype (Table S7). Second, genotypic variation in bract and leaf monoterpenoid-sesquiterpenoid balance is substantial (Fig. 1, Table 2), indicating that the relative composition of volatile profiles in these organs could be altered by targeted breeding efforts. Third, the proportional contribution of individual major compounds to the overall profile is highly variable.

**Genetic variation in major compounds across sunflower organs**

While 196 unique compounds were detected and identified in this study, only sabinene was present in every sample analyzed. Major compounds present in all organs of all genotypes were the monoterpenoids alpha-pinene, sabinene, gamma-terpinene, and o-cymene (Table S8). In the reproductive structures (petals, disc florets, and bracts), the monoterpenoids alpha-pinene and sabinene together comprised on average 60–72% of total volatile abundance, with the remaining portion of the profile up to > 85% made up of a combination the monoterpenoids beta-pinene, D-limonene, alpha-terpinene, gamma-terpinene, terpinene-4-ol, o-cymene, and bornyl acetate, along with the sesquiterpenoids beta-gurjunene, beta-cubebene, and beta-elemene, and the non-terpenoid methoxyphenyloxime and desmethoxyencecalin (Table 3). Variation in the composition of these compounds in each organ varied substantially among genotypes (Table 3). Alpha-pinene varied from 33–77% in petals, 35–62% in disc florets and 40–64% in bracts, while sabinene varied from 11–31% in petals, 9–24% in disc florets, and 4–12% in bracts (Table 3). The other major compounds varied at least 2-fold to as high as 50-fold among genotypes in each of these three organs (Table 3). Among genotypes, there was a strong negative correlation between the proportions of alpha-pinene and sabinene in both petals ($R^2 = 0.81$) and disc florets ($R^2 = 0.65$), though not in bracts where these two compounds are less dominant (Fig. S1).

In leaves, the most abundant compounds were the monoterpenoids D-limonene, sabinene, alpha-pinene, gamma-terpinene, and endo-borneol, the sesquiterpenoids beta-cubebene, alpha-cadinene, beta-cadinene, gamma-cadinene, alpha-muurolene, gamma-muurolene, and caryophyllene, and the non-terpenoids methoxyphenyloxime and 1,5,9,9-tetramethyl-Z,Z,Z-1,4,7-cycloundecatriene (Table 3). Among genotypes, D-limonene varied from 19–32%, beta-cubebene from 8–23%, sabinene from 6–14%, and alpha-pinene from 6–14% (Table 3). The other major compounds varied at least 2-fold and as high as 18-fold among genotypes in leaves (Table 3).

**Diversity of volatile profiles compared with other cultivated and wild Helianthus**

The results obtained here for a systematic cross-section of cultivated *Helianthus* germplasm are consistent with previous research on scattered varieties of cultivated sunflower. An assessment of essential oils derived from dried leaves and whole capitula of two varieties (‘Carlos’ and ‘Florom 350’) identified 51 and 49 compounds, respectively, of which 84–88% were terpenoids, with slightly more sesquiterpenoids than monoterpenoids (Ceccarini et al., 2004). Another assessment of essential oils derived from fresh leaves of two different cultivars (‘Mammoth’ and ‘Chianti’) identified 64 compounds, of which 95.1% were terpenoids with about twice as many sesquiterpenoids as monoterpenoids (Lawson et al., 2019). The most abundant compounds in these studies overlapped heavily with those identified here, including alpha-pinene, sabinene, limonene, bornyl acetate, terpinene-4-ol, beta-pinene, beta-gurjunene, and camphene. This indicates that a core set of terpenoid

compounds in cultivated sunflower are present across most germplasm, and can be captured by assessing even a few accessions, but that there are many additional non-core compounds that are unlikely to be detected without screening far more genotypes.

Research conducted on wild *H. annuus*, the progenitor to cultivated sunflower, demonstrates a similar degree of qualitative phytochemical diversity to that observed in the Core 12 genotypes assessed here. Assessment of volatiles in snap-frozen tissues from greenhouse-grown plants of one accession of wild *H. annuus* from Konza Prairie, Kansas using identical analytical methods identified 79 compounds in leaves and 67 compounds in petals (17 shared between organs), only slightly less than observed in the Core 12 lines here (Table S10, Table S11; Bahmani et al., 2022). In both petals and leaves, terpenoids made up a higher proportion of identified compounds in wild *H. annuus* than in the Core 12 lines here (Table S10, Table S11). In petals, there were fewer monoterpenoid and non-terpenoid compounds and more sesquiterpenoid and diterpenoid compounds than observed in the Core 12 lines here (Table S10). In leaves, there were more monoterpenoid and diterpenoid compounds, and fewer sesquiterpenoids and diterpenoids than observed in the Core 12 lines here (Table S11). A broader assessment of essential oil extracts derived from air-dried leaves sampled in the field from 20 populations distributed across the native range of *H. annuus* identified 83 total compounds, 20 of which were shared across all populations, with a very similar average breakdown of compounds as those from Konza Prairie (Table S11; Adams et al., 2017; Bahmani et al., 2022). Considering total volatile abundance estimated from mass-normalized peak areas, wild *H. annuus* from Konza Prairie had over six-fold higher volatile abundance in both petals and leaves than observed on average in the Core 12 lines here (Table S12), though the proportional abundance was similar between wild and cultivated sunflower for petals (> 90% monoterpenoids in wild). In leaves, this proportional abundance was slightly shifted toward monoterpenoids in wild *H. annuus* from Konza Prairie (66% monoterpenoids, 31% sesquiterpenoids) relative to cultivated sunflower (Table S12). The average proportional abundance identified in leaves across the range of wild *H. annuus* was further shifted toward monoterpenoids (72% monoterpenoids, 15% sesquiterpenoids) (Table S13), suggesting that domestication and improvement have increased the relative abundance of volatile sesquiterpenoids in cultivated genotypes of *H. annuus*. However, given that total volatile production is far lower in cultivated sunflower than wild *H. annuus*, even accounting for these shifts total monoterpenoid and total sesquiterpenoid abundances are quantitatively on average 6-8 times lower in both petals and leaves (Table S3, Table S12). The abundance of nonvolatile sesquiterpene lactones has been previously demonstrated to be far higher in wild *H. annuus* accessions than in cultivated accessions (Prasifka et al., 2015), such that our findings here extend this pattern to volatile sesquiterpenoids and monoterpenoids as well.

A broader assessment of leaf and petal volatile profiles across 40 species of wild *Helianthus* using identical sampling and analytical methods identified approximately 500 compounds, with no single compound shared among petals of all species, and only four compounds shared among leaves of all species (Bahmani et al., 2022). Across the genus, total volatile abundance varied over 130-fold in leaves and 320-fold in petals, with the proportion of terpenoids varying from 9–99% of volatile abundance in petals and 29–99% in leaves (Bahmani et al., 2022). The balance of terpenoid subclasses varied from 2–92% monoterpenoids and 0–91% sesquiterpenoids among species in both petals and leaves (Bahmani et al., 2022).

**Table 3.** Proportional contribution (based on mass-normalized peak area) of major compounds identified across the Core 12 genotypes in each of the four organs, as assessed by SPME-GC-MS. Values represent the mean percentage for each listed compound, calculated across all replicate samples, and the grand mean across all genotypes. Percentages may not sum to 100% due to rounding

| | Mean | SAM 020 | SAM 022 | SAM 027 | SAM 093 | SAM 094 | SAM 176 | SAM 185 | SAM 191 | SAM 203 | SAM 237 | SAM 240 | SAM 262 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Petal | | | | | | | | | | | | | |
| Alpha-Pinene | 49.7 | 44.6 | 36.2 | 40 | 53.7 | 37.7 | 77.1 | 52.2 | 53.3 | 57.6 | 33.2 | 51.8 | 59.4 |
| Sabinene | 22.6 | 25.9 | 27.4 | 27.9 | 18.8 | 31.3 | 10.9 | 23.8 | 23.8 | 11.2 | 29.5 | 21.7 | 19.3 |
| Gamma-Terpinene | 5.1 | 6.1 | 6.0 | 5.6 | 4.5 | 6.5 | 2.5 | 4.5 | 4.8 | 4.1 | 8.0 | 5.4 | 2.9 |
| Terpinen-4-ol | 3.7 | 5.0 | 5.8 | 3.3 | 5.0 | 3.9 | 1.5 | 2.5 | 2.9 | 2.1 | 6.5 | 2.7 | 2.9 |
| Beta-Gurjunene | 2.6 | 2.2 | 1.8 | 5.2 | 4.5 | 2.5 | 1.6 | 3.5 | 1.4 | 0.1 | 4.8 | 1.5 | 1.8 |
| Alpha-Terpinene | 2.4 | 3.2 | 3.2 | 3.0 | 1.2 | 3.2 | 0.7 | 2.3 | 2.0 | 1.3 | 4.1 | 2.6 | 1.6 |
| O-Cymene | 2.1 | 2.1 | 2.5 | 2.1 | 2.4 | 2.2 | 1.0 | 1.9 | 2.3 | 2.3 | 3.7 | 1.7 | 1.4 |
| Disc Floret | | | | | | | | | | | | | |
| Alpha-Pinene | 52.6 | 45.1 | 51.7 | 48 | 54.3 | 40.6 | 62.1 | 55.4 | 62.2 | 60.6 | 34.7 | 57 | 59.9 |
| Sabinene | 16.3 | 22.5 | 12.2 | 15.3 | 15.1 | 25 | 13.8 | 15.2 | 10.7 | 9.0 | 24.1 | 13.8 | 19.2 |
| D-Limonene | 5.3 | 6.0 | 6.0 | 7.2 | 6.1 | 3.6 | 5.7 | 2.3 | 4.6 | 6.6 | 7.1 | 4.7 | 4.1 |
| Gamma-Terpinene | 4.1 | 5.6 | 3.2 | 4.6 | 3.6 | 7.4 | 3.6 | 3.2 | 3.1 | 2.5 | 5.9 | 4.0 | 3.0 |
| Beta-Gurjunene | 3.1 | 4.4 | 6.1 | 4.7 | 2.7 | 3.2 | 1.2 | 3.1 | 2.3 | 0.3 | 5.2 | 2.6 | 1.6 |
| Terpinen-4-ol | 2.1 | 2.2 | 1.4 | 2.8 | 1.7 | 2.6 | 1.9 | 2.0 | 1.5 | 1.6 | 3.2 | 1.6 | 2.2 |
| Alpha-Terpinene | 2.0 | 2.7 | 1.6 | 2.1 | 1.8 | 3.7 | 2.0 | 1.5 | 1.3 | 1.0 | 3.3 | 1.7 | 1.5 |
| Bornyl-acetate | 1.4 | 0.6 | 3.6 | 1.8 | 0.5 | 2.1 | 0.3 | 1.1 | 1.1 | 2.7 | 0.6 | 1.8 | 0.6 |
| O-Cymene | 1.4 | 1.5 | 1.2 | 1.4 | 1.3 | 1.7 | 1.2 | 1.5 | 1.3 | 1.5 | 2.0 | 1.2 | 0.7 |
| Methoxyphenyloxime | 1.2 | 0.7 | 1.8 | 1.9 | 1.3 | 0.5 | 0.4 | 2.7 | 0.6 | 0.7 | 1.3 | 0.4 | 1.6 |
| Beta-Cubebene | 1.1 | 1.7 | 1.5 | 0.5 | 0.7 | 0.4 | 1.2 | 1.3 | 2.2 | 0.5 | 1.5 | 0.4 | 0.7 |

*Table 3 (continued)*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bract | | | | | | | | | | | | | |
| Alpha-Pinene | 51.7 | 50.8 | 55.3 | 51 | 49.7 | 39.5 | 63.7 | 44.8 | 55.8 | 54.5 | 44.5 | 47.9 | 63 |
| Sabinene | 8.7 | 10.4 | 9.8 | 7.8 | 8.7 | 12.4 | 7.3 | 8.7 | 4.0 | 7.7 | 9.2 | 9.5 | 8.7 |
| Beta-Gurjunene | 8.0 | 10.7 | 8.8 | 11.5 | 7.0 | 10.5 | 4.0 | 11.3 | 10 | 0.8 | 9.9 | 6.0 | 5.8 |
| D-Limonene | 4.7 | 3.7 | 5.7 | 5.3 | 3.9 | 3.0 | 4.9 | 1.9 | 4.2 | 6.9 | 7.5 | 4.1 | 5.0 |
| Bornyl acetate | 3.7 | 0.6 | 1.8 | 3.4 | 6.6 | 4.9 | 0.6 | 6.6 | 1.1 | 5.2 | 3.5 | 6.6 | 3.1 |
| Beta-Pinene | 3.6 | 3.2 | 0.2 | 4.3 | 3.9 | 5.1 | 1.5 | 4.9 | 3.1 | 7.0 | 4.9 | 2.6 | 2.3 |
| Beta-Cubebene | 1.8 | 2.8 | 2.5 | 0.5 | 0.4 | 1.9 | 2.3 | 1.2 | 3.1 | 1.3 | 2.2 | 2.5 | 1.4 |
| Beta-Elemene | 1.4 | 1.8 | 1.2 | 0.8 | 1.2 | 1.3 | 0.9 | 1.4 | 2.5 | 1.5 | 1.6 | 1.3 | 1.2 |
| Gamma-Terpinene | 1.1 | 1.3 | 1.2 | 1.4 | 1.0 | 1.6 | 0.7 | 1.2 | 0.4 | 1.1 | 0.6 | 1.7 | 1.4 |
| Desmethoxyencecalin | 1.0 | 0.4 | 0.1 | 0.4 | 0.6 | 3.7 | 0.5 | 0.3 | 4.1 | 0.2 | 1.1 | 0.1 | 1.0 |
| Leaf | | | | | | | | | | | | | |
| D-Limonene | 23.6 | 20.8 | 29.5 | 23.1 | 23.8 | 21.3 | 32.4 | 19.0 | 19.2 | 23.8 | 19.3 | 23.6 | 27.1 |
| Beta-Cubebene | 13.8 | 15.4 | 14.6 | 12.2 | 8.1 | 13.4 | 10.4 | 13.3 | 22.5 | 13.4 | 17.2 | 15.8 | 9.0 |
| Sabinene | 10.5 | 9.1 | 9.8 | 11.3 | 12.8 | 13.6 | 11.3 | 13.7 | 6.0 | 8.3 | 8.3 | 6.9 | 14.4 |
| Alpha-Pinene | 9.2 | 6.3 | 8.2 | 6.5 | 10.1 | 8.1 | 12.4 | 11.7 | 6.5 | 10.8 | 7.0 | 9.3 | 13.6 |
| Methoxyphenyloxime | 4.1 | 8.0 | 5.9 | 3.1 | 8.6 | 0.8 | 2.6 | 3.3 | 3.1 | 5.4 | 1.9 | 0.8 | 5.8 |
| Beta-Cadinene | 3.7 | 5.0 | 3.4 | 4.0 | 3.9 | 3.4 | 3.4 | 4.6 | 5.2 | 2.6 | 4.0 | 3.7 | 1.9 |
| Caryophyllene | 3.1 | 4.2 | 2.7 | 2.7 | 3.4 | 2.8 | 3.1 | 3.3 | 4.3 | 2.1 | 4.1 | 2.8 | 1.8 |
| Gamma-Terpinene | 2.6 | 1.5 | 2.3 | 2.4 | 2.5 | 4.0 | 2.7 | 2.9 | 2.0 | 2.4 | 2.3 | 2.9 | 3.6 |
| Gamma-Cadinene | 2.5 | 3.0 | 2.5 | 2.8 | 2.6 | 2.3 | 2.4 | 2.8 | 3.5 | 1.7 | 2.5 | 2.4 | 1.3 |
| Endo-Borneol | 2.4 | 0.5 | 1.8 | 4.4 | 1.5 | 2.0 | 0.6 | 1.2 | 1.9 | 9.1 | 0.8 | 3.8 | 1.0 |
| Gamma-Muurolene | 1.5 | 2.1 | 1.6 | 1.6 | 1.4 | 1.5 | 1.4 | 1.7 | 1.9 | 1.5 | 1.6 | 1.4 | 0.7 |
| *Cycloundecatriene | 1.3 | 1.4 | 1.6 | 1.6 | 0.9 | 1.3 | 1.3 | 1.5 | 1.7 | 1.3 | 1.4 | 1.2 | 0.8 |
| Alpha-Muurolene | 1.3 | 1.9 | 1.1 | 1.1 | 1.2 | 1.5 | 1.2 | 1.2 | 1.8 | 1.2 | 1.5 | 1.4 | 0.7 |
| Alpha-Cadinene | 1.2 | 2.0 | 1.0 | 1.3 | 0.5 | 1.2 | 1.3 | 1.1 | 1.7 | 0.9 | 1.5 | 1.4 | 0.8 |

*Cycloundecatriene is an abbreviation of 1,5,9,9-tetramethyl-Z,Z,Z-1,4,7-Cycloundecatriene.

This dramatic variation in volatile abundance and composition indicates that the secondary and tertiary germplasm of sunflower is rich in quantitative phytochemical diversity that could be leveraged for cultivar improvement, far beyond that that exists in the primary *H. annuus* germplasm alone (Kantar et al., 2015).

**Contributions of monoterpenoids and sesquiterpenoids to volatile profiles**

Within and among organs, overall total volatile abundance was heavily influenced by the total production of monoterpenoids (Fig. 2). In reproductive structures, monoterpenoids dominated total volatile production, such that variation in total volatile abundance was not significantly correlated with the production of sesquiterpenoids or other compounds. In leaves, however, both total monoterpenoid and total sesquiterpenoid abundance contributed significantly to variation in total volatile production among genotypes, with $R^2$ values of 0.88 and 0.71 respectively (Fig. S2). In leaves, monoterpenoid and sesquiterpenoid production were weakly correlated ($R^2 = 0.39$), indicating that among genotypes increasing leaf volatile abundance is associated with a general increase in both major classes of terpenoids, but that monoterpenoid and sesquiterpenoid abundance do not move in lockstep.

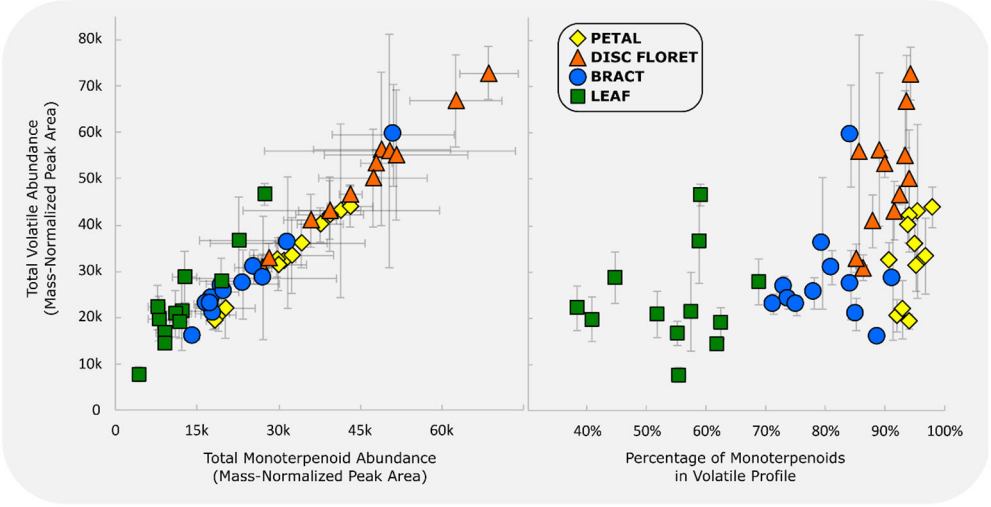


**Figure 2.** Contribution of total monoterpenoid abundance to total volatile abundance across organs and genotypes (left panel), and distribution of the organs and genotypes for total volatile abundance and proportion of monoterpenoids in the volatile profile (right panel). Points represent the mean across replicates of a single organ-genotype combination, with error bars representing the standard error of the mean.

Among genotypes, the proportion of the volatile profile comprised of monoterpenoids was highly correlated between leaves and bracts ($R^2 = 0.68$), as was the proportion of sesquiterpenoids ($R^2 = 0.80$), indicating that profile composition is not independent across these two organs and that genotype variation affects both organs simultaneously (Fig. S3). No significant correlations were observed between petal and disc floret proportions, or between proportions in these two organs and those in bracts or leaves, likely attributable to the low variation in sesquiterpenoid abundance in petals and disc florets.

**Underlying secondary metabolism and applications in biotic interactions**

The strong gradient in monoterpenoid-sesquiterpenoid balance observed between vegetative and reproductive structures across all Core 12 genotypes, as driven by variation in monoterpenoid production, is a major underlying driver of phytochemical differentiation among sunflower organs. Variation in this same monoterpenoid-sesquiterpenoid balance is observed within and among organs in wild *H. annuus* (Adams et al., 2017) as well as diverse *Helianthus* species (Bahmani et al., 2022), indicating that it is an inherent property of terpenoid secondary metabolism common to all sunflowers. High monoterpenoid production in reproductive structures is likely related to sunflower floral fragrance and its role in pollinator attraction, a trait that has to date received very little attention other than documenting variation among a few cultivars (Pham-Delegue et al., 1990; Bertoli et al., 2011). While the role of traits like floret morphology and nectar rewards in pollinator attraction have been systematically studied in cultivated sunflower using diverse germplasm (Mallinger & Prasifka, 2017; Portlas et al., 2018), the role of floral volatiles has been limited to valuable but narrow comparisons of few cultivars and honeybee choice and conditioning experiments with compounds derived therefrom (Pham-Delegue et al., 1986; Pham-Delegue et al., 1989; Pham-Delegue et al., 1990). The substantial quantitative variation observed among the Core 12 lines for disc floret volatile abundance, as well as relative composition of major compounds, indicates that the chemical signaling provided to pollinators is diverse among cultivated sunflower germplasm. Given this, broader screening of germplasm has the potential to identify particularly attractive floral fragrance profiles, either leveraging existing honeybee choice data or expanding this to wild bees given the critical pollinator services they provide to hybrid seed production where outcrossing is required, and potential to increase yield in oilseed production despite self-compatibility (Greenleaf & Kremen, 2006; Portlas et al., 2018). Optimization of cultivar floral fragrance would be highly facilitated by description of the genetic architecture of disc floret volatiles in sunflower (Dudareva & Pichersky, 2006; Pichersky & Dudareva, 2007).

The documentation provided here that cultivated sunflower exhibits substantially lower volatile abundance than wild *H. annuus* or other wild *Helianthus* may be an important factor related to the observation that cultivated sunflower is more susceptible to a wide range of pests and pathogens than wild *H. annuus* (Rogers et al., 1987; Chen & Welter, 2002; Charlet et al., 2008; Michaud & Grant 2009; Mayrose et al., 2011). While sesquiterpene lactones have been shown to contribute to resistance against head-feeding insects like the sunflower moth *Homeosoma electellum* (Rogers et al., 1987; Prasifka et al., 2015), few assessments of the anti-herbivore or anti-pathogen effects of volatile monoterpenoids or sesquiterpenoids have been conducted in sunflower. However, adjusting the relative ratios of five major sunflower monoterpenoids (alpha-pinene, beta-pinene, limonene, camphene, and bornyl acetate) substantially alters lure attractiveness to the red seed weevil *Smicronyx fulvus*, and substitution of sabinene reduces attraction further (Roseland et al., 1992). Ontogenetic variation in relative ratios of these same sunflower volatiles has also been demonstrated to alter attractiveness to the brown marmorated stink bug *Halyomorpha halys* (Wong et al., 2021). These examples indicate that at minimum, manipulation of volatile profiles is a potential route to developing cultivars that have reduced attractiveness to pests. The lack of tight phenotypic integration in volatile profiles among sunflower organs reported here suggests that volatile metabolism can be independently optimized in vegetative and reproductive structures.

Now that a substantial diversity of volatiles has been documented in a representative cross-section of cultivated sunflower germplasm, an obvious next step is to leverage the full Sunflower Association Mapping panel to identify the genetic architecture of volatile abundance and composition in sunflower. The genetic resources now exist to identify the genetic basis of this phytochemical variation (Badouin et al., 2017; Hübner et al., 2019), and permit optimization of sunflower volatile profiles through either genetic engineering or traditional breeding approaches. Furthermore, expanding investigation of the genetic basis of volatile production to wild *H. annuus* through use of the sunflower pangenome (Hübner et al., 2019) is a likely avenue for increasing volatile production beyond the range currently described in cultivated sunflower, whether for improvement of floral fragrance output, repelling pests, or other goals. Beyond this, leveraging the much broader qualitative and quantitative diversity available for volatiles in wild *Helianthus* is a yet another route if wild *H. annuus* diversity proves insufficient for a desired application (Kane et al., 2013; Kantar et al., 2015). Sunflower is already a more sustainable choice compared to many other crops, producing substantially lower greenhouse gas emissions than cereals or rapeseed both per hectare and per ton of yield (Debaeke et al., 2017), and the development of improved host plant resistance and improved pollinator-mediated seed set through phytochemical optimization of cultivars can further improve agricultural sustainability under a changing climate. In addition, the substantial genetic variation within sunflower in the composition of volatile terpenes deserves more attention in the context of terpenoid-based specialty biofuel production (Pausas et al., 2016; Mewalal et al., 2017).

## CONCLUSION

Volatile terpenoids in cultivated sunflower mediate biotic interactions and are of value for cultivar improvement. To clarify the level of terpene diversity in cultivated sunflower, terpene profiles were evaluated across four vegetative and reproductive organs in twelve cultivated sunflower genotypes (known as ‘Core 12’) that capture about 50% allele diversity in a sunflower association mapping population. Results indicated a significant compositional diversity of volatiles among the studied lines, though substantial reduction in total volatile abundance relative to wild *H. annuus*. In the Core 12 genotypes, leaves produce a mixture of mono- and sesquiterpenoids, while reproductive organ composition is monoterpenoid dominated, although absolute sesquiterpenoid production is roughly similar across organs. Across the Core 12 genotypes, there is limited qualitative but substantial quantitative variation in volatile profiles, suggesting that for breeding increased volatile production the use of wild *H. annuus* and other wild *Helianthus* germplasm may be necessary.

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AUTHOR CONTRIBUTIONS. CMM and JAD designed the study. CMM and JAD led the greenhouse experiment and JAD led sampling of tissue. JAD and KB prepared samples for analysis. KB conducted GC-MS. MG and KB quantified metabolomic data from raw GC-MS output. KB and CMM performed data analysis and created figures. KB and CMM wrote the manuscript with input from MG and JAD.

DATA AVAILABILITY. Data used for this study are included in the supplement (Appendix 1) as well as the Dryad Digital Repository (Dryad: 10.5061/dryad.6wwpzgn31).

SUPPLEMENTAL MATERIAL: https://agronomy.emu.ee/index.php/category/running-issue/?aid=9386&sa=0#abstract-9379

Appendix1: https://agronomy.emu.ee/index.php/category/running-issue/?aid=9387&sa=0#abstract-9379

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## SUPPLEMENTAL MATERIAL

**Table S1**. The 12 cultivated sunflower genotypes (inbred lines) for which volatile phytochemistry was assessed in this study.
**Table S2.** Total number of volatile compounds detected and identified via SPME-GC-MS in each genotype (pooling all four organ types assessed), as well as proportional breakdown of compounds.
**Table S3.** Volatile profiles as assessed by SPME-GC-MS in the four organ types assessed (averaged across the twelve plant genotypes).
**Table S4**. Volatile profiles as assessed by SPME-GC-MS in the twelve plant genotypes assessed (averaged across the four organ types).
**Table S5.** Fold-change variation in volatile compound profile metrics across the Core 12 genotypes and four organ types (48 genotype-by-organ combination means).
**Table S6.** Fold-change variation in volatile compound profile metrics across the Core 12 genotypes within the four organ types.
**Table S7.** Fold-change variation in volatile compound profile metrics across the four organs within each Core 12 genotype.
**Table S8.** The four most abundant compounds identified by SPME-GC-MS as a percentage of the overall volatile profile across the Core 12 genotypes and four organ types (48 genotype-by-organ combination means).
**Table S9.** The most abundant compounds identified by SPME-GC-MS as a percentage of the overall volatile profile in each of the Core 12 genotypes across the four organ types.
**Table S10.** Total number of volatile compounds detected and identified via SPME-GC-MS in *Helianthus* petals in recent studies, as well as the proportional breakdown of compounds.
**Table S11.** Total number of volatile compounds detected and identified via SPME-GC-MS in *Helianthus* leaves in recent studies, as well as the proportional breakdown of compounds.
**Table S12.** Volatile profiles as assessed by SPME-GC-MS in leaves and petals of one population of wild *Helianthus annuus* from Konza Prairie, Kansas (KON) by Bahmani et al. (2022).
**Table S13.** Volatile profiles as assessed by SPME-GC-MS in leaves of 20 wild populations of *Helianthus annuus* by Adams et al. (2017).
**Table S14.** Volatile profiles as assessed by SPME-GC-MS in leaves ($n$ = 37) and petals ($n$ = 24) of 40 species of wild *Helianthus* by Bahmani et al. (2022).
**Figure S1**. Negative correlations among the Core 12 genotypes between alpha-pinene and sabinene in petals and disc florets.
**Figure S2.** Positive correlations among the Core 12 genotypes between the total monoterpenoid abundance, total sesquiterpenoid abundance, and total volatile abundance in leaves.
**Figure S3.** Positive correlations among the Core 12 genotypes between the proportion of monoterpenoids and sesquiterpenoids in bracts and the proportion of monoterpenoids and sesquiterpenoids in leaves.