Floral Scent Analysis: a primer for the collection and characterization of fragrance.

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Introduction:
Until recently, the majority of studies on pollination biology, plant reproductive ecology and floral evolution have fallen into one of two categories: those that provide brief verbal or metaphorical descriptions of floral scent, and those that ignore it completely. However, the remarkable diversity of floral odor “quality” (to human perception) within and between angiosperm families suggests that non-random patterns of scent chemistry exist, with potential links to specific plant lineages or pollinator classes. These patterns, combined with the general, well-documented importance of olfactory cues and signals to most flower-visiting animals (particularly insects), suggest that floral biologists who continue to ignore fragrance do so at the risk of misinterpreting selection on floral phenotypes in their systems.

Recent advances in analytical chemistry have made it possible to collect and identify the blends of chemical compounds that comprise floral odors. A thorough analysis of fragrance chemistry yields a list of such compounds, their relative abundance within an odor blend, and their absolute emission rates per unit floral mass, over time. These odor blends do not represent a fixed property of a given species; instead, they constitute complex floral phenotypes with spatial and temporal dimensions, and may vary within and between populations of the same plant species. The dynamic nature of floral scent demands that researchers define the spatial and temporal scales at which they wish to analyze fragrance for any plant species. This document is written to help motivated biologists to add fragrance analysis to their bailiwicks using sensitive, reproducible and modern methods.

1. Before you begin: know your flowers!
Floral scent analysis can be expensive and time-consuming, especially when your research requires that multiple sample replicates be analyzed. There are several non-technical preparatory steps that serve to simplify or focus the eventual chromatography.

A. Describe the flower’s odor in perceptual terms: is it powerful or weak? Does it smell like other flowers, fruit, animal waste or fungi? Can specific qualities be discerned, such as the presence of “mint” or “clove” notes? The answers to these questions are informative to an experienced chemical ecologist, as they suggest which classes of scent compounds will be encountered, and which analytical methods should be employed to identify them.

B. Determine the tissues of origin for the flower’s fragrance by dissecting flowers into individual organs (calyx, petals, androecium, etc.). The amount of floral material used depends upon the intensity of odor. Place specific flower parts into their own glass vials, cap them with parafilm or aluminum foil and let the odors equilibrate within. The odor-laden air above living tissue is known as headspace, and will be discussed repeatedly in this document. After 15-30 minutes, remove the stopper/cap and sniff the air within the vial, describing what you smell for each flower part, as above. If all parts smell the same, odor emission is diffuse across the flower, and spatial patterns in scent emissions are unlikely. Neutral red histological stain can be used to localize odor glands (“osmophores”), trichomes or secretory tissues within a specific floral organ. However, this technique produces “false positives” when nectaries, oil bodies or lipophilic tissues take up stain, and should be interpreted with caution.

C. Determine when odor is produced, and whether it follows a diurnal or nocturnal cycle. Many flowers remain open for several days and have staggered sexual stages, or remain turgid after pollination. Note the times of day when pollinators or florivores visit your flowers; odors produced during this time are most likely to be influenced by selection. Does odor intensity or quality change during the course of the day/night? Do flowers remain scented over several days, or after flowers have been pollinated? These observations provide guidelines for the interval in a flower’s lifetime during which odors should be analyzed. Circadian rhythms are strictly defined as rhythms that are maintained, with a slight shift in timing, under pure light or pure dark conditions, a response known as “free cycling”. Other, more plastic rhythms may simply result from light perception by the plant and are ablated by pure darkness.

2. Qualitative scent analysis: maximizing signal-to-noise ratios
You are now ready to collect floral scent and determine its chemical composition. This qualitative goal is distinct from calculating the amount of odor emitted by your flower, and requires that you maximize the amount of scent-producing floral tissue while minimizing the volume of headspace air above it. It is likely that no flower is truly scentless, so the key is to determine the threshold of detection for your plant’s odors. This statement implies
one of the most important maxims of fragrance research; there is no single, standard method by which all floral scents can and should be analyzed.

One excellent method for qualitative scent analysis involves passive adsorption of odors on the surface of a special fiber, known as Solid Phase Micro Extraction, or “SPME”. SPME fibers are modified syringes with retractable, adsorbent “needles” that are directly injected into the GC for thermal desorption (see diagram below). Here, floral tissue is enclosed in a clean glass flask or oven bag only slightly larger than the flowers, and odors reach equilibrium in the floral headspace. Weak odors (or those with high molecular weight) generally require about 30 minutes to equilibrate, whereas strong odors (or those with low molecular weight) tend to saturate after 15 min. You should increase equilibration time until you no longer detect additional compounds. I sometimes excise flowers or flower parts and place them into 50 mL glass beakers or seal them (with an impulse heat sealer) within tiny headspace “sachets” cut from oven bags. This approach, when combined with SPME, is a very effective way to maximize odor signal. 1 mL autoinjector vials are ideal for small flowers or dissected parts.

After equilibration, SPME fibers are inserted into the headspace chamber and exposed to its fragrance-rich air for an additional 15-30 minutes, then placed directly into the injection port of the gas chromatograph (GC), where the adsorbed odors are thermally desorbed onto the GC column. Some tinkering will be required to determine the amount of equilibration time, SPME exposure time and floral material necessary to collect and identify all odor compounds in your system. Start with a single flower and 15 minutes each for equilibration and exposure, then increase each of these independently until you no longer detect additional GC peaks. You can graph peak number against time or tissue and stop when the curve reaches an asymptote. Be aware that intense odors typically require very little equilibration and exposure time. Overexposure produces large, asymmetrical GC peaks that obscure the presence of other, less abundant compounds and may be difficult to clean from the GC column. Please try to avoid overloading the GC column with trapped odors.

SPME is a rapid, sensitive and immensely satisfying analytical technique, and the novice may be tempted to rush to publish their data. However, some finer points deserve consideration:

Be sure that you collect control SPME samples from the empty headspace chamber; do this in as clean a setting as possible, avoiding proximity to strong odors such as solvents, naphthalene, latex and rubber bands, hand lotions and perfumes. Beware: sunscreen and insect repellent stink, as do breath mints and chewing gum. These odors will find their way into your data, if you let them.

Collect control samples from vegetation, calyces and any specific floral tissues that prior observations suggest are responsible for odor production. Dissected flower parts produce wound artifacts (see below), which should be omitted from analyses.

Beware of co-eluting compounds (two peaks that overlap); repeat the GC-MS analyses using slow GC oven temperature ramps (3-5°C/min) and see if any new peaks appear (see below).

Try another GC column with different polarity. Some compounds elute poorly on polar columns (carbowax) but do nicely on non-polar ones (DB1, DB5) or vice-versa.

When you mix a solution of the identified compounds, does it smell like the flower? If not, you may have to use different methods to identify the full blend of compounds. A common example of this is ammonia, which does not chromatograph well, but is an obvious component of hawthorn (Crataegus) flower scent.
3. Quantitative fragrance analysis: integrating chemistry with emission rates

The qualitative methods used above will give you the best opportunity to identify fragrance components, especially those that represent minor fractions of the total blend. However, odors vary tremendously between species, and over time within species, in the rates at which they are emitted. Emission rates, in general, are responsible for the differences between strongly and weakly scented plants. Emission rate data are important for at least three reasons:

-(1) Behavioral assays with pollinators require that you use appropriate odor concentrations in manipulated natural or artificial flowers.
-(2) Phylogenetic comparisons of odor as a floral character require that you standardize its intensity (emission rate per mass of floral tissue, per unit time) as well as its chemical composition. This standardization makes it possible to identify interesting physiological phenomena (allometry, ectopic expression, up-regulation and compensatory patterns) in an evolutionary context, especially of habit or inflorescence structure vary in a lineage.
-(3) Investigations of environmental plasticity or heritable variation in odor as a character will require repeatable measurements of its emission over time, under specific environmental conditions.

The previous section described passive odor collection in saturated headspace using SPME. This is a “static headspace” method, in which scent compounds reach equilibrium between the gas phase in headspace air and the liquid phase in the floral tissues. This method offers little to no insight on the quantitative aspects of floral scent. Instead, we must use a “dynamic headspace” method, in which headspace air is replaced as it is sampled over time, using an adsorbent material such as activated charcoal, Tenax or Porapak polymers, packed within a glass or steel trap. This

Headspace chamber (A) is an inverted glass flask with aluminum foil gasket around stem, supported by ring stand (B). Oven bags also could be used as headspace chambers. During the equilibration period, fragrance compounds are emitted until vapor pressures equilibrate within headspace, SPME fiber (C) is introduced into headspace through a septum or gasket (arrow) and exposed for an empirically determined length of time. After exposure, the fiber is sheathed, removed from the chamber and inserted directly into the GC-MS injection port. Ambient (chamber only) and vegetative controls should be performed, as always.
Dynamic headspace apparatus for collection of floral scent from living, intact flowers. Headspace chambers are inverted glass sidearm flasks with aluminum foil gaskets around stems (ring stand support not shown). Incoming air is filtered through charcoal traps (1), enriched with plant volatiles and trapped by cartridges packed with adsorbent polymers (2). Y-tubes (3) and tubing connect multiple floral samples (A, C), vegetative (B) and ambient (D) controls to a single, portable vacuum source (4) at a controlled flow rate. Variations on this theme include using individual pumps for each replicate (if plants are widely dispersed), pushing air through pre-filters (1) with compressed air, or using light, flexible, disposable bags as headspace chambers.

can be done by “pushing” clean air through the headspace chamber, over the flower and through the trap with a filtered compressed air cylinder, or by “pulling” clean air through the apparatus with a vacuum pump. The trick is to do this at a flow rate sufficient to replace headspace air relatively frequently (at least once per 5 min.) without creating a vacuum that would alter natural emission rates. Flow rates are not absolute, but are determined relative to headspace volume. Flow rates of 200-250 ml air/min with headspace bags of ½ to 1L volume are sufficient to trap most “typical” floral scents perceived by humans.

How long should scent be collected? The answer to this question depends upon the biological questions you are asking. If the goal is to compare the scent profiles of different species for ecological or evolutionary reasons, then sampling duration should provide enough fragrance for minor components to be identified and temporal variation to be averaged. If the goal is to examine shorter-term phenomena, such as photoperiodic or post-pollination changes in odor, then collections of shorter duration are required. The exact time intervals must be determined experimentally for each species, and should be standardized per flower, or per unit fresh or dry floral mass for comparative studies.

How much adsorbent should one use in traps? There are several schools of thought on this, but the most important point is that the eventual method that you choose is sufficiently sensitive to address your question and leads to reproducible results. I initially chose 100 mg of Tenax and charcoal (later switched to Porapak Q) to trap Clarkia odors because that amount was suggested in the literature and I found it to be appropriate for the amount of odor emitted by my flowers over 12 hours. I used different volumes of hexane to elute my traps and found that 3 ml was required to completely flush the trapped odor compounds from them; this is called the “void volume”. However, I could not simply inject 1 µl from this 3 ml solution; it was too dilute. Solvent desorbed samples can be concentrated to much smaller volumes using a flow of gaseous nitrogen. Attach a flow regulator to a nitrogen tank, run Tygon and/or Teflon tubing to a clean Pasteur pipette, and use a ring stand to clamp it above the sample vial, using a flow that gently boils off the solvent. This kind of setup is attainable in most university laboratories. Unfortunately, some amounts of trapped odors may be lost during this phase, especially if the sample is weak. Also, relative ratios between compounds may be altered by selective evaporation.

An alternative approach is to make a much smaller trap, with 1-10 mg of adsorbent material, which is then eluted with a smaller volume (100-300 µl) of solvent. These samples require lower flow rates, shorter collection times and little to no concentration before injection into the GC. Roman Kaiser and others have used this approach to great advantage, and it is ideal if you can accurately measure and assemble traps with such small amounts of adsorbent. Orbo, Inc. makes such traps, which are sold by Supelco. HOWEVER, you must experimentally determine the optimal amount of time for odor collection using small traps. If you collect for too long, some compounds might “break through” and be lost, such that your samples will underestimate true emission rates. An easy way to do this is to position two traps in series, and elute from both. When you see scent compounds in the second trap, the vacuum pumps have been on too long.

Which adsorbents and solvents should one use? Olle Pellmyr and I performed head-to-head comparisons and found that Poropak Q and Tenax TA were comparable in the range of chemicals that they trapped, and each was better than charcoal, which is known to catalyze rearrangements of trapped odors. Poropak Q comes in smaller mesh sizes, so it tends to trap more efficiently than Tenax due to its larger surface area per unit mass. Now a modified form called “SuperQ”, which adsorbs less water vapor during trapping, has become available from Alltech Associates and other chromatography suppliers. Solvent choice is more of a compromise, in my view. I use hexane because it is a good solvent for most scent compounds, does not dissolve my adsorbents and is not too volatile for quantitative analyses. Its major drawbacks are universal impurities and the poor solubility of compounds like indole and vanillin in it. Pentane is cheaper and purer, but is easily lost to evaporation. Diethyl ether is a better solvent for vanillin but is frequently impure, flammable (!) and vaporizes in seconds, along with your sample. Methylene chloride is less volatile, but it
dissolves Tenax (!) and is quite toxic. Roman Kaiser, who is responsible for so much innovation in this field, regularly elutes very small adsorbent traps with a 9:1 mixture of hexane and ultra-pure acetone, to ensure maximum solubility.

**How should samples be stored before analysis?** One of the greatest challenges of scent analysis is how to collect and transport thermally sensitive trapped odors from remote field settings back to the lab. For many years my collaborators and I have struggled with diverse sources of ambient contamination, from Ziploc bags and oily aluminum foil to Styrofoam coolers and nasty rental-car air, not to mention sun-block, deodorant and insect repellent odors. My present strategy is the following:

1. Clean and flush super-Q traps with hexane, let dry in an empty fume hood, then wrap and seal in groups of 4 within fresh oven bags.
2. Pack these cartridges with dry ice or “blue” ice packs in a plastic (not foam) cooler.
3. Keep the cooler insulated from sun, heat, any strong odors; avoid plant samples and food!
4. Bring small aliquots of elution solvent (e.g. hexane) into the field with you, and elute the scent traps immediately after you finish collecting odor from the flowers. If you bring a small plastic rack, Pasteur pipettes and a bulb with you, this can be done quickly. Elute samples into 4ml (“1 dram”) borosilicate glass vials with Teflon lined screw caps, label them and store in cooler or -20°C if possible. The samples are safer this way than on the traps.
5. If humidity or rain water accumulated during the scent collection, it will interfere with your sample. Before concentration or injection into the GC, you can add small amounts of MgSO₄ or NaSO₄ to dry the sample. Unfortunately, this will remove some odors.
6. In addition to the ambient and vegetative controls, plan to elute additional traps to control for the storage conditions alone, and subtract these volatiles from your samples.

**How are emission rates calculated?** I use 2 methods to estimate odor emission rates:

1. The use of an **internal standard** results in an approximation of total scent emission based on the response factor for one compound that is added to the sample. While crude, this method acknowledges the reality that fragrances often consist of tens to hundreds of distinct compounds, and is preferable for comparative studies between several species. I add a known amount of toluene to each sample after concentration but before injection into the GC, then integrate all peaks and use algebra to convert peak areas to concentrations.

**Example:** Collect odor from 2 open flowers, each weighs 0.65 g in fresh mass, over 4 hours. Elute with 3 ml of hexane, concentrate down to 75 µl using gaseous N₂. Add 5 µl of 0.03% solution of toluene in hexane to the sample (now 80 µL total). Inject 1 µl of sample into GC-MS.

Density of toluene = 0.867 g/ml

0.03% toluene = 3 µl of toluene in 1 ml total (hexane) = 3 x 0.867 x 0.001 = 2.6 mg x 0.1 (for 1:10 dilution in hexane) = 0.26 mg x 0.0625 (for 5:75 dilution in sample) = 0.016 mg

Thus, each sample vial contains 16 µg of toluene before injection into GC. Total scent emission (µg) per sample = sum of all peak areas x 16 µg toluene x 80 µL peak area toluene

This number is divided by 4 (= µg scent per hr), then either divided by 2 (µg scent per flower per hr) or by 1.30 (µg scent per g fresh floral mass per hr).

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**Total ion chromatogram for *Nicotiana alata* floral headspace; only first 6 minutes are shown. Toluene internal standard peak is at far left, other peaks are floral compounds. Red lines indicate integration marks placed by the software program, and peak areas are generated in an Excel-like spread sheet.**
Why toluene? Internal standards usually are chosen arbitrarily, by virtue of what your plant of interest is producing as odors. I chose toluene because it is absent in most plants, and because it is chemically similar to benzaldehyde and other small aromatic compounds that my plants produce. Ideally, one should choose 2-3 internal standards that bracket the retention times and chemical properties of your volatiles, but are not likely to show up in your target species’ floral scent. Methyl stearate is used for this purpose by some chemical ecologists. Toluene would be a poor surrogate for estimating the emission rates of a large, aliphatic hydrocarbon like n-octadecane, which elutes at high GC oven temperatures on most columns. Likewise, linalool or benzyl alcohol would be poor choices because they are ubiquitous in floral scents.

2. The use of **external standards** allows you to generate dose-response or dilution curves for each scent compound that is important to you, avoiding the assumptions (and error) associated with internal standards. This approach is preferable for any focused study on one plant species, especially if you are tracking change in amount of odor due to post-pollination processes, genetic crosses or population level variation.

**Example:** Your studies of thistle show that 4 scent components (2-phenyl ethanol, benzyl alcohol, methyl salicylate and phenylacetaldehyde) are important in honey bee attraction, and you wish to track their changes pre- and post-pollination. You integrate peak areas in each chromatogram, as above, and you may even include an internal standard to control for inaccuracies in injection or concentration volume; this step is worthwhile if you hand-inject your samples into the GC using a syringe. You then prepare several dilutions of the 4 compounds in the same solvent you use for sample elution and inject them, smallest concentration first, into the GC. Integrate peak areas for each dilution and graph them vs. concentration in log scale; responses should be linear if the concentrations are within range of the mass spectrometer’s sensitivity.

Now, fit the peak areas from your chromatograms to the dilution curves and back calculate their concentration from these graphs (see red dashed line above). These dilution curves should be generated from pure standards in the same batch of analyses as the plant samples. If additional samples are run weeks to months later, another round of dilution curves should accompany them, because mass spectrometers are notorious for electronic drift. Clearly this involves more work than an internal standard, but it is far preferable when the compounds of interest are few and known. Nobody who has worked with eugenol (viscous fluid) or methyl cinnamate (solid crystal) in floral samples would be comfortable using toluene as a surrogate for their emission rates.

**How do I identify unknown scent compounds?** This question is a flash point for analytical chemists, who are trained not to rely on single analytical methods to identify unknown compounds. There is a fair bit of chance involved here; you
may encounter simple blends of less than 20 compounds or one major biosynthetic theme, or complex blends that would challenge any chemist to characterize fully. Here I describe a first pass at using GC-MS to identify unknowns. However, rigorous identification of all volatile compounds and their absolute molecular configuration may require much time, money and a battery of analytical approaches. This problem stems from unexpected dimensions of chemical diversity; some compounds may be new to science, with mass spectra that don’t match MS library entries or the offerings of suppliers such as Aldrich, Fluka or Sigma. Other compounds may appear as pairs of enantiomers that are not resolved using standard GC methods. If scent identification were easier, we’d probably know much more about fragrance at this point, and it would not represent such a frontier (and opportunity!).

One mouse click reveals the mass spectrum for geraniol, the boxed GC peak (bold arrow). GC peaks are populations of molecules eluting from the column at a given time. Mass spectra really are histograms of the ion fragments resulting when populations of molecules of a chemical species are fragmented by ionizing energy. The MS detects positively charged ion fragments (cations) that sometimes (esp. with CI) include the un-fragmented molecular ion, which indicates the molecular weight. The most abundant ion fragment (highest histogram bar) is referred to as the “base peak” (41 m/z in the geraniol spectrum above). A diagnostic ion in this sample is m/z 69, which results from the isoprene-like cation (C₅H₉) and indicates that the compound probably has a terpenoid carbon skeleton.

The interpretation of mass spectra through first principles (McLafferty’s rules) is a lost art, due to the availability of on-line MS libraries offered by NIST, Wiley and Adams. MS libraries are useful tools for learning what your spectra might be. The NIST, in particular, has a very user-friendly interface program with which you can browse the library by common name, IUPAC name or molecular formula; it is a powerful educational tool. However, most algorithms used to search MS libraries don’t incorporate retention times into their searches, often resulting in incorrect matches. MS libraries should be used to generate lists of potential matches for each GC peak, desidereata to be obtained from chemical suppliers or colleagues. In this way, I have accumulated a library of several hundred authentic standards over the past decade, stored in a -20 C freezer.

How does one winnow down the lists of possible matches? You must now co-inject standards with your floral samples to confirm that they co-elute, preferably on 2 different GC columns. This is laborious even for less than 10 peaks! A better alternative is to generate your own libraries of relative retention times using standard compounds. The logic of this approach, known as the Kovats Index, is that all GCs vary - and the temperature, pressure and flow parameters of your GC will not be universally comparable to those in other labs - but the relationship of unknowns to standard alkane
hydrocarbons on a given column should be universal. You can standardize the performance of your GC by creating a retention index based on when different scent compounds elute from the column vis a vis such standards.

Example: You are using a non-polar DB5 column, from which compounds generally elute in increasing size order, with sesquiterpenes and other compounds with molecular weights > 200 daltons retained until oven temperatures exceed 200 C. You first inject a mix of standard alkanes, increasing in carbon chain length from 8 (octane) to 21 carbons (heneicosane), using a GC oven temperature program that increases from 50-275 C at 10 C per minute. These compounds elute in a highly regular pattern, with roughly 1.15 minutes elapsing between peaks. You arbitrarily assign index numbers to each hydrocarbon, such that octane = 800, dodecane = 1200, eicosane = 2000, etc. Then, you inject a series of standard blends containing compounds likely to be found in floral scent, such as linalool, limonene, methyl salicylate, trans-β-ocimene and α-humulene. Using the following formula, you generate a retention index for each standard as a function of which two alkane hydrocarbons (HC) bracket its retention time (RT):

Kovats Index for compound X = (log RT X) – (log RT lower HC) / (log RT upper HC) – (log RT lower HC) + Number of Carbons for lower HC x 100

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>RT min.</th>
<th>Index</th>
<th>Standard</th>
<th>RT min.</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonane</td>
<td>7.792</td>
<td>900</td>
<td>dl-limonene</td>
<td>10.370</td>
<td>1043</td>
</tr>
<tr>
<td>decane</td>
<td>9.658</td>
<td>1000</td>
<td>linalool</td>
<td>11.492</td>
<td>1107</td>
</tr>
<tr>
<td>undecane</td>
<td>11.392</td>
<td>1100</td>
<td>E-B-ocimene</td>
<td>12.000</td>
<td>1056</td>
</tr>
<tr>
<td>dodecane</td>
<td>13.017</td>
<td>1200</td>
<td>m. salicylate</td>
<td>13.233</td>
<td>1215</td>
</tr>
<tr>
<td>tridecane</td>
<td>14.540</td>
<td>1300</td>
<td>a-humulene</td>
<td>17.225</td>
<td>1494</td>
</tr>
<tr>
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<td>1400</td>
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<td>pentadecane</td>
<td>17.300</td>
<td>1500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hexadecane</td>
<td>18.567</td>
<td>1600</td>
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For linalool, the lower HC would be undecane, the upper would be dodecane, and its Kovats index would be 1107. This relationship should hold for any DB5 column with a constant rate of oven temperature increase. Now repeat for a polar column like DBwax.

What if you can’t obtain a certain compound? This is a common dilemma, especially for sesquiterpenes and for most enantiomers. There are at least two solutions. The more respectable solution is to persuade a synthetic chemist to collaborate with you, and test several candidate structures for co-retention and other chemical properties consistent with your sample. A quicker approach is to reproduce the chromatographic conditions used by researchers who have published analyses of other odors that contain your desired compound, and compare your retention index with theirs. The risk inherent to this approach is that the comparison is only valid if the work is reputable; read closely to determine whether and how these authors authenticated their standards. Robert Adams’ Kovats indices and the analyses published in the Journal of Essential Oil Research (particularly by Brian Lawrence) are reliable for these purposes. However, GC-MS alone will not allow you to separate chiral compounds, identify the atomic composition of compounds new to science or determine their absolute configuration (e.g. the location of a double bond within an alkene, and whether the compound is cis or trans). Sooner or later, all scent biologists require the services of analytical chemists. The rewards include the thrill of finding novel compounds and the opportunity to test their importance in behavioral assays.

Reading List: In a technology-dependent field such as plant volatile analysis, reading lists tend to change dramatically from year to year. However, the following books and journal articles provide good coverage of fundamental questions related to floral scent analysis.

Basics: The essential techniques, described by the masters.
Application of Methods: Options and comparisons, with relevance to floral odor.


Biological Considerations: Reminders that fragrances have histories and functions.


