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REVIEW ARTICLE

THE SMELL OF ENVIRONMENTAL CHANGE: USING FLORAL SCENT TO EXPLAIN SHIFTS IN POLLINATOR ATTRACTION[1]

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As diverse environmental changes continue to influence the structure and function of plant–pollinator interactions across spatial and temporal scales, we will need to enlist numerous approaches to understand these changes. Quantitative examination of floral volatile organic compounds (VOCs) is one approach that is gaining popularity, and recent work suggests that floral VOCs hold substantial promise for better understanding and predicting the effects of environmental change on plant–pollinator interactions. Until recently, few ecologists were employing chemical approaches to investigate mechanisms by which components of environmental change may disrupt these essential mutualisms. In an attempt to make these approaches more accessible, we summarize the main field, laboratory, and statistical methods involved in capturing, quantifying, and analyzing floral VOCs in the context of changing environments. We also highlight some outstanding questions that we consider to be highly relevant to making progress in this field.

Key words: climate change; flower; headspace sampling; multivariate analysis; plant–pollinator interactions; volatiles.

Historically, pollination biologists have focused on visual plant traits to help explain patterns in plant–pollinator interactions. These plant traits include flower color, size, symmetry, and reward type, among others. Typically these traits are easily quantifiable, and have formed the basis for "pollination syndromes" (Faegri and Pijl, 1979). Floral scents, as qualitatively perceived and described by biologists, were also included in some pollination syndromes. Flower odors were described as "fruity," "pleasant," "strong," "perfumy," "of decay," and so on, with each broad scent indicative of attracting different pollinator types (Baker and Hurd, 1968; Waser and Ollerton, 2006). In the past quarter century, with the development of new technologies, it has become far easier to collect, identify, and quantify plant volatile organic compounds (VOCs), especially under field conditions. Advances have occurred in detector sensitivity (e.g., mass spectrometers) and especially in headspace sampling (i.e., the airspace surrounding plants), which offer a more accurate picture of VOCs emitted by plants than older techniques (e.g., solvent extractions or steam distillation). To date, these techniques have been most extensively applied to the examination of leaf VOCs in the context of plant–herbivore interactions, in which leaf VOCs strongly mediate interactions across multiple trophic levels (i.e., tritrophic interactions; Dicke, 2015). Thus, although studies of floral VOCs predate those focused on leaf VOCs and plant–herbivore interactions by 30 years, pollination ecologists have been slow to rigorously incorporate chemically based perspectives in research approaches to understand plant–pollinator interactions (Raguso, 2008a, 2008b).

Although the field of plant–herbivore interactions has benefited from a quantitative understanding of chemically mediated signaling across trophic levels, most studies of plant–pollinator interactions do not invoke floral VOCs, although this is rapidly changing. The majority of studies on floral VOCs, especially early investigations, aimed only to catalog the VOCs produced and not the functional consequences of those VOCs for pollinator foraging (Raguso, 2008a). Recent studies have shown that the combinations of VOCs presented by plants via their flowers—not surprisingly—send signals to pollinators and influence plant–pollinator interactions (e.g., Suchet et al., 2011; Farré-Armengol et al., 2015; Larue et al., 2016). Floral VOCs can vary diurnally (e.g., Loughrin et al., 1990; Majetic et al., 2007), spatially among populations (e.g., Majetic et al., 2008), spatially across environmental conditions and geographic ranges (e.g., Majetic et al., 2009a; Soler et al., 2011), with floral genders (e.g., Ashman et al., 2005; Ashman, 2009), and with floral color (e.g., Odell et al., 1999). Pollinator discrimination among plants with variable floral VOCs can also influence plant fitness (e.g., Majetic et al., 2009b) and patterns of selection (e.g., Parachnowitsch and Kessler, 2010; Parachnowitsch et al., 2012). In fact, floral VOCs may be subject to stronger selection pressures than other floral traits in some plant species (Parachnowitsch et al., 2013). Patterns of floral VOCs, their influence on pollinator attraction, and subsequent effects on plant reproductive success and selection on plant traits are complex and context dependent (see Junker and Parachnowitsch, 2015 for a review). There are now clear, quantified roles for floral VOCs in the formation (or breakdown) of pollination syndromes (e.g., Schiestl and Dötterl, 2012; Schiestl and Johnson, 2013) and plant reproductive isolation (e.g., Peakall and Whitehead, 2013; Byers et al., 2014; Bischoff et al., 2015; Schiestl, 2015; Campbell et al., 2016), as well as a growing

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understanding of the interplay between pollinators and herbivores in mediating selection on floral VOCs (e.g., Kessler and Halitschke, 2009; Schiestl, 2010; Theis and Adler, 2012; Kessler et al., 2013).

The use of portable volatile collection systems in combination with gas chromatography–mass spectrometry (GC-MS) and sophisticated statistical analyses allows for in-depth investigation of how complex blends of floral VOCs influence pollinator attraction and structure plant–pollinator interactions. In particular, these methods have recently been used to quantify how different components of environmental change, either singly or in combination, affect floral scent (e.g., Farré-Armengol et al., 2014), and some studies extend the effects to include implications for pollinator attraction (e.g., Burkle and Runyon, 2016). Despite the fact that scientists worldwide are trying to understand the ecological effects of global environmental change on species interactions, we find it notable how few are using a chemical ecology approach to understand how volatile organic compounds may contribute to a predictive understanding of shifts in species interactions.

Several potential reasons underlying a lack of enthusiasm for this approach include the view that quantifying VOCs requires expensive equipment and sophisticated knowledge about how to use it and interpret the results. Time can be an additional barrier to the study of floral VOCs. Time is an expensive and often limited resource, but substantial time is required to collect an adequate number of samples; furthermore, GC analysis—particularly peak identification and sometimes quantification—cannot be automated and therefore can take much time to complete. To some extent, we recognize that some of these potential barriers pose real hindrances to adoption and frequent use. However, the development of relatively inexpensive headspace techniques and sensitive benchtop GC-MS systems has improved the accessibility of VOC analysis. Moreover, pollination ecologists can overcome many of these barriers by simply collaborating with chemists (Raguso et al., 2015). Despite these barriers, the study of floral VOCs is an exciting and rewarding endeavor and is more important than ever if we are to understand and mitigate pollinator declines and climate change impacts. Here, we summarize some of the main methods used to quantify and analyze floral volatiles in the context of plant–pollinator interactions under environmental change to facilitate the use of these approaches.

In this paper, we describe how these methods can be used to better understand how global environmental changes affect plant–pollinator interactions, emphasizing the merits and limitations of the most commonly used techniques. First, we provide an overview of the observational and manipulative methods that can be used to emulate and explore effects of environmental change on plants. Next, we summarize practical approaches to capturing and quantifying floral VOCs. We then discuss statistical methods that can be used to link environmental changes to shifts in complex blends of floral volatiles and plant–pollinator interactions. Lastly, we highlight several of the numerous outstanding questions that remain in our understanding of floral volatiles and how we may incorporate them into investigations of plant–pollinator interactions to better understand the current and projected effects of environmental changes at different spatial and temporal scales.

EARLY HISTORY OF VOLATILE COLLECTION METHODS

For centuries, it has been suspected that flowers emit odors to attract pollinators (e.g., Sprengel, 1793; Müller and Darwin, 1883). Despite this long-held notion, interactions between plants and pollinators—until very recently—were perceived as being largely visually guided (Raguso, 2008b). The ease of measuring visual cues and the technical difficulties of capturing and identifying VOCs have clearly contributed to this visual bias. This is in contrast to studies on interactions between plants and herbivores, which have long focused on plant chemistry as a key mediating factor (Ehrlich and Raven, 1964). Early studies on floral VOCs focused on orchids and attraction of male euglossine bees (Knudsen and Tollsten, 1993). In fact, the first headspace collections from plants were by Dodson and Hills (1966), who placed orchid flowers into plexiglass boxes, withdrew scent-laden headspace with a gastight syringe, and directly injected it into a GC. A key finding by Dodson and Hills (1966) was that floral scent is composed of mixtures of many volatile compounds. Hills et al. (1968) used this method to survey fragrances of 150 orchid species and identify 11 of the most abundant floral VOCs. The following year, Dodson et al. (1969) experimentally demonstrated behavioral consequences of orchid VOCs for pollinators by showing that the VOCs attracted orchid bees. Even with these early advances in elucidating the importance of floral VOCs, their inclusion in our conceptualization and understanding of plant–pollinator interactions has been slow (Raguso, 2008b). However, this is changing rapidly as studies of floral VOCs have increased substantially in recent years and have demonstrated that these olfactory cues play important roles in plant–pollinator relationships.

APPROACHES

Plants experiencing environmental changes**—**There are numerous ways investigators may choose to study the effects of environmental change on floral volatiles and pollinator attraction, and these will likely be dictated by their specific study questions and system in which they work. For example, the environmental conditions that a plant experiences can be manipulated at small scales by growing plants in growth chambers or in greenhouses to impose specific CO_2, temperature, or drought treatments. These potted plants can then be placed in the field for pollinator observations to link shifts in floral volatiles with pollinator attraction (e.g., Burkle and Runyon, 2016). Investigators may also use small-scale field experiments to manipulate environmental conditions of pre-existing or newly recruiting plants. For instance, there are numerous methods, including open-top chambers, snow fences, and rain diverters, that are regularly used to alter temperature, precipitation, or disturbance regimes (e.g., prescribed fire) in the field. Larger-scale manipulative studies are possible, for example, using free-air carbon dioxide enrichment (FACE) plots, although we are not aware of any such studies that measure floral VOCs and compare them to those produced in control plots. Other abiotic factors that can be experimentally manipulated and are known to alter floral scent include air pollution (McFrederick et al., 2008; Fuentes et al., 2016) and nutrient availability (Majetic et al., 2016). Biotic interactions, such as herbivory or disease, experienced by plants can be manipulated by caging caterpillars or other herbivores on leaves, applying plant hormones like jasmonic acid, or infecting plants with pathogens (e.g., Kessler and Halitschke, 2009; Schiestl et al., 2014; Hoffmeister et al., 2016). As with any ecological experiment, these manipulations provide the advantage of isolating the effects of specific mechanism(s) on plant traits and floral VOCs while maintaining control over many of the other conditions that the plants experience, but offer a limited scale of inference.

Alternatively, natural variability in environmental conditions across time or space can be used to investigate the potential effects of environmental change on floral volatiles (Fig. 1). For example, one could field sample floral VOCs of plants growing along environmental gradients or use time-for-space substitutions to sample floral volatiles of plants over multiple years that vary in environmental conditions. Research investigating genetic and geographic patterns in plant secondary chemistry also offers methodological insights to those interested in conducting similar studies with compounds related to floral scent (e.g., Keefover-Ring and Linhart, 2010; Holeski et al., 2013; Bravo-Monzón et al., 2014; Keefover-Ring et al., 2014; Pratt et al., 2014). Although taking advantage of naturally occurring environmental conditions offers a broader scale of inference, it is more difficult to attribute any shifts in floral volatiles or pollinator behavior to a particular environmental factor, and any patterns in pollinator attraction may be related to differences in local pollinator communities (e.g., Devoto et al., 2005; Bates et al., 2011). Exhaustively detailing the numerous experimental and observational approaches for investigating the effects of environmental change on plant traits and floral volatiles is beyond the scope of this paper, and we focus on the methods used to quantify floral volatiles once the study system has been identified.

***Collecting and quantifying floral VOCs*—**Quantification of floral VOCs is important because the main effects of environmental changes on floral VOCs of a plant species are likely to be shifts in the relative abundances of compounds emitted, and not necessarily changes in the identity of which volatiles are produced. This consistency in volatile production is presumably due to strong selection pressure for minimal intraspecific variability in floral VOCs to maximize signal fidelity (Wright and Schiestl, 2009). In addition, environmental change, notably temperature, can alter the rate of emission of some compounds more than others due to differences in volatility or activity of temperature-specific enzymes (e.g., terpene synthases; Farré-Armengol et al., 2013, 2014). Alternatively, the indirect effects of environmental change on floral VOCs are likely to be complex and may involve shifts in the identity of volatiles produced. For example, herbivory is expected to increase with global change (e.g., Huberty and Denno, 2004; Scherber et al., 2013), and insect herbivory is known to induce qualitative changes in floral VOCs (Kessler and Halitschke, 2009). However, we found that plants experiencing an experimental drought treatment produced the identical suite of floral VOCs as plants in control or herbivory treatments, but drought strongly affected the quantities emitted of each of these compounds (Burkle and Runyon, 2016). Importantly, visual floral traits of the four forb species examined responded in the same way to experimental treatments, but floral VOCs and pollinator visitation changed in species-specific ways; these results suggest that VOCs may be relatively more informative to pollinators than visual traits in a changing environment (Burkle and Runyon, 2016). Increasing temperatures have been found to have similar quantitative effects on floral VOCs (reviewed in Yuan et al., 2009; Scaven and Rafferty, 2013; see also Sagae et al., 2008; Farré-Armengol et al., 2014) and biogenic VOCs in general (reviewed in Peñuelas and Staudt, 2010). Thus, quantitative examination of floral VOCs is likely required to fully understand and predict how environmental change will alter plant–pollinator interactions.

Plant VOCs are most appropriately investigated by sampling the headspace surrounding plants. Compared with older methods like steam distillation and solvent extraction, headspace sampling of living plants is nondestructive and provides the most realistic representation of the VOCs naturally emitted from plants and encountered by pollinators (Raguso and Pellmyr, 1998; Knudsen et al., 2006). Another clear advantage of headspace sampling is that emission rates of floral VOCs can be determined. Headspace sampling is accomplished by enclosing flowers of living plants in a container to concentrate the VOCs and by pulling the air out across an adsorbent to trap VOCs, which are later eluted with solvent or heat for GC-MS analysis. Glass and plastics such as Teflon are commonly used materials for headspace collection chambers, but can be expensive and put undesirable weight or constriction on flowers; clear polyethylene cups with dome lids (Appendix 1) work well and are inexpensive, lightweight, available in several sizes, and relatively free of contaminants (e.g., Kessler and Halitschke, 2009; Burkle and Runyon, 2016). To use these polyethylene cups, a hole is drilled in the bottom to accommodate the adsorbent VOC trap, and cotton is used to surround the stem or peduncle where it enters the dome lid to fill the airspace and protect plants from damage (Fig. 2). For taller plants, these cups can be supported using laboratory stands (in greenhouses) or garden stakes (in the field) to avoid undue bending or stress on plants (Fig. 2).

The most commonly used method of trapping plant VOCs from the headspace of a plant or flower utilizes adsorbent porous polymers that are packed into narrow glass tubes (Fig. 2, inset). There are many types of porous polymers available, and reviews exist to help guide selection (Raguso and Pellmyr, 1998; Dettmer and Engewald, 2002; Tholl et al., 2006). Available evidence indicates that VOCs trapped by the most commonly used porous polymers (e.g., Tenax TA, Porapak Q, HayeSep Q) are qualitatively similar (i.e., collect the same ratios of compounds), but absolute amounts of VOCs can vary (Agelopoulos and Pickett, 1998; Raguso and Pellmyr, 1998). Therefore, it is important to use the same adsorbent for all treatments and samples within a study and report as much information as possible to allow repeatability and comparison across studies. Porous polymers can be purchased in bulk, and premade volatile collection traps containing one or more porous polymers are commercially available (Appendix 1). Solid-phase microextraction (SPME; i.e., fibers coated with adsorbent[s] that are inserted into the plant headspace) is another option for VOC sampling. However, SPME is best suited for surveys of VOC blends or determining presence/absence of compounds because quantifying VOCs with SPME is difficult, and ratios of some compounds can vary compared to porous polymers (Agelopoulos and Pickett, 1998; Tholl et al., 2006).

An important consideration when collecting floral VOCs is whether to push filtered air into the system (termed "dynamic headspace"; Tholl et al., 2006). Pushing clean air into the headspace alleviates the potential for VOC artifacts caused by changes in plant physiology resulting from enclosing flowers within containers, which can lead to a buildup of oxygen and increases in humidity or temperature. The shorter the collection time, the less likely such effects will occur; however, the length of VOC collection depends on the study question and plant species involved. If VOCs must be collected for extended periods of time (e.g., >1 h), examining the degree to which enclosure affects VOC emissions would be worthwhile. The disadvantages of supplying air includes the challenge of assuring that only clean air is introduced and, especially for field applications, the portability of such systems. Benchtop volatile collection systems are commercially available as are battery-operated portable systems with push-pull airflow capabilities (Appendix 1). For pull-only applications, small battery-powered vacuum pumps, initially designed for personal sampling of airborne contaminants, work well and are relatively inexpensive (Appendix 1). For all headspace sampling techniques, but especially for pull-only setups, it is critical to sample from empty cups (i.e., the entire setup without the flowers) positioned nearby to identify possible background impurities in the ambient air; one disadvantage is that this can complicate detection and quantification of minor floral VOCs.

The most popular technique for plant VOC analysis is GC-MS. GC-MS technology and especially sensitivity have advanced greatly in recent decades. Low detector sensitivity hampered early studies on floral VOCs because very long sampling periods or sampling of many flowers were required to obtain adequate quantities of VOCs (Raguso and Pellmyr, 1998). Today, MS detector sensitivities are in the picogram or lower range, which—for many species—permits short sampling times. This combined with nondestructive headspace techniques allows for examining environmental effects on floral VOCs at fine temporal resolutions. The development of large mass spectral databases with search functions (e.g., the National Institute of Standards and Technology [NIST] Mass Spectral Library [https://www.nist.gov/srd/nist-standard-reference-database-1a-v14] and the Wiley Registry of Mass Spectral Data [McLafferty, 2016]) has aided identification of floral VOCs. These databases and search engines provide suggestions of VOC identity; further evidence of compound identity can be obtained by comparing retention times and mass spectra with commercially available authentic standards, when available. Additional confidence about VOC identity can be obtained using the Kovats retention index (Tholl et al., 2006). Knudsen et al. (2006) provide an overview of the distribution and a checklist of known floral VOCs, and online databases can further inform identification (e.g., http://www.pherobase.com). Due to the diversity of floral VOCs and the potential for false identifications, it is important to describe how each compound was identified when reporting methods in a publication. VOCs can be quantified relative to one or more internal standards and standardized on a per-flower or flower mass basis. Several excellent reviews are available that contain further information on plant volatile collection and analysis methods (Raguso and Pellmyr, 1998; Tholl et al., 2006; Tholl and Rose, 2006).

Statistical methods for analyzing floral volatile profiles—Once the volatile profiles of focal flowers have been quantified, statistical methods can be used to investigate patterns in VOCs and detect differences among treatments or along gradients (Fig. 1). It is important to emphasize that it is the suites of volatile compounds emitted by a flower *in combination* (i.e., the floral "bouquet" or "blend") that are thought to influence pollinator attraction, and rarely the presence or quantity of single compounds taken individually (when single compounds prove important for pollinator attraction, it is typically in highly specialized systems or species interactions). Thus, we advocate for analytical methods that fully consider the "multi-species" nature of volatile data.

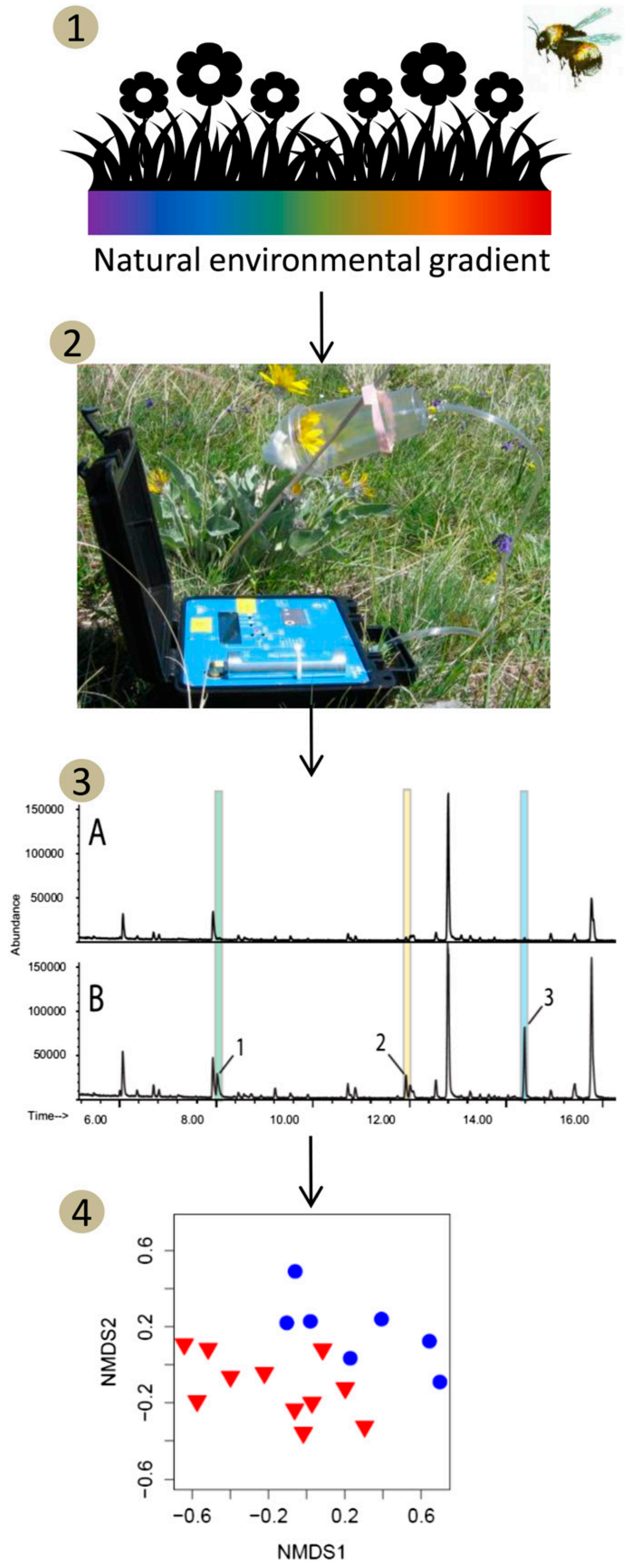

Fig. 1. Example workflow to investigate how the floral VOCs produced under changing environmental conditions may influence plant–pollinator interactions. (1) Across a natural environmental gradient, or through experimental manipulations of environmental conditions (not shown), measure plant traits and pollinator visitation. (2) Quantify floral VOCs by first enclosing flowers in a container and trapping emitted VOCs on an adsorbent trap using a portable volatile collection system. (3) Then elute, identify, and measure the VOCs using GC-MS. (4) Analyze and visualize patterns in VOCs.

Fig. 2. Collecting VOCs from flowers of arrowleaf balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt.) in the field. This setup is being used by the authors to study the effects of environmental change on floral scent and pollinator attraction. The inset shows a volatile collection trap containing a bed of the adsorbent HayeSep Q. Details of this setup are provided in Appendix 1.

Many types of analyses can be borrowed from community ecology (Van Dam and Poppy, 2008), in which volatile compounds can be viewed as "species" and the plants from which the volatiles were sampled as "sites." For example, Jaeger et al. (2016) recently used the random forest classification algorithm (Breiman, 2001) to identify different sagebrush species and subspecies using their volatiles, instead of difficult-to-discriminate morphological features or molecular markers. Random forest has advantages over other classification methods for selecting the minimum subset of volatiles for discrimination and other classification goals, and will rank these VOCs based on their importance for discrimination (Ranganathan and Borges, 2010). Principal component analysis, cluster analysis, and discriminant analysis have also been suggested as useful for volatile analysis (Ozawa et al., 2000; Degen et al., 2004; Van Dam and Poppy, 2008). When testing for differences in floral VOCs that may result from treatments manipulating environmental conditions, we have used permutational analysis of variance (such as that which can be performed by the 'adonis' function in the vegan R package) (Burkle and Runyon, 2016). Notably, further analysis can be performed to determine which compounds contribute most to the observed overall differences in volatile profiles between plant species, treatments, etc. (Sumner et al., 2003; Jansen et al., 2005). We have used similarity percentage analysis (SIMPER) to complete this task (Burkle and Runyon, 2016). Prior to analysis, numerous types of study designs may benefit from visualizations of patterns in floral volatile data using ordination, such as nonmetric multidimensional scaling (NMDS; Fig. 1).

***Linking VOCs and pollinator attraction*—**Whenever possible, it will be useful to quantify pollinator visitation (i.e., the identity and frequency of pollinator visitors per flower, per unit time) to the plants for which floral volatile data has been collected, to make direct connections between volatile composition and pollinator attraction (e.g., Kessler et al., 2008; Majetic et al., 2009b). The molecular basis for variation in floral VOCs can be simple in some plant species for some compounds, and manipulating compounds by experimentally adding them to flowers (e.g., Larue et al., 2016) or removing them through gene silencing are potentially powerful techniques that have shed light on attractive and repellent functions of VOCs (reviewed in Schiestl and Johnson, 2013). In factorial environmental manipulations or along environmental gradients (see "Plants experiencing environmental changes," above), genomics could also be used to link environmental change to shifts in gene expression, floral VOCs, and plant–pollinator interactions (Clare et al., 2013). These approaches, in combination with measurement of other plant traits known to be important for pollinator attraction, will help to quantify the importance of and control for individual plant variation, as well as to better understand implications for plant fitness and potential selection on traits (e.g., Ômura and Honda, 2005; Junker and Parachnowitsch, 2015).

It is important to note, however, that if the study design involves manipulating the environmental conditions experienced by individual plants, such that the scale of the arrangement of these plants in the field is (1) at a smaller, and possibly more patchy, spatial or temporal scale than exists in the natural variability for that environmental condition, or (2) at a smaller scale than the perception of and foraging range of the pollinators in your system, then you have essentially created a foraging choice experiment (e.g., Klinkhamer et al., 2001; Steffan-Dewenter et al., 2002; Westphal et al., 2006). Visitation patterns obtained from a pollinator choice experiment may not easily translate to predicted shifts in pollinator attraction if environmental changes occur more evenly across wider spatial or temporal scales (e.g., Burkle and Irwin, 2010). Creative manipulation of the spatial and temporal arrangement of manipulated plants, however, may provide unique insights to the effects of volatile plumes, neighborhood context, and scales of perception of foraging pollinators (e.g., Rusch et al., 2016). We also recognize the difficulties inherent in manipulating environmental conditions at large scales or using natural gradients in conditions (e.g., Farré-Armengol et al., 2016).

FUTURE DIRECTIONS

The effects of environmental change on floral VOCs and pollinator attraction are just beginning to be investigated. We do not yet know the conditions under which we might expect to see the largest shifts in volatiles with the greatest implications for plant–pollinator interactions. In fact, there are numerous, basic gaps in our biological knowledge of patterns in floral VOCs for most species. Increased understanding of these fundamental patterns in floral VOCs of species and their interactions with pollinators, as well as other mutualists or antagonists across their geographic ranges, is needed to predict which populations, species, and communities may exhibit shifts in species interactions most strongly influenced by environmental change via changes in floral VOCs. Here, we summarize some of the questions that we consider most pressing to answer to better incorporate floral VOCs into our understanding of the effects of environmental change on plant–pollinator interactions. Some of these questions are basic and do not directly address environmental change by themselves, but we feel that answering these questions will provide a critical foundation for increased understanding of environment–floral VOC–pollinator interactions.

Intraspecific variability in floral volatiles**—**What is the natural intraspecific variability in floral volatiles of different species (e.g., Azuma et al., 2001)? What explains intraspecific variability in the composition of floral volatiles (e.g., Svensson et al., 2005)? We might expect there to be a genetic signal in floral volatiles, in which individuals of a species that are more closely related to one another have more similar volatile profiles than distantly related individuals (sensu Karban et al., 2014). But we might also expect the past and current environmental conditions in which a plant is growing to influence its floral volatiles (e.g., Majetic et al., 2008, 2009a). Additionally, floral volatiles of an individual might change over the course of a day, over the lifetime of a flower (e.g., after ovules within a flower become fertilized), over the flowering season of an individual, or over the lifetime of a plant individual (e.g., Schiestl et al., 1997; Burdon et al., 2015). There may be interactions between typical temporal rhythms of floral volatile production and composition of a plant and the environmental conditions that it is experiencing, but this is poorly understood. Partitioning the variability in intraspecific floral volatiles into genetic and environmental components, and evaluating the speed by which these effects can manifest and shift will provide a baseline from which to make comparisons and will allow a better understanding of the plasticity and potential for adaptation under future environmental change.

Interspecific variability and context-dependency of floral volatiles**—**Across species, how variable are floral volatiles, what explains interspecific variability in floral volatile composition, and what are the implications for pollination (e.g., Füssel et al., 2007)? If conserved biochemical pathways are involved in the production of floral volatiles, we might expect closely related plant species to have similar floral volatiles. That is, some amount of the variation in floral volatiles is likely explained by phylogenetic relatedness among species (e.g., Raguso et al., 2006; Schiestl, 2010). As with intraspecific variation in floral VOCs, there is also likely to be some signal of environmental conditions that creates variation in floral volatiles across species (e.g., Majetic et al., 2009a). Plant species may respond in species-specific ways to environmental gradients, or there may be fundamental biochemical pathways that are affected by environmental conditions and reflected in similar shifts in floral VOCs across species (e.g., sensu Coley et al., 1985). As the community context in which a plant is growing changes (i.e., when the composition of co-occurring species relative to a focal plant differs across space or time), we may also want to know the implications for plasticity and local adaption of floral volatiles to optimize pollinator attraction (sensu Soler et al., 2011; Filella et al., 2013; Pierik et al., 2014; Ninkovic et al., 2016). One way to address these questions is to investigate floral VOCs across communities that span environmental gradients (e.g., Majetic et al., 2009a). As the homogenization of plant communities proceeds as a result of landscape management practices, the consequences for pollinator attraction and plant–pollinator interactions across landscapes with potentially homogenized volatile plumes is unknown. In other words, shifts in plant community composition for pollinator perception of volatiles across scales have potentially important, but unexplored, implications.

Floral VOCs of invasive species**—**Plant invasions are a major driver of environmental change, and the introduction of alien species into an existing community can disrupt the interactions among native species (e.g., Olesen et al., 2002; Morales and Aizen, 2006; Lopezaraiza-Mikel et al., 2007). Investigating the floral VOCs of nonnative plants and of native plants in invaded communities may provide insight into the potential mechanisms by which species interactions are disrupted. For nonnative plant species, how do their floral volatiles differ in their native vs. invasive ranges? To what degree are floral volatiles produced by nonnative plants similar to or distinct from the volatile profiles of flowers produced by native communities that have been invaded (sensu Llusià et al., 2010), and how do these similarities (or differences) influence patterns of pollinator attraction to native vs. invasive species? We might expect that nonnative flowers that smell similarly to native flowers may receive pollinator visits “by accident,” especially if other floral traits (e.g., flower color and shape) are also similar between the native and nonnative; in this scenario, native species could facilitate the pollination of nonnative species. It is also possible that the floral VOCs of nonnative species may present unique combinations of compounds that allow nonnatives to stand out and draw pollinators away from native flowers, thus contributing to invasiveness. As with native plants, the floral VOCs of nonnatives may present signals not only to pollinators but also to other mutualists or antagonists. For example, in its invaded range, a species of thistle (*Cirsium arvense* (L.) Scop.) produced compounds in its floral bouquet that attracted both pollinators and florivores (Theis, 2006).

Relationships between floral volatiles and other plant traits**—**Plant species often differ in their responses to environmental changes, and, taken individually, these studies can indicate an overwhelming array of species-specific effects with little predictive power. Perhaps even more discouraging for the synthesis of these idiosyncratic results is that the magnitude and even direction of responses to environmental context can be variable among plant traits being investigated. Therefore, a functional trait approach may provide a useful perspective for a more immediate understanding of the effects of environmental change on plant–pollinator interactions, via shifts in floral VOCs and other plant traits (e.g., McGill et al., 2006). To develop this functional trait perspective, it would first be useful to know the degree to which properties of floral volatiles are correlated with plant traits that are known to be important cues for mutualists and antagonists (i.e., plant height, biomass, floral size/display, seed production, etc.) within a species or across species (e.g., Dormont et al., 2010; Doubleday et al., 2013). To what degree are floral and leaf volatiles similar to each other (e.g., Kotze et al., 2010)? What are the patterns in floral volatile compounds produced by different parts within flowers (e.g., petals, sepals, anthers, stigmas) (reviewed in Muhlemann et al., 2014)? We might expect trade-offs between investment in floral volatiles vs. other traits. For example, smaller plants or those with smaller, less colorful flowers may produce larger quantities of floral VOCs or evolve floral volatile bouquets that are particularly noticeable and attractive to pollinators. Alternatively, if there is correlational selection or if particular suites of traits (including volatiles) are over-represented and attractive to different pollinator groups (i.e., syndromes), then we would expect positive relationships between floral volatiles and other traits. A better understanding of the relationships between floral VOCs and other plant traits, as well as the degree to which these relationships shift in concert under different environmental conditions, will help form a framework for predicting species and interactions that may be most vulnerable to environmental changes. Because floral VOCs can influence plant relationships with both mutualists and antagonists, the balance of attracting (or repelling) these players can have strong implications for plant growth and reproduction.

Effects of environmental changes on floral volatiles**—**Because floral volatiles appear to be immediately responsive to fluctuations in environmental conditions, we can view them as current and accurate signals to other organisms. However, there may also be longer-term or delayed effects of environmental shifts on floral VOCs, including effects that may span multiple generations. For example, to what degree does a drought in one year result in seeds and offspring that reflect ("remember") those conditions when they flower in some future year? This type of transgenerational memory has been shown for plant defenses (Holeski et al., 2012), but is just beginning to be explored for floral VOCs. Floral volatiles produced by perennial plants may carry signals of environmental conditions experienced in previous years, but the degree to which lags in the effects of environmental shifts across a growing season or across seasons is unknown. For instance, in a year when snowmelt is early, does a species that blooms mid- to late summer reflect those early-season conditions in the floral volatiles produced? Furthermore, we lack an understanding of how long these effects could persist.

CONCLUSIONS

In summary, floral VOCs hold substantial promise for better understanding the effects of environmental changes on plant–pollinator interactions, and in particular, may provide a framework for predicting the implications of future environmental changes. Indeed, recent research suggests that environmental-induced changes in floral VOCs are more important for pollinators than visual cues, at least for some plant species (Burkle and Runyon, 2016). We recommend that collection and analysis of floral VOCs be incorporated in studies seeking to understand plant–pollinator interactions in general, and how global change affects plants and pollinators in particular. Examination of information-rich floral VOCs opens numerous avenues for future research that provide exciting opportunities to integrate chemical and pollination ecology.

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Appendix 1. The setup used in this study to collect floral VOCs (shown in Fig. 2).[a]

Equipment	Function	Supplier(s)	Notes
Polyethylene cups with dome lid (clear)	Floral headspace container	Dart Container Corporation, Mason, Michigan, USA	Several sizes are available
Cotton balls	Fill airspace and protect plants from damage where they enter the cup	Numerous	
VOC trap containing porous polymer adsorbent (HayeSep Q)	Captures and concentrates VOCs from floral headspace	A diversity of porous polymers are available from numerous chemical suppliers. Premade VOC traps (porous polymer packed into narrow glass tube) are available from: Volatile Assay Systems, Rensselaer, New York, USA; Sigma Scientific LLC, Micanopy, Florida, USA; Markes International, Pontyclun, United Kingdom.	Porous polymers can be purchased in bulk which allows custom-made VOC traps. The most commonly used for plant VOCs include Tenax TA, Porapak Q, and HayeSep Q (all available from Sigma-Aldrich, St. Louis, Missouri, USA), but others are available.
Volatile collection system (vacuum pump)	Pulls air out of headspace container through VOC trap	Portable systems with automatic timers are available from Volatile Assay Systems (Rensselaer, New York, USA). Small, manual, battery-powered vacuum pumps are a cheaper (e.g., AirLite sampling pumps, SKC, Eighty Four, Pennsylvania, USA).	Benchtop push-pull volatile collection systems are available from Sigma Scientific LLC (Micanopy, Florida, USA) and Volatile Assay Systems (Rensselaer, New York, USA).

[a] Our intent is not to endorse these products or suppliers, only to specify what we used in this study. There are numerous alternative VOC collection methods that can be tailored to individual plant species and research questions. Alternative VOC collection methods are reviewed in Tholl and Röse (2006) and Tholl et al. (2006).