Review

Floral volatile organic compounds: Between attraction and deterrence of visitors under global change

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A B S T R A C T

Plants produce and emit a large variety of volatile organic compounds that play key roles in interactions with abiotic and biotic environments. One of these roles is the attraction of animals (mainly insects) that act as vectors of pollen to ensure reproduction. Here we update the current knowledge of four key aspects of floral emissions: (1) the relative importance and interaction of olfactory signals and visual cues, (2) the spatial and temporal patterns of emission in flowers, (3) the attractive and defensive functions of floral volatiles and their interference, and (4) the effects of global change on floral emissions and plant–pollinator interactions. Finally, we propose future lines of research in this field that need to be addressed or investigated further.

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Introduction

Previous reviews on floral emissions have provided a good basis on the biochemical processes involved in the interactions of flowers with their flower visitors (Dudareva et al., 2000; van Schie et al., 2006), their action over pollinators’ behaviour (Riffell, 2011) and the ecological processes that drive their evolution (Raguso, 2008b). Here we update previous reviews on floral BVOCs and address diverse complementary and less considered ecological aspects of floral volatile emissions. We review their coexistence and association with visual signals, their patterns of emission and their underlying causes, their attractive and defensive functions and their interference, and finally we discuss the potential effects of global change on plant–pollinator interactions through the induction of changes in these floral emissions.

Plants produce and emit a large array of biogenic volatile organic compounds (BVOCs) that are useful in their interactions with their immediate environment. BVOCs include terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives (Dudareva et al., 2004, 2006). These emissions of BVOCs to the atmosphere have significant biological effects on the relationships of plants with other organisms and also environmental effects on atmospheric physicochemical properties (Peñuelas and Llusia, 2003; Peñuelas and Staudt, 2010). These volatile substances serve diverse functions in plants, including interactions with both abiotic (Sharkey and Singsaas, 1995; Peñuelas and Llusia, 2002; Peñuelas and Munné-Bosch, 2005; Niinemets, 2009) and biotic factors (Dudareva et al., 2006; Pichersky and Gershenson, 2002; Seco et al., 2011; Regge and Pierik, 2010; Peñuelas et al., 1996). As sessile organisms, plants do not have the capacity to move to escape from detrimental organisms and conditions to which they are exposed. Plants have therefore evolved a great diversity of chemicals to deal with those detrimental factors. BVOCs, and especially terpenoids, are among the most relevant compounds used by different tissues of the plant to interact with their abiotic and biotic environments (Peñuelas and Llusia, 2004; Schiestl, 2010). Benzenoids are ubiquitous in floral scents (Knudsen et al., 2006) and they are similarly important and abundant than terpenes (van Schie et al., 2006).

This capacity to chemically interact with their environment emerged early and diversified extensively in the evolution of the plant kingdom (Chen et al., 2011; Paul and Pohnert, 2011). The protection of plant tissues from its consumption by other organisms (herbivory) might be one of the first needs that the ancestors of terrestrial plants had to cope with (Van Donk et al., 2011). One of the mechanisms that plants have evolved to resolve this need was the production and eventual release of deterrent compounds from their tissues. Also competition has been one of the most common biotic interactions experienced by plant ancestors, and in response to this they have evolved allelopathic substances (Rasher et al., 2011). Primitive BVOCs may have served diverse other functions related to the interaction with abiotic agents, as primitive plants have been exposed to diverse environmental stresses. With the appearance of terrestrial plants and phanerogams, diverse plant lineages developed other biological interactions, like those established with pollinators (Bronstein et al., 2006). The establishment of these interactions is mediated at least in part by chemical communication channels (Negre-Zakharov et al., 2009). At this point, the large array of pre-existing chemical substances may have assumed new biological functions, such as the attraction of pollinators (Pellmyr and Thien, 1986; Armbruster, 1997; Schiestl, 2010), which is one of the most relevant functions of BVOCs (Dudareva et al., 2006). The evolution of these compounds experienced a new impulse with the radiation of flowering plants, as it has been stated that biotic pollination has contributed to the diversification of flowering plants and their floral traits (Grimaldi, 1999; Niet and Johnson, 2012).

More than 85% of the species of flowering plants depend on insects for pollination (Ollerton et al., 2011). Pollinators see communities of flowering plants as “biological markets” that offer a wide variety of flowers from which they can choose those with the best rewards (Chittka and Raine, 2006). The distribution of visitors among flowers is strongly affected by competition between plants, mechanisms of facilitation for the attraction of pollinators (Ghazoul, 2006; Duffy and Stout, 2011), and competition between pollinators for the exploitation of floral resources (Pleasants, 1981). Plants need to attract and compete for the attention of pollinators to receive their services. At this point, floral recognition by pollinators plays a key role in plant–pollinator systems.

Olfactory vs. visual cues

Floral recognition is mainly mediated by colour vision and olfaction (Chittka and Raine, 2006). The visual and olfactory display of flowers includes thus the floral traits that play the most important roles in the attraction of pollinators ( Kunze and Gumbert, 2001). Plant–pollinator relationships have been historically regarded to be mostly mediated by vision. The study of communication between plants and pollinators has therefore focused mostly on visual traits; little consideration has been given to the contribution of the chemical traits of floral phenotypes (Raguso, 2008a). Visual cues, though, may act in concert with olfactory cues to allow pollinators to find plants (Burger et al., 2010; Leonard et al., 2011a,b). The presence of floral odours may enhance the discrimination of colours by improving attention towards visual cues, and the combination of chromatic and aromatic cues may enhance the formation and retrieval of memories in pollinators ( Kunze and Gumbert, 2001). The relative importance of each sense may vary in the various plant–pollinator interactions. Olfactory signals are particularly important in plants that bloom at night when visual characteristics are less important for their pollinators (Jürgens et al., 2002; Carvalho et al., 2012); however, some nocturnal pollinators may rely in both visual and olfactory cues to locate and feed on night-blooming flowers (Raguso and Willis, 2005). In fact, investment in the production of scent as an advertisement of reward provides a net fitness benefit to plants (Majetic et al., 2009a). Olfactive signals can constitute a more reliable signal for pollinators to detect the presence of rewards and find them than visual traits (Raguso, 2004a). Ample evidence shows that pollinators such as bees are able to detect pollen and nectar in flowers via olfactory cues (Wright and Schiestl, 2009, and references therein). Floral scents thus occupy a relevant place in the hierarchy of stimuli that drive floral selection (Parachnowitsch et al., 2012); honey bees and bumble bees learn odours faster and with a higher retention than colours, and odours evoke a stronger discrimination between flowers (Kugler, 1943; Menzel, 1985; Dobson, 1994; Leonard et al., 2011a,b). Many pollinators learn the particular scents of different species of plants to recognise those flowers offering the highest quality rewards (Chittka et al., 1999). The learning of olfactory cues in pollinators strongly contributes to forming the networks of interactions established in plant–pollinator communities, which are dynamic in time and space (Riffell, 2011), and represents an important component of the selective environment determining the evolution of floral signals through their impact on plant fitness (Wright and Schiestl, 2009).
Spatial and temporal variation of floral emissions of BVOCs

The scent of different floral organs

Certain BVOCs are commonly emitted by both flowers and vegetative parts of plants (Dudareva and Pichersky, 2000). Some compounds produced only by the flower, however, may serve flower-related functions, such as the attraction of pollinators or the deterrence of nectar thieves. Different floral parts such as petals, sepals, pollen, and nectar can emit diverse blends of BVOCs (Dötterl and Jürgens, 2005; Mena et al., 2005; Jullien et al., 2008; Filella et al., 2011). These blends may serve different functions developed by diverse floral organs. Some organs may preferentially attract the attention of visiting animals or present particular chemical defences. In many cases, differential emission patterns along different flower organs serve pollinators to find reward-offering structures (Flamini et al., 2002; Dötterl and Jürgens, 2005).

Petals and sepals

In many species of plants, scents from whole flowers are predominantly composed of volatiles emitted from petals, mainly benzenoids, phenylpropanoids, nitrogen-bearing compounds, and terpenoids, such as the common floral monoterpenes β-ocimene (Dötterl and Jürgens, 2005; Bergström et al., 1995; Mena et al., 2005; Knudsen et al., 2006). Petals, though, are not always the only organs of flowers with the highest emissions (Mactavish and Menary, 1997a; Jullien et al., 2008).

While leaves store BVOCs in a variety of different structures (trichomes, idioblasts, cavities, and ducts), depending on the species (Weker, 1993; Gershenzon et al., 2000; Turner et al., 2000; Gang et al., 2001), flowers usually produce their blends of BVOCs in osmophores or in conical cells located in the petals (Bergougnoux et al., 2007; Whitney et al., 2011) and sometimes in other floral structures such as sepals (Cabrai et al., 2010). Vogel (1962) established the term osmophore for an enclosed area of floral tissue that specialises in the emission of scents. Osmophores consist of a multilayered glandular epithelium (Vogel, 1962; Stern et al., 1987; Hadacek and Weber, 2002). Cabral et al. (2010) found evidence that the volatiles in species of Acianthera are released by the cells of the osmophores and stored in periplasmic and intercellular spaces. They suggest that these compounds are probably volatilised by daytime temperatures and are released through the stomatal pores in sepals. Petal emissions have been observed to correlate well with endogenous concentrations along floral maturation, which leads to the conclusion that at least in many cases petal emissions are released more or less readily depending on their volatility and their internal concentration (Bergougnoux et al., 2007). The diffuse emission of BVOCs is probably a pleiomorphic character of flowers, while the spatial pattern of emission, characterised by the distribution of osmophores, is most likely an apomorphic character (Vogel, 1962; Bergström et al., 1995). In some cases, concentration gradients of BVOCs along petals indicate the path to reach floral nectaries (Bergström et al., 1995; Dötterl and Jürgens, 2005).

Pollen

The characterisation of chemical constituents present in the odour of pollen has been largely ignored because of difficulties in sampling and analysis. Samples of odours from pollen analysed with headspace techniques have been found to be chemically different from scent from whole flowers (Dobson et al., 1996; Flamini et al., 2002), and the diversity of compounds identified is often lower in pollen (Knudsen and Tollsten, 1991; Dobson et al., 1996). Odours from pollen are probably detected at short distances by insects in those cases in which pollen emissions of BVOCs are quantitatively less abundant than those from the entire flower. However, many species may present stronger pollen odours than others (Dobson and Bergström, 2000).

Plants with pollen that has an odour significantly different from that of other floral structures are able to advertise the existence of pollen as a reward, providing these plants with an additional level of specific differentiation from other plants. The benefits offered by plants that present a characteristic odour from pollen may include providing pollen-foraging insects with a higher efficiency to locate food, and a higher fitness for the plant due to an increased export of pollen and the more effective transport of pollen to stigmas (Dobson and Bergström, 2000). Pollen applies two main pressures on plants: the need for protection from non-pollinating insects that exploit it without providing any benefit for the plant (Hargreaves et al., 2009), and the need to make this reward more attractive to pollinators. This conflict may play a key determining role in the evolution of pollen’s odour and of other floral traits, such as nectar, that also suffer from over-exploitation by non-pollinating insects.

Knoll (1930) was the first to study the origin of odours from pollen and proposed that scents came from the pollen-kitt. This term refers to the oily, and often sticky, coloured substances coating pollen grains that cluster the grains into aggregates. Knoll (1930) enumerated and extensively discussed the diverse functions attributed to the pollen-kitt, among which are those that enhance adhesion, confer the yellow pigmentation that protects pollen from UV radiation, provide nutritional value through fatty oils, and confer odour. Pollenkitt thus has a large variety of functions in addition to the provision of odour (Dobson, 1989; Pacini and Hesse, 2005), which may imply the application of a great diversity of evolutionary pressures driving the traits of the pollen-kitt. Dobson et al. (1987) confirmed that all the main compounds found in pollen headspace can be found in the pollenkitt. Measurements conducted by Dobson et al. (1990) suggested that the pollenkitt adsorbs some volatile compounds from the surrounding air, which is impregnated with diverse odours from petals. Pollen constitutes an important nutritional resource for many insects that visit flowers, especially as a source of proteins and lipids (Roulston and Cane, 2000). Some studies have investigated how signals from pollen (basically olfactory and visual) affect the localisation and identification of pollen by insects (Dobson and Bergström, 2000; Lunau, 2000). The need to explore the behavioural responses of insects to BVOCs constitutes an added difficulty of this field of research. Various studies provide evidence that pollen-seeking insects such as bees (Dobson et al., 1999; Dobson and Bergström, 2000), beetles (Bartlet et al., 1997; Cook et al., 2002), and syrphids (Golding et al., 1999) rely on gradients of odour during their search for food. BVOCs from pollen may be required to induce pollinators to land on flowers. An example is found in foraging-naive bumble bees, in which landing is most effectively elicited when combining olfactory signals from pollen with visual stimuli from anthers (Lunau, 1992). Dobson (1999), in a series of behavioural field studies of bumble bees foraging for pollen on Rosa rugosa, provided the strongest evidence that bees use scents from pollen to distinguish between flowers that have different amounts of pollen.

Nectar

In many plants, pollination requires the help of nectar-feeding pollinators. Floral nectar is the most common reward that plants offer to their pollinators (Simpson and Neff, 1983). The chemical composition of nectar is complex; it contains primary metabolites, such as sugars and amino acids that are used to attract pollinators, but also secondary metabolites, such as alkaloids, phenolics, and nonprotein amino acids (Baker, 1977) that repel nectar thieves and also have undesired negative effects on pollinators’ visits (Stephenson, 1981; Kessler and Baldwin, 2007). Although we know
that the sugars in nectar come from nectaries (Fahn, 1979), little is known about the origin of the secondary metabolites found in nectar. The set of BVOCs present in nectar comes both from the volatiles released by the surrounding floral tissues and from those released by the nectaries into the nectar solution (Balao et al., 2011). Raguso (2004b) found that some volatile compounds were taken up by artificial nectar applied to petals of Magnolia grandiflora, while others were not. Volatile compounds providing information about the existence of nectar often come from other flower structures, such as the above-mentioned nectar guides present in petals and other floral organs. The function of those BVOCs associated with the presence of nectar is probably complex, given that these scents have both attractive (Honda et al., 1998; Raguso and Willis, 2002) and deterrent effects on nectar consumers (Ömura et al., 2000).

Spatial diffusion and distribution of floral scent

Once BVOCs are released from flowers, they are rapidly mixed and diluted by physical atmospheric processes creating a dynamic olfactory environment (Riffell et al., 2008). They generate different patterns of diffusion depending on which type of transport predominates, advection or turbulence. Advection transport generates a continuous concentration gradient near the source of emission, while turbulent transport creates filaments of odour of intense concentration. Pollinators may perceive these scent trails differently and may adapt their navigational strategies (Cardé and Willis, 2008) and sensory systems to them. Moreover, the dynamics of odours may be affected by the size and position of the source of emission above the substrate. The size of the sources (flowers) and their position relative to the ground, then, may be floral traits available to selection via patterns of diffusion. These traits elicit different efficiencies in the detection and location of a signal’s source by the pollinator. Atmospheric dynamics influencing the distribution of odours vary among different habitats (e.g., grassland, shrubland, or dense forest). Habitats to which plants and insects are adapted may exert diverse selective pressures on floral traits and the sensory systems of pollinators through the influence of atmospheric dynamics and the diffusion of odours. The environment of odours experienced by animals is also affected by their own body size and translational speed (Riffell et al., 2008).

The distance at which BVOCs can be perceived may depend on both the physicochemical traits of the BVOCs and the sensitivity of the sensory systems of the pollinators (Chittka and Raine, 2006; Riffell, 2011). BVOCs have different chemical properties that confer different reactivities and longevities (Atkinson and Arey, 2003). These properties affect the persistence of BVOCs in the air and their capacity to be transported large distances from their source (Blande et al., 2010).

Temporal variation in floral scent

During their lifespan, flowers may vary their scent both quantitatively and qualitatively for many reasons. Temporal patterns of emission can become apomorph traits that reflect convergent evolution based on particular relationships with certain groups of pollinators (Morinaga et al., 2008; Okamoto et al., 2008; Dötterl et al., 2012). Plants may benefit from emitting higher amounts of BVOCs when the principal pollinator is active and also from saving resources by reducing emissions when the pollinator is not active. These factors may affect the circadian variation of such floral emissions, as occurs in moth-pollinated flowers, emitting maximal scent in early evening and night (Raguso et al., 2003; Okamoto et al., 2008), although not always (Pichersky et al., 1994). Apart from the conservation of resources, a reduction in volatile emissions when specialist pollinators are inactive may partially prevent the visitation of generalist pollinators, which can result in less effective pollination. Nevertheless, when specialist pollinators are rare, plants may take advantage of visits from generalist pollinators. Plants that mainly attract specific pollinators may change floral blends to attract generalist pollinators and ensure pollination when flowers remain unpollinated for a long time (Dudareva and Pichersky, 2000). Individual flowers may also change or reduce their emissions once they are pollinated (Negre et al., 2003) to prevent more visits that can cause damage to flowers, direct visits to flowers that are still unpollinated (Rodriguez-Saona et al., 2011), and prevent visits from florivores or seed-feeders (Muhlemann et al., 2006). Floral emissions may vary with floral ontogeny due to differences in floral processes and stages of maturity of different floral parts (Mactavish and Menary, 1997b). In plants with unisexual flowers, emissions may vary between male and female flowers with floral maturation (Proffitt et al., 2008; Ashman, 2009). Many hermaphroditic flowers may experience changes in their scent profile during their lifetime due to a temporal differentiation in the male and female phases (Goodrich et al., 2006; Goodrich and Raguso, 2009). The emission of BVOCs by plants, including floral scents, may represent a heritable component of phenotypic plasticity that may be species (or population) specific and may be modulated by environmental conditions (Majetic et al., 2009b).

Functions of floral scent: attraction

Floral scents are composed of a mixture of BVOCs with characteristically high vapour pressures and low molecular weights (Knudsen et al., 2006; Copolovici and Niinemets, 2005). Benzenoids, whose biosynthetic pathways are related to the synthesis of floral pigments mainly found in floral tissues (van Schie et al., 2006), can serve attractive functions in flowers, while various terpenoids, which can be also found in emissions from vegetative tissues, have both attractive and deterrent effects on facultative visitors. Pollinators and other visitors may exert different selective pressures on flowers that may affect quantitative and qualitative differences in floral blends of BVOCs. To date, many studies have addressed the difficult task of revealing the differential traits of floral scents that are typical of particular pollination syndromes (Knudsen and Tollsten, 1993; Andersson et al., 2002; Raguso et al., 2003; Pettersson et al., 2004; Dobson, 2006).

Most pollinators rely on floral rewards offered by flowers, such as nectar, pollen, or oil products (Steiner et al., 2011). Floral BVOCs provide information about the location, abundance, and quality of floral rewards (Wright et al., 2009; Howell and Alarcón, 2007). Flowers attract pollinators by exploiting their species-specific innate preferences and their abilities to learn the association between scent and floral reward (Farina et al., 2007; Raguso, 2008b; Arenas and Farina, 2012). Plants that do not offer nectar may mimic the scents and colours of neighbouring flowers that do attract pollinators (Kunze and Gumbert, 2001; Schiestl, 2005). While some BVOCs are known to be generalist attractants of a great diversity of pollinators (e.g. Li et al., 2008; Johnson and Hobbah, 2010), others may be specific attractants of particular species of insect (e.g. Eltz et al., 1999; Schiestl et al., 2003; Schiestl and Glaser, 2012). For example, some orchids attract only males of their pollinator species by emitting analogues of feminine pheromones, thereby tricking the males into believing that the flowers are females (sexual deception) (Schiestl, 2005; Gaskett, 2011; Gögl et al., 2011). Some flowers deceive insects that feed on other animals by emitting BVOCs that mimic prey-related odours (Shuttleworth and Johnson, 2009; Stökl et al., 2010) or the emissions of herbivore-infested plants (Brodmann et al., 2008, 2012). Other flowers emit volatile blends that resemble the odours of carrion or dung to attract
pollinators (Niet et al., 2011; Urrutia et al., 2011). Male euglossine bees are attracted to and collect floral volatiles from particular species of orchids to attract females (Eltz et al., 1999; Bembé, 2004). Some of these relationships are very specific and rare but together highlight the capacity of floral scents to attract pollinators by providing information about very different types of resources, even though these may not exist. Most of these plant–pollinator interactions involving only one plant species and one or few species of insects are mediated by private communication channels, consisting on the emission of unusual BVOCs, the emission of specific ratios of more ubiquitous compounds, and the use of volatiles that act as filters of particular floral visitors (Raguso, 2008b).

The importance of BVOCs in the ecology of several insect groups suggests that selective pressure on floral scent by pollinators is widespread in entomophilous plants (Schiestl, 2010). Entomophilous plants (pollinated by insects) emit more pronounced scents than ornithophilous (pollinated by birds) and anemophilous plants (pollinated by wind) (Wragg and Johnson, 2011; Magallhães et al., 2005). This tendency suggests that emission of BVOCs from flowers evolved mainly to attract insect pollinators. Dobson (1988) observed that species pollinated by insects (e.g. Lepidoptera) that consume rewards other than pollen tended to have pollen with relatively fewer BVOCs than species pollinated by bees that rely more on pollen as a food source.

Functions of floral scent: defence

Plants may experience detrimental effects from being visited by some non-pollinating flower visitors that consume floral rewards (Burkle et al., 2007), disturb pollinators (Tsuij et al., 2004; Junker et al., 2007), or feed on floral tissues (McCall and Irwin, 2006). The detrimental effects caused by antagonistic visitors can exceed the benefits from mutualists (Morris et al., 2007). Plants may benefit from selecting visitors of flowers, and they present a variety of defensive properties that include some compounds of floral scents (Kessler and Baldwin, 2007; Junker and Blüthgen, 2008; Kessler et al., 2008; Willmer et al., 2009; Galen et al., 2011). Junker and Blüthgen (2010) demonstrated that some floral BVOCs act as “filters” that select effective pollinators and deter detrimental (thieves and herbivores) and neutral floral visitors (generalist pollinators carrying heterospecific pollen). From this viewpoint, obligate visitors, either mutualistic or antagonistic, may have evolved a tolerance to deterrent and toxic compounds present in floral structures and scents and can use these compounds as specific signals to find their host plants. Some floral volatiles have antimicrobial properties to protect floral structures by preventing colonisation by bacterial communities that can alter floral tissues and the chemistry of nectar (Tholl et al., 2005; Junker et al., 2011).

Defensive functions of the odour of pollen

The presence of volatile compounds in pollen, the variation in their composition and relative abundance, and the repulsive and antimicrobial function of some of these compounds suggest that BVOCs of pollen have additional functions besides attracting pollinators. One of the main functions of these compounds is to protect the male gametophyte from pollen-consuming animals and pathogens that do not provide any benefit to plant fitness. BVOCs that repel thieves may be found in diverse floral parts (Mullin et al., 1991). Plants with deterrent chemicals in non-pollen floral parts might avoid the need to have them in the pollen. Anthers may repel unwanted visitors that consume pollen by presenting deterrent compounds (Belcher et al., 1983; Rossiter et al., 1986). Conversely, attractive volatiles released by sterile pollen of heterantherous flowers (Faden, 1992) or by highly attractive food structures, such as staminodes (Bergström et al., 1991; Endress, 1994), may keep pollen-feeders away from the fertile pollen. The two abundant α-methyl ketones in the odour of pollen in R. rugosa, namely 2-undecanone and 2-tridecanone (Dobson et al., 1990), are deterrent and even toxic to several insects (Kennedy et al., 1991; Maluf et al., 1997), and some α-methyl ketones have antifungal activity (Cole et al., 1975). Anemophilous plants are not expected to suffer disadvantages from presenting deterrent compounds in their flowers, because they do not need to attract pollinators (Dobson and Bergström, 2000). They are then expected to present more chemical defences in the scent of their pollen.

Some BVOCs in pollen have concurrent multiple functions. A variety of essential-oil volatiles identified in the scent and considered to be attractants to pollinators might also have microbial and fungal defensive functions (e.g. Knobloch et al., 1989; Kubo et al., 1995). An example is eugenol, a common volatile of pollen found in R. rugosa, that has both the potential to attract an array of insects and antimicrobial activity (Zaika, 1988). The lactone parthenin, a sesquiterpene, has at least three functions: defence, attraction of specialist herbivores, and pollen allelopathy (Jayanthi et al., 1993). Pollen allelopathy is a phenomenon that has been rarely documented among the functions of BVOCs. The allelopathic effects of BVOCs in the pollen of one species deter the germination of pollen from other species, conferring a competitive advantage to the species with these compounds (Murphy, 1999). Pollen allelopathy has been documented in few species, and the magnitude of its effects on ecosystems requires investigation (Murphy, 2000).

Interference of pollination by defensive volatiles

The need to inform pollinators about the presence of floral rewards is in conflict with the potential detrimental effects of non-pollinating visitors that may also be attracted by these compounds. To solve this problem, some plants pollinated by specialists emit defensive BVOCs from flowers that act as “filters” by selecting some visitors while inhibiting others (Fig. 1A; Junker and Blüthgen, 2010; Junker et al., 2010; Ömura et al., 2000). The emission of 2-phenylethanol by the flowers of Polemonium viscosum causes different responses in visitors depending on the dosage (Galen et al., 2011). When released at high rates, 2-phenylethanol repels both ant thieves and pollinators, triggering negative effects on pollination and plant fitness. When released at intermediate rates, 2-phenylethanol deters thieves and reduces the consumption of nectar per visit by pollinators, enhancing the number of visits of pollinators per volume of nectar and stimulating the movement of pollinators between different flowers, which may encourage out-crossing. The presence of nicotine in nectar has also been observed to deter thieves and to optimise the number of pollinators’ visits per volume of nectar consumed (Kessler and Baldwin, 2007; Kessler et al., 2012). Another frequent strategy is to present qualitatively different floral bouquets of BVOCs. While some BVOCs are efficient at attracting pollinators (Pleys et al., 2002; Cunningham et al., 2004), other compounds with deterrent functions can negatively affect plant fitness by repelling pollinators (Kessler et al., 2011). By assuming a heritable component of floral scent, we can expect that the evolution of floral blends of BVOCs may be driven by positive selection on BVOCs that attract pollinators and negative selection on those that repel pollinators (Schiestl et al., 2011). Pollinators can exert stronger selection pressures on floral traits than herbivores or florivores (e.g. Bartowska and Johnston, 2012). Adler et al. (2012) have shown that entomophilous Nicotiana species present lower amounts of chemical defences in their flowers than their autogamous relatives, and that floral contents of these
compounds shown a good correlation with those of leaves, suggesting a pleiotropic effect among the contents in these different tissues and indicating a selective effect of pollinators on deterrent floral compounds and indirectly on leaf compounds. BVOCs that deter herbivores and thieves may also be positively selected (Fig. 2). The rarity of some defensive floral volatiles can provide floral scents with a higher level of specificity that favours the

Fig. 1. Effects of BVOCs on plant–pollinator interactions and the interferences of herbivory. BVOCs are classified as attractive (those related to the attraction of pollinators and therefore to plant reproduction) or deterrent (involved in plant defence and avoidance of detrimental visitors). Notice that a particular compound can cause different behavioural responses in diverse visitors belonging to different insect groups, as they have different olfactory preferences and can therefore develop both attractive and defensive functions simultaneously. This receiver-specific effect of BVOCs becomes useful for plants to select a particular visitor profile that constitutes an efficient pollination vector while keeping detrimental visitors away. Figure shows the different effects of BVOCs emitted by attacked and non-attacked plants on visitors. (A) Flowers from non-attacked plants constitutively emit BVOCs that attract a wide variety of insects, including both pollinators and thieves. Flowers from non-attacked plants may also constitutively emit specific compounds that act as filters that deter thieves and some generalist pollinators while allowing specialist pollinators to visit flowers. Leaves from non-attacked plants can constitutively emit BVOCs to deter herbivores. Constitutive emissions from leaves can also be involved in pollinator attraction (Dufay et al., 2003; Caissard et al., 2004). (B) In flowers of herbivore-attacked plants, moreover, production and emission of herbivore-induced plant volatiles (HIPVs) may be elicited by the transduction of signals from damaged leaves to flowers (systemic response). These HIPVs may repel visitors (both pollinators and thieves) and thus interfere in pollination. Leaves and flowers from herbivore-attacked plants emit HIPVs to repel herbivores. However, specialist herbivores (monophagous and oligophagous herbivores) that have coevolved with their nutritious plants can use HIPVs to find the plants.

Fig. 2. Selective pressures exerted by plant visitors on functional groups of BVOCs. Constitutively emitted attractive floral BVOCs are mainly selected to attract pollinators (1, 4). Attractive BVOCs, however, may have negative effects on plant fitness by attracting detrimental visitors that may consume rewards or feed on flowers and fruits (6, 8). Constitutive deterrent BVOCs from flowers are selected to repel unwanted visitors such as thieves (7) and herbivores (9). Obligate pollinators may have coevolved with the chemical defences of plants and can tolerate deterrent BVOCs and even use them to locate their host plant (2), while such BVOCs may inhibit visits by generalist pollinators (5). HIPVs are selected to deter herbivores (10). HIPVs may have detrimental effects on pollinators visiting flowers and even inhibit their visits (3). Numbers indicate the references supporting the selective pressures presented in figure: 1. Majetic et al. (2009a), Wright and Schiestl (2009), Filella et al. (2011), Wragg and Johnson (2011), Pleps et al. (2002), Schiestl et al. (2011). 2. Junker and Blüthgen (2010), Junker et al. (2010). 3. Adler and Irwin (2005), Kessler et al. (2011), Kessler and Halitschke (2009). 4. Wright and Schiestl (2009), Wragg and Johnson (2011), Schiestl et al. (2011). 5. Adler and Irwin (2005), Schiestl et al. (2011). 6. Okamoto et al. (2008), 7. Ómura et al. (2000), Junker and Blüthgen (2008), Shuttleworth and Johnson (2009), Willmer et al. (2009), Junker and Blüthgen (2010), Junker et al. (2010). 8. Theis (2006), Muhlemann et al. (2006). 9. Adler et al. (2001). 10. Adler et al. (2001).
identification of host-plant flowers by pollinators. Examples are the array of defensive chemicals, such as the lactone protoanemonin in the array of pollen chemicals (Bergstrøm et al., 1995) and α-methyl ketones in the pollen from R. rugosa (Dobson et al., 1990, 1999). Detrimental BVOCs might also enhance the selection of pollen by pollinators (Schmidt, 1982). Blends of BVOCs that include unusual volatiles with defensive functions may assist pollinators to become specialised on particular species, as these taxon-specific compounds may become key signals in host recognition (Junker and Blüthgen, 2010).

When leaves or flowers suffer attacks from herbivores or pathogens, the chemistry of flowers may change. Many plants may react to herbivory by inducing the production of toxins in nectar and floral tissues (Adler et al., 2006; McCall, 2006) and by producing herbivore-induced plant volatiles (HIPV) in leaves and flowers (Kessler and Halitschke, 2009; Röse and Tumlinson, 2004; Peñuelas et al., 2005; Zangerl and Berenbaum, 2009). Ample evidence confirms that HIPVs can affect several members of the insect community, including pollinators, herbivores, and predators (Fig. 1B; reviewed by Lucas-Barbosa et al., 2011). Few studies, though, have investigated the induction of systemic emissions of BVOCs after the consumption of leaves by insects and the potential effects of HIPVs on plant fitness and the behaviour of pollinators (Effmert et al., 2008; Kessler and Halitschke, 2009; Theis et al., 2009; Kessler et al., 2011). Systemic phytochemical responses to herbivory have been observed in undamaged leaves (Turlings and Tumlinson, 1992; Mattiacci et al., 2001; Rodriguez-Saona et al., 2009; reviewed by Paré and Tumlinson, 1999). Damaged leaves might also induce emissions of HIPVs from flowers. Even if floral emissions did not vary in response to attacks from insects (Effmert et al., 2008), HIPVs produced and emitted by attacked leaves may modify the chemical mixture of compounds in the air surrounding the plant and have the potential to alter the behaviour of pollinators visiting flowers. Systemic responses have been observed to operate in the opposite direction, from damaged flowers to undamaged leaves (Röse and Tumlinson, 2004).

BVOCs of plants can influence the foraging behaviour of pollinators (Kessler and Halitschke, 2007, 2009; Kessler et al., 2008; Raguso, 2008a), and the role played by HIPVs in the behavioural changes observed in pollinators warrants further investigation (Dicke and Baldwin, 2010; Kessler and Halitschke, 2007). In Cucurbita pepo subsp. texana plants subjected to herbivory, no effects were seen on the behaviour of pollinators when herbivory caused changes in the number of flowers, display, or quality of the rewards, but visits from pollinators were reduced as a result of changes in the rates of floral emissions of BVOCs (Theis et al., 2009). Phytophagous attacks can cause divergent consequences in the attraction of pollinators. Root herbivory in Sinapis arvensis increased the visits to flowers by pollinators (Poveda et al., 2003), but the combined herbivory of leaves and roots induced a reduction in the flowering period and the number of fruits produced, although seed production was not affected. In most studies, however, herbivory of both flowers and leaves decreased visitation by pollinators (Adler et al., 2001; Cardel and Koptur, 2010; Danderson and Molano-Flores, 2010; Kessler and Halitschke, 2009; Strauss et al., 1996). Flowers of wild tomato plants received fewer and shorter pollinator visits when the leaves of the plant were damaged by an insect herbivore (Kessler and Halitschke, 2009). These results indicate that local and systemic emissions of HIPVs may influence the foraging behaviour of pollinators, and when visitation is negatively affected, a negative selective pressure is exerted on these emissions (Dicke and Baldwin, 2010). Hare (2010) found that the production of HIPVs in Datura wrightii was especially high in spring during the vegetative growing phase, but production declined after the plants began to flower and produce fruit. This timing may avoid the interference between the release of HIPVs and the attraction of pollinators and seed dispersers. The induction of HIPVs during the flowering phase can have a major effect on community dynamics (Kessler and Halitschke, 2007; Poelman et al., 2008). Moreover, the emission of floral HIPVs can influence different groups of the local insect community, such as nectar thieves and florivores (Baldwin, 2010).

Effects of global change on emissions of BVOCs

Environmental conditions are changing globally as a result of human activities. Changes in temperature, precipitation, land use, concentrations of atmospheric CO₂ and ozone, and UV radiation, among others, are expected to affect emissions of BVOCs by plants (Fig. 3; Peñuelas and Staudt, 2010; Loreto and Schnitzler, 2010). These changes are quite variable in intensity, timing, BVOCs, and species but are generally likely to increase emissions of BVOCs (reviewed by Peñuelas and Staudt, 2010). Higher emissions might increase the efficiency of reproduction by enhancing plant–pollinator interactions. The global changes may not only induce quantitative variations in emissions, but may also cause qualitative variations in species–specific blends due to differential responses of the different compounds to the changes. Changes in the rates of emission induced by an increase in temperature can be compound specific (Llusia and Peñuelas, 2000; Loreto and Schnitzler, 2010). Some BVOCs may experience a more pronounced increase in their rates of emission than others. The rates of emission from plant tissues depend in large part on the physicochemical properties of the BVOCs, mainly their volatility. Henry’s law constants (Hₚs) of diverse substances respond differently to increases in temperature (Copolovic and Niinemets, 2005; Allou et al., 2011). This compound-specific increase in volatility will result in an increase in the rate of emission of stored BVOCs that are also compound specific (Llusia and Peñuelas, 2000). This response to temperature involves factors other than those directly related to physicochemical properties, such as factors involved in physiological responses.

Rates of emission also depend on the activities of particular temperature-dependent enzymes (Monson et al., 1992). The enzymatic activities of various terpene synthases may present enzyme-specific temperature curves. Even though the positive effects of temperature on the volatility and biosynthesis of BVOCs are clear, changes in temperature may be accompanied by changes in the availability of water or in other environmental conditions whose effects on rates of emission may interact with or even neutralise those of temperature (Fortunati et al., 2008). In the end, though, the relative composition of a scent is likely to change as a result of all these interacting environmental changes, with likely significant consequences for the plant–pollinator relationship. Of the interactions that may be affected by altered emissions, the plant–pollinator interaction is probably the most susceptible to interference, especially in plants that rely on only one or a few species of pollinators. This situation may have serious consequences on reproduction and the functioning of ecosystems.

In addition to warming, other components of global change varying widely on a local scale, such as air pollution and high concentrations of ozone, might induce a reduction in the efficiency of pollinators to forage for flowers by reducing the longevity of BVOCs once released and the distance at which they can be perceived by pollinators (McFrederick et al., 2008; Blande et al., 2010). Nevertheless, the effects of the various drivers of global change on floral emissions are difficult to predict, because they can variously affect emissions by altering different processes occurring in the plant or by interacting with BVOCs once they are released. The particular
responses of plant–pollinator interactions to these altered emissions may depend on traits particular to the species of both plant and pollinator. Assuming that some plant–pollinator interactions may be more affected than others within the networks present in biological communities, changes in the emission of BVOCs will therefore probably translate into variations in the competitive abilities of pollinators and plants.

**Perspectives**

The unknowns highlighted in this review warrant much more research effort for the characterisation (spatial and temporal) of emission of BVOCs from different floral parts, such as sepals, carpels, and osmophores, the specialised cellular and glandular structures that produce and emit BVOCs in flowers. Some studies have confirmed that the functions of different floral organs are actually reflected in different profiles of emissions. However, future studies should address the composition and function of floral scent bouquets on a finer spatial scale within the flower. Static headspace sampling techniques may serve to characterise the emission profiles of the diverse organs, including scarce compounds emitted in low rates. On the other hand, dynamic headspace sampling techniques may be indispensable to quantify total and compound specific emission rates, to compare signal size of diverse floral organs and see the particular contribution of each one to the whole floral scent. Diverse volatile sampling methods have been employed to analyse flower scent with different advantages and disadvantages over other methods (Stashenko and Martínez, 2008) that must be considered when designing an experiment.

The temporal variations of floral scent generated by floral rhythms or maturation clearly warrant further investigation. An interesting phenomenon, which needs more evidence, is the suggested capacity of the flowers of specialist-pollinated plants to shift their emissions to attract a more generalist range of visitors when they remain unpollinated for a long time (Dudareva and Pichersky, 2000).

A key question that arises from the high phenotypic variability observed in emissions of floral BVOCs (e.g. Wright and Thomson, 2005; Majetic et al., 2009b) is whether this plasticity is similar in plants with different levels of selective pressure acting on floral scent (such as specialist- and generalist-pollinated plants). Less variability in the profiles of floral scents is expected in plants experiencing higher selective pressures on floral scent as a reliable signal for pollinators. Therefore, it is warranted an extensive and intensive comparison of the phenotypic variability in the ratios of compounds emitted by generalist-pollinated plants with those of specialist-pollinated plants that use private communication channels with their specialist pollinators. Some works have observed that deceptive species present a higher variability in traits associated with pollinator attraction, including floral scent, than rewarding species (Salzmann et al., 2007; Ackerman et al., 2011; reviewed by Juillet and Scopece, 2010).

The interaction between pollinators and herbivores constitutes an interesting line of research. The changes in floral emissions induced by systemic responses to herbivorous attacks and the implications for behavioural responses in the visitors of flowers clearly warrant investigation. Electroantennographic detection coupled with gas chromatography and mass spectrometry (GC–MS/EAD) allows the identification of volatiles from a sample while testing the recognition of its compounds by pollinators (e.g. Balao et al., 2011; Gögl et al., 2011; Brodmann et al., 2012). Once the compounds that stimulate insect sensory system are identified, the response they elicit over insects can be tested by using behavioural assays (Chittka and Thomson, 2001). The attractive or deterrent effect of BVOCs can be verified by developing preference tests with the help of diverse olfactometer systems. The stimulus of proboscis extension reflex (PER), associated with the motivation of nectar consumption, is another response from
insects to floral BVOCs that can be tested by exposing them to individual volatiles or particular blends and recording the responses they elicit (e.g. Honda et al., 1998; Reinhard et al., 2010; Giurfa and Sandoz, 2012).

Little evidence supports the negative selective pressure that pollinators may exert on HIPVs. Future experiments may include measurements of plant fitness for comparing plants with and without induction of HIPVs. New studies may address the effects of HIPVs on the community through their interference of plant–pollinator interactions.

Parachnowitsch et al. (2012) have provided an interesting work on phenotypic selection on floral scent. They demonstrate that selection towards higher floral emissions can be stronger than selection acting on other floral traits also related with pollinator attraction, such as flower colour and size. However, they did not make measurements to identify the agents of selection acting in their system and the specific importance of each one. Diverse selection agents act on flower traits; many of them are mutualists while others are antagonists and usually exert opposed selection pressures (Fig. 2). In their recent work, Bartowska and Johnston (2012) have provided evidence in favour of a higher selection pressure exerted by pollinators on floral traits than the selection exerted by herbivores, although they do not include floral scent among the floral traits they consider in the work. New experiments should try to reveal the agents driving floral scent selection and the relative intensity of the selection pressures they exert.

The use of native genotypes in studies trying to understand the function of single floral volatiles or other floral traits limits the conclusions that can be obtained from these experiments, because several, frequently unmeasured, traits differ among individuals. The use of genetically transformed plants with RNAI constructs silencing the expression of many genes to avoid traits that add noise and confusion to the trait that is the focus of the study becomes a strong tool for researchers that deal with natural variation in floral scents. Plant phenotypes can be silenced to target the single expression of one volatile compound that constitutes the object of the study, and this might confer an experimental advantage to these experiments.

The drivers of global change can act on at least two levels in the role of BVOCs in plant–pollinator communication. Firstly, by affecting floral status and emissions, and secondly, by affecting the properties of BVOCs once they are released into the air. These two levels can synergistically, additively, or antagonistically affect the signals in floral scents for pollinators. These effects of global change need urgent research since a reduction in the capacity of pollinators to find flowers would have serious consequences on plant communities and agriculture. Temperature effect on flower emissions needs to be investigated. If floral emission is a non-controlled process (Bergougnoux et al., 2007) then floral emissions may be regulated only by tissue internal pools of BVOCs and by compound specific volatilities. Internal pools of BVOCs are regulated by biosynthesis processes that are temperature dependent because enzymatic activity increases with temperature. At this point only limiting availability of the biosynthetic precursor may limit this increasing effect of temperature over biosynthesis of BVOCs. On the other hand, volatility is also positively related with temperature. Temperature-volatility curves may be compound-specific; then, the ratios of emitted compounds may vary with temperature. Floral emission profiles and rates at different temperatures need to be measured to find out how temperature can affect floral signal size and composition.

Plant exposition to ozone can also change floral BVOC emissions from flowers by causing damage to the plant and eliciting the emission of stress-induced volatiles. Ozone effects over emitted flower blends must be investigated, and the consequent effects over flower visitor’s behaviour need to be elucidated. Ozone is expected to react with volatiles coming from flowers and reduce their lifetime, while leading to the formation of new products. It might be interesting to investigate the potential of ozone to change floral odours perceived by pollinators and affect their capacity to find and identify flowers. This can be achieved by using reaction chambers to expose volatiles coming from flowers to ozone and by capturing the resulting mixture of volatiles to analyse them with GC–MS and more dynamically with PTR-MS-TOF, and also to apply them on behavioural tests against non ozone-exposed floral emissions and against control air free of VOCs.

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