6 Localization of the Synthesis and Emission of Scent Compounds Within the Flower

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6.1 INTRODUCTION

Previous chapters dealt with the types of compounds found in floral scent and how these compounds are synthesized inside the cell. However, it has been known for many years that different parts of a flower can emit different mixtures of scent compounds or no scent at all. This chapter reviews our present knowledge regarding the spatial distribution of scent biosynthesis and emission within the flower, and the organization of certain structures that are sometimes associated with such localized scent production.

6.2 OSMOPHORES

More than 100 years ago, Arcangeli reported the observation that the release of scent can be connected with certain parts of an inflorescence.[2] He specified the upper part of the spadix of *Arum italicum* (Araceae) as the site of odor evaporation and called it *osmoforo*, the "odor bearer," whereas the lower part was called *antoforo*, the "flower bearer" (greek: *osmo*, odor; *antos*, flower; *pherein*, to bear). The relevance of this phenomenon for scent emission and pollination remained unvalued until Vogel[3] reintroduced the term osmophore to describe floral volatile production and emission via specialized defined tissue areas with glandular characteristics.

6.2.1 Definition of Osmophores

Osmophores, also called floral scent glands, possess the ability to emit volatiles and comprise defined floral organs.[3,4] They can be found within the whole inflorescence as part of the perianth, bracts, appendices of peduncles, or anthers. Although osmophores might vary in shape and habitus, being plane-, whip-, brush-, club-, or palp-shaped, they have features in common. They usually face toward the adaxial side of the perianth and display a bullate, rugose, pileate, conical, or papillate epidermis (Figure 6.1).[3,5–10] Subjacent are several cell layers that form the glandular tissue, which merge into normal parenchyma cell layers.[4,6] Cells of the glandular tissue show enlarged nuclei compared to cells of nearby tissue and a dense cytoplasm.[4,6] A dispersed vacuome observed before and at anthesis, often turns into a large vacuole after anthesis.[6] Transmission electron microscopy revealed that cells of the glandular layers are supplied with an abundant rough or smooth endoplasmic reticulum (rER or sER, respectively), sometimes dictyosomes, many mitochondria, and lipoid droplets.[3–5] These droplets, probably lipid-protein mixtures, are surrounded by a monolayer of phospholipids and embedded in the cytosol.[11,12] They contain essential oils to be released and lipids like fatty acids and triacylglyderides.[3,4]

Hudak and Thompson[12] identified in cytosolic lipid-protein particle fractions of *Dianthus caryophyllus* petals (carnation, Caryophyllaceae) several volatile components of the carnation flower fragrance including hexanal, (*E*)-2-hexenal, nonanal, 2-hexanol, 3-hexen-1-yl benzoate, benzyl alcohol, and benzyl benzoate. As a result of pulse-labeling experiments with [^{14}C]-acetate, the authors suggest that the cytosolic lipid-protein bodies originate from membranes, indicating hydrophobic subcompartmentation. Most remarkable is the presence of enormous starch deposits in osmophores, which obviously ensures a carbon and energy supply for volatile production.[3,6] This is supported by the fact that the deposits disappear due to utilization while volatiles are emitted.[3] In the adaxial epidermis cells of osmophores, however, amyloplasts are usually absent. Based on these observations, Vogel distinguished the glandular cell layers as the site of volatile production and the osmophore epidermis as the site of emission.[3] As a consequence, inter- and intracellular transport toward the osmophore epidermis has to be postulated. Trafficking of lipoids is probably accomplished by close association of the rER with the plasma membrane, creating channels and multitubular structures closely related to lipoid bodies.[11,13] In addition, the transformation of homogeneous lipoid droplets into a multivesicular body just

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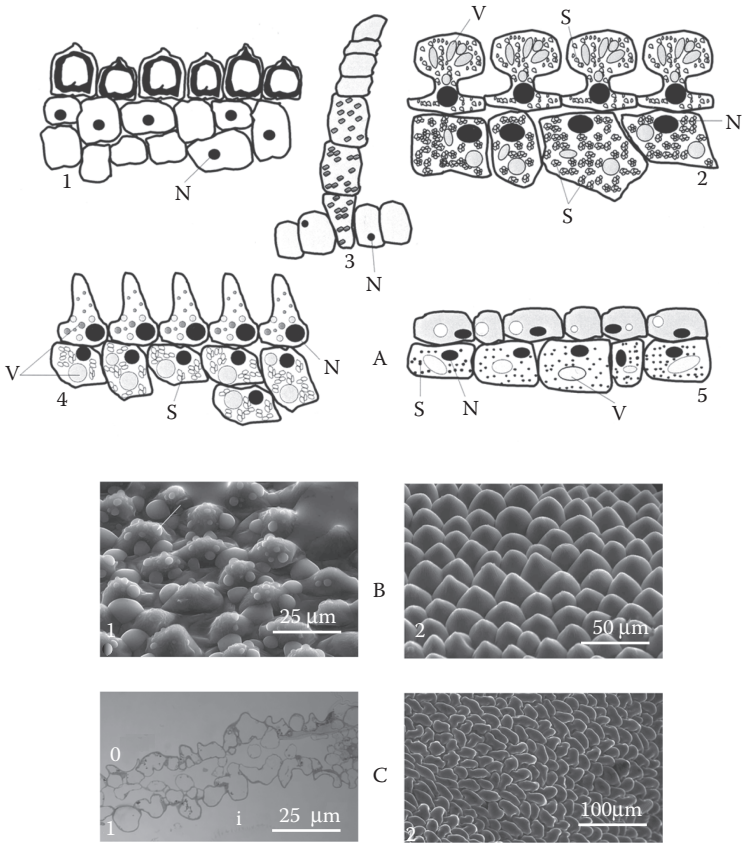

FIGURE 6.1 Epidermal surfaces of scent emitting flower tissue. (A) Schematic presentation of epidermal cells that are often observed in emitting floral tissue: (1) rugate to conical type; (2) pileate type; (3) trichome; (4) conical type; (5) flat to bullate type. N, nucleus; S, starch grains; V, vacuole. (B) Scanning electron microscope (SEM) picture of petals from scented and unscented rose (*Rosa hybrida*). (1) Adaxial epidermal cells with secretions (arrow) and (2) conical adaxial epidermis cells without secretions. (Courtesy of F. Ehrig.) (C) (1) Semithin cross section (light microscope) of the petal lobe of *M. jalapa*. Both epidermata exhibit bullate appearance. Loose mesophyll cells and many intercellular spaces are visible. (2) Bullate adaxial epidermal cells of *M. jalapa* (environmental scanning electron microscope).

before fusion to the plasma membrane was reported.[14] Osmophores are often supplied with an extended vascular system and fragile intercellular net with phloematic bundles pervading an aerenchymatic tissue, and stomata are frequently found on the abaxial side of osmophores.[3,4,14]

6.2.2 Detection of Osmophores

In the middle of the last century, the human nose was the most important measure for determining osmophores in floral tissue.[3] A somewhat better, nonbiased indication for the presence of osmophores is based on neutral red staining of floral

tissue.[3,15,16] Intact osmophore tissue is able to selectively take up and retain this vital stain. This phenomenon is attributed to the increased permeability of the cell wall in osmophores and the long-lasting storage ability of vacuoles. Applied nonionic neutral red molecules (aqueous solution, 0.01%, pH 8) penetrate the tonoplast and enter the vacuole. Since vacuoles act like an ion trap because of their slight acidic environment, a migration of neutral red cations is not possible. Therefore permeability of the cell wall and cuticle, as well as the presence of vacuoles are the prerequisites for the distinct staining of floral tissue with neutral red, which often correlates with the presence of osmophore tissue in the flower. Sudan black B, Sudan III, and Sudan IV are useful dyes for the detection of triacylglycerides and protein-bound lipids. Osmium tetroxide and Nile blue A will also stain lipids and NADI reagent indicates terpenoids.[16,17] In addition to staining, morphologic, and anatomic investigations, the objective evidence of volatile emission determined by headspace and gas chromatography-mass spectrometry (GC-MS) are indispensable for the identification of osmophore tissues.

6.2.3 Examples of Osmophores

Vogel[3] revived research interest in volatile emanation, and since then interest in the phenomenon of scent emission has increased. Species that have been investigated belong to the families of Alliaceae,[18] Aristolochiaceae,[3] Asclepiadaceae,[3] Fabaceae,[19] Rutacae,[20] Solanacee,[21] and the intensively studied Araceae[3,11,13,14,22] and Orchidaceae[3–8,15,16,23]. In the following paragraphs, some selected representative examples are described.

One of the most comprehensive investigations within the Araceae were performed with *Sauromatum guttatum* (voodoo lily). As typical for Araceae, the inflorescence of *S. guttatum* is made up of an unbranched spadix subtended by a colored bract called a spathe. Tiny female flowers are located at the base of the spadix and are crowned by small club-shaped organs, whereas male flowers are found in the upper part of the spadix, which develops a prominent apical appendix. GC-MS analysis proved that both the club-shaped organs and the appendix represent osmophores responsible for volatile emission.[11,13,14] The upper and lower half of the appendix produce 163 different volatile compounds, whereas 43 volatiles are solely assigned to the upper and 4 to the lower half of the appendix. A total of 105 volatiles are emitted by the club-shaped organs, and 29 of them are exclusively released by these extraordinary organs. The most prominent constituents are monoterpenes like α- and β-pinene, mainly released by the lower part of the appendix and α-phellandrene and limonene, mainly released by the club-shaped organs. α-Terpinolene and linalool are almost exclusively emitted by the latter.[14]

Papillose epidermis cells of the appendix contain an extremely fine, dispersed vacuome and large nuclei.[3] At the beginning of volatile emission, the formerly compact epidermal cell layer generates large intercellular channels. The same applies to the subjacent production layer.[11] The cells of this layer contain numerous mitochondria and amyloplasts. Electron microscopic investigations of the appendix reveal the presence of rER associated with Golgi dictyosomes present before volatile emission. The rER and Golgi network seems to play a key role in the production,

accumulation, and secretion of osmiophilic lipoid droplets.[11,13,22] Lipoid droplets are also observed in the cells of the glandular layers of the club-shaped organs, but are not present in epidermis cells.[14]

Flowers of the Orchidaceae show high morphologic differentiation, thus this family has the most polymorphic osmophore structures. Two striking examples are presented. The *Restrepia* inflorescence is single-flowered, with one slender dorsal and two fused lateral sepals, two antennae-forming petals with broadened apices, and a narrow, short column. The osmophore of *Restrepia antennifera* was originally identified as a palp-shaped adaxially thickened segment that separates the basal and distal parts of the dorsal sepal.[3] Detached dorsal sepals retrieves the foul odor of the entire inflorescence, while the other flower parts are scentless to the human nose. More recent investigations of *Restrepia hemsleyana*, *Restrepia muscifera*, and *Restrepia shuttleworthii*, however, assigned the osmophore character to the adaxial apex of the dorsal sepal and to the adaxial and abaxial apices of the petals.[15]

Is polylocular correct?

The osmophore epidermis expresses very conspicuous pilei-forming cells (Figure 6.1). The cell head contains dense cytoplasm with a polylocular vacuome and some small starch grains, whereas the neck contains an enlarged nucleus. In older buds, epidermis cells undergo increasing vacuolation and some starch grains are associated with plastoglobuli. At anthesis, small lipoid droplets and osmiophilic aggregates appear inside the tonoplasts. These observations could be supplemented by electron microscopic investigations.[15] Cuticular pores of irregular size, shape, and arrangement are found in all adaxial osmophore areas, whereas the abaxial side of the dorsal sepal and all other perianth areas lack these pores. Cells of the subjacent glandular layers contain a smaller vacuome, and toward the epidermis, enlarged nuclei embedded in a dense cytoplasm. These cells develop rER and sER close to the plasmalemma and possess large numbers of amyloplasts. The starch agglomerations fade away during volatile evaporation, accompanied by vacuole enlargement and cytoplasm depletion. Only a thin peripheral layer of cytoplasm with very few amyloplasts, mitochondria, and rER/sER remain. Some large lipoid osmiophilic droplets can still be observed.

Osmophores are also found in *Stanhopea* sp. (Orchidaceae). The inflorescence consists of narrow petals, a lip divided into a basal hypochile, the horn-bearing mesochile, a distal epichile, and an elongated column flexed toward the lip. The osmophore lines the pouch of the hypochile and shows species-dependent epidermal structures. A flat to bullate surface is typical of *Stanhopea pulla*, a papillate surface is found in *Stanhopea candida*, a papillate to rugate surface is found in *Stanhopea tigrina*, a papillate surface with unicellular trichomes is found in *Stanhopea saccata*, and papillae with short, unicellular trichomes are present in *Stanhopea martiana*.[8] The ultrastructures of epidermal and subepidermal cells resemble mainly those of *Restrepia*.[3,6–8] Myrcene, α-pinene, β-pinene, α-terpineol, 1,8-cineol, methyl salicylate, phenylethyl acetate, and indol are frequently found scent compounds in *Stanhopea*.[24]

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An osmophore might also be present in *Boronia megastigma* (brown boronia, Rutaceae), which is native to southwestern Australia. Whereas the tissues of various flower organs contain lysigenous oil glands, stigma, and androecium lack these characteristic glands. Instead, the adaxial surface of the stigma and the tips of the

stamina show a papillate epidermis. Subjacent cell layers of all three organs are characterized by a dense cytoplasm and intracellular spaces. Fifty percent of the total scent is emitted by the stigma and the androecium. The compounds are almost exclusively β-ionone, dodecanol acetate, and *cis*-n-heptadecene, while the other flower parts emit mostly monoterpenes.[20,25,26]

Finally, a recently described example of a putative osmophore structure is found in *Gilliesia graminea* (Alliaceae). The striking similarity between flowers of this species and sexually deceitful orchids (e.g., *Ophrys*) caused closer investigation of this inflorescence, which also mimics insect shapes. Two thick structures extending abaxial from the staminal column (appendage 1) imitate the insect abdomen, and several narrow structures surrounding the staminal column appear as legs (appendage 2). The latter are thought to posses osmophores. Both appendage types display an epidermis with papillose cells (Figure 6.1).[18]

6.3 EPIDERMAL EMISSION

Osmophores with characteristic epidermal cells and subjacent glandular cell layers are found in a few, often highly evolved genera. Although typical osmophores are predestined for volatile production and emission, they are not always needed for scent emission. For example, the conspicuous absence of typical osmophores and papillate or rugose epidermis was observed for the well-scented *Clarkia breweri* (Onagraceae) and *Stephanotis floribunda* (Asclepiadacae)[27] (Figure 6.2). Consequently, the question arises, how does a flower that apparently lacks a typical osmophore emit volatiles? The most striking difference is that the epidermis cells are involved in both biosynthesis and the emission of volatiles. Thus the osmophores' characteristic starch agglomerations and lipoid droplets are primarily found in epidermis cells and not in the underlying cell layers.[3,28] The adaxial epidermis often displays delicate bullate, conical, or papillose cells in order to facilitate volatile emission, but the multilayered secretory production and releasing tissues present in

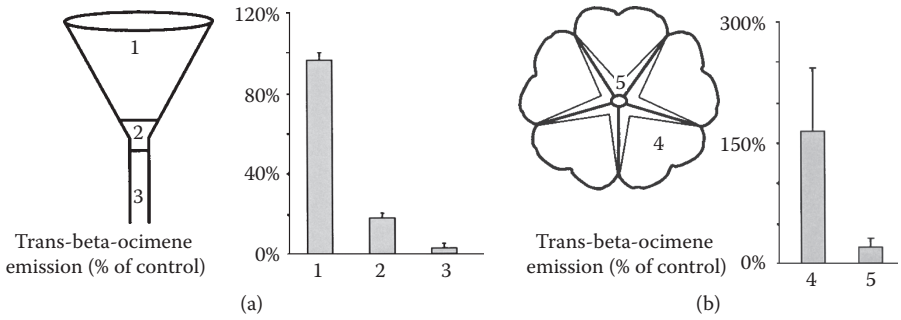

FIGURE 6.2 Localization of scent emission from *M. jalapa* flowers. (A) Schematic of the *M. jalapa* flower into (1) limb, (2) transition zone, and (3) tube. Relative emission of trans-β-ocimene in each floral part is presented. (B) Schematic of (4) petaloid lobe and (5) star-shaped center. Relative emission of trans-β-ocimene in each part is presented. The petaloid lobes are the primary source of ocimene emission.

typical osmophores are missing (Figure 6.1).[29–31] Therefore emitting floral tissues of this type are often very fragile, comprising only a very few loosely connected mesophyll cells and layers that are dispersed by an extensive intercellular system (Figure 6.1).[29,31,32]

In this context, two methods of volatile emanation are discussed. Lipoid secretions emerge on the adaxial surface of the emitting tissue and subsequently evaporate into the atmosphere (Figure 6.1B). Such secretions have been observed in *Rosa hybrida*. Often, an apparent secretion cannot be observed (e.g., *C. breweri*, *Antirrhinum majus* [snapdragon, Scrophulariaceae], and *Mirabilis jalapa* [four o'clock, Nyctaginaceae]) (Figure 6.1C), which indicates that evaporation is an immediate process.[29,31,33] Further details on the localization of scent synthesis in these species is presented in Section 6.5.

6.4 ORGAN- AND TISSUE-SPECIFIC EMISSION

More recent investigations have demonstrated that all flower organs are not equally employed in scent emission. Spatial differences within a flower (perianth, gynoecium, androecium) are quite common. Although petals are often the main source, and decisive for the whole flower bouquet, the stamen, pistil, and sepals contribute or are dominantly or even solely responsible for the emission of certain compounds. Examination of spatial emission patterns of *C. breweri* revealed that petals are mostly responsible for S-linalool, methyl eugenol, and methyl isoeugenol emission, whereas linalool oxide is released from the pistil. Although the benzenoid esters benzyl acetate, benzyl benzoate, and methyl salicylate are released from all flower parts, petals are responsible for most of the benzyl acetate/methyl salicylate emission, while the pistil is the primary source of benzyl benzoate release.[34,35,36] In *Chrysanthemum coronarium* (garland, Asteraceae), the compounds camphor and *cis*-chrysanthenyl acetate are primarily emitted by tubular and ligulate florets. The involucral bracts are responsible for sesquiterpene release, such as *trans*-α- and *trans*-β-farnesene, but also emit considerable amounts of myrcene and *cis*-ocimene.[37] Sesquiterpenes are also only found in sepal and gynoecium samples of *Rosa rugosa* (hedgehog rose, Rosaceae), whereas the constituents of the petal scent resemble the dominating compounds of the rose bouquet (citronellol, nerol, geraniol, 2-phenylethanol). In part, these petal volatiles are also retrieved in other flower parts, but only geranial and citronellol emission seems to be restricted to the petals.[38] Comparative analysis of volatiles emitted from different flower organs of *Ranunculus acris* (buttercup, Ranunculaceae) showed that petals, stamens, sepals, and gynoecium comprise identical volatiles, although they contribute different amounts; petals and stamens contribute the most.[39]

Remarkable also is the observation that emitting tissue sometimes comprises only certain parts of a floral organ. The basal (nectariferous) region of petals of *R. acris* is characterized by a higher emission of α-farnesene and 2-phenylethanol and an increased diversity of volatiles than in the apical region.[39] *M. jalapa*, a tropical plant that primarily emits *trans*-β-ocimene and small amounts of β-myrcene, *cis*-ocimene, *trans*-epoxy-ocimene, and benzyl benzoate, exhibits a perianth that consists of a tube and a five-lobed limb.[31,40,41] This corolla-like calyx is divided into four

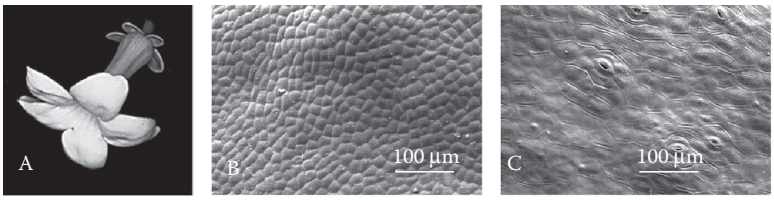

FIGURE 6.3 Petal epidermis of *S. floribunda*. (A) An individual *S. floribunda* flower. (B) The adaxial epidermis of the lobe exhibiting flat to slightly bullate cells (SEM). (C) The abaxial side of the lobe with a flat appearance of the epidermis. Some stomata are observed (SEM).

sections (tube, transition zone between tube and limb, petaloid lobes, and a star-like center of the limb). The segments were separately examined by GC-MS analysis for scent emanation, revealing that the petaloid lobes are the region of highest emission of *trans*-β-ocimene, which correlates with a bullate epidermis on the adaxial side (Figure 6.1C and Figure 6.3).[31]

Scent released by defined areas of flower organs are described, for example, for the wings of *Spartium junceum* (Spanish broom, Fabaceae), the vexillum of *Lupinus cruckshanksii* (Lupine, Fabaceae), or the paracorolla of *Narcissus jonquilla* (jonquil, Amaryllidaceae).[3,4] These areas are also stainable with neutral red and often such fragrance-emitting areas are congruent with ultraviolet (UV) light absorbing (visible) nectar guides, which represent an important cue to direct pollinators toward nectaries.[3,4] Lex[42] established the term "odor guide" for these fragrance-emitting areas and showed in her study that nectar guides were always correlated with odor guides.

Volatiles emitted by pollen are often remarkably different from those emitted by the other flower parts.[43] Pollen volatiles are significant constituents, but sometimes account for only a small part of the whole flower fragrance.[38] This phenomenon is reported for *R. acris*[39] as well as for *R. rugosa*.[38] *C. coronarium*,[37] *Filipendula vulgaris* (dropwort, Rosaceae), and *Lupinus polyphyllus* (lupine, Fabaceae).[43] Pollen volatiles comprise the same major classes of compounds known for floral scent, but most pollen odors are dominated by a few specific volatiles.[44] Protoanemonin is one of the typical compounds almost exclusively detected in pollen.[39] Furthermore, carbonylic compounds and long-chained linear hydrocarbons are present.[37,38,43]

6.5 GLANDULAR TRICHOMES

Volatile production and emission from vegetative tissue is linked to compartments such as glandular trichomes, oil glands, oil ducts, and cavities. Glandular and nonglandular trichomes have been well described for vegetative tissues.[45,47] These structural barriers and chemical weapons are important components of resistance to herbivores for plants in general.[48–51] Various types of glandular trichomes have been described (e.g., peltate, capitate, conoidal, and digitiform) that can be present on the same plant organ or tissue. Many plant species respond to insect damage by increasing the density or number of trichomes on new leaves.[52–60] Recent studies demonstrate that a jasmonic acid-dependent pathway also regulates the systemic

increase in physical defenses such as trichomes in *Arabidopsis thaliana* or *Lycopersicon esculentum*.[61,62] Exogenously applied jasmonic acid up-regulates and salicylic acid down-regulates trichome formation on new leaves, while gibberellin and jasmonic acid exhibit a synergistic effect on trichome production. The *jai1* tomato mutant (homolog to the F-box protein coronative-insensitive *COI* from *A. thaliana*), which is defective in jasmonic acid signaling, shows several defense-related phenotypes, including the inability to express jasmonic acid-responsive genes, severely compromised resistance to two-spotted spider mites, and abnormal development of glandular trichomes.

Since floral organs are metamorphogenized leaves, it can be assumed that floral glandular trichomes exist that harbor volatile secondary metabolites that may function in defense as well as attraction. However, it turns out that the yield of essential oils and fragrances is generally low in floral tissue (e.g., rose, 0.075% w/v; acacia, 0.084% w/v; and jasmine, 0.04% w/v), which may be the result of a different primary emanation process in flowers compared to vegetative tissue.[45] Presently it remains unclear how much trichome volatiles contribute to floral fragrance compositions. In contrast to our knowledge of glandular and nonglandular trichomes in vegetative tissues, the literature is limited about the presence, absence, and distribution of glandular trichomes/glands in floral organs and tissues. Microscopic studies of the adaxial and abaxial epidermis of various plant species were performed. The well-scented *S. floribunda*, which emits at least 27 different compounds, with methyl benzoate, linalool, α-farnesene, benzyl benzoate, and 1-nitro-2-phenylethane as major volatiles, does not exhibit trichomes on either the adaxial or the abaxial petal epidermis (Figure 6.2).[63] Capitate hairs, but no peltate glands, are present on the abaxial, but not the adaxial petal site of *Nicotiana alata* and *Nicotiana suaveolens* (Figure 6.4 and Figure 6.5). Both Solanaceae species emit terpenoid-rich (e.g., sabinene, β-myrcene, limonene, *trans*-β-ocimene, 1,8-cineole) and benzenoid-rich (e.g., methyl benzoate, methyl salicylate) scents nocturnally.[64] Headspace scent collection of separated petal lobes and petal tubes followed by GC-MS analysis revealed that fragrances are only emitted from lobe tissue (Figure 6.4 and Figure 6.5) (Piechulla B. et al., unpublished results). Since the same type of trichomes are present on lobe and tube tissue, it seems very likely that they are not involved in the synthesis or emission of fragrance compounds.

Mattern and Vogel[65] showed that many Laminaceae have glandular trichomes on the corolla. *Plectranthus ornatus* (Laminaceae), cultivated as an ornamental or as a source of essential oils, shows an unusual conoidal trichome with long unicellular conical heads on the calyx and the corolla.[17,66] On stamens and carpels, peltate trichomes are numerous. On the calyx, which is two-lipped (an upper and lower lip with four small teeth) capitate, digitiform, and conodial trichomes are clustered on the abaxial calyx surface, while peltate trichomes are scare and are restricted to the periphery of the lips. Digitiform and conoidal trichomes are the most conspicuous trichome types on the adaxial calyx surface. Histochemical studies reveal the presence of hyaline, a slightly viscous or orange-brown secretion in these glandular trichomes. The capitate and conoidal trichomes stain positively for lipophilic and hydrophilic substances, the digitiform types give positive reactions for hydrophilic substances, and peltate trichomes give positive reactions for lipophilic and terpenoid

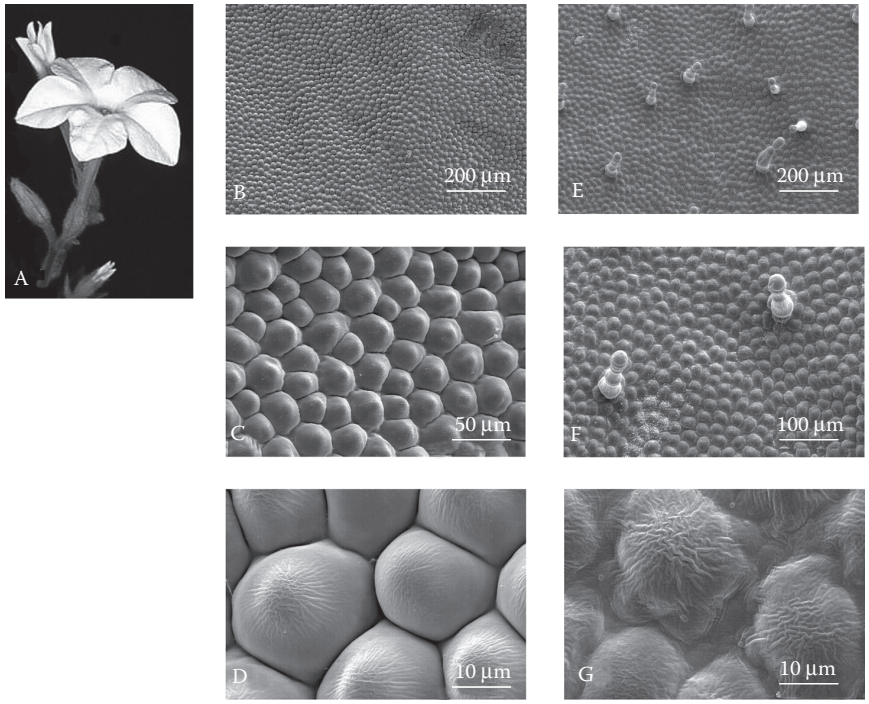

FIGURE 6.4 Petal epidermis of *N. alata*. (A) An individual *N. alata* flower. (B–D) The adaxial epidermis of the lobe exhibiting conical cells with a wrinkled cuticle at the tip (SEM, increasing magnification). (E–G) The abaxial epidermis of the lobe exhibiting bullate epidermis cells and several capitate trichomes. Higher magnifications show the wrinkled cuticle in the center of the epidermis cells.

compounds. Thus the trichomes of *P. ornatus* produce various amounts of secretory materials, however, volatiles have yet not been reported.

A large number of variable glandular hairs are found on the reproductive organs of *Salvia* species (Laminacee).[67] *Salvia dominica* peltate hairs, almost exclusively on the abaxial site of the calyx, are responsible for the secretion of neryl acetate, α-terpineol, and α-terpinyl acetate as major compounds, while myrcene, 1,8-cineole, and β-pinene are minor compounds. The stalky hairs are present on both sites of the corolla and produce relatively large amounts of linalyl acetate. Linalool and linalyl acetate appear in large amounts in abaxial hairs on the calyces, bracts, and peduncles of *Salvia sclarea*. The different morphological structures of the prominent scent-secreting hairs on the inflorescence rather than the peltate hairs on the vegetative tissue led Werker et al.[67] to suggest that they may have a different function (e.g., in luring specific pollinators).

The adaxial epidermis of *Phragmopedilum grande* (Orchidaceae, hybrid: *Phragmopedilum caudatum* × *Phragmopedilum longifolium*) of the distal part of two extremely elongated and twisted petals is supplied with unique uniseriate trichomes.[3] The trichomes consist of seven to eight cells and were found to be the source of the foul-smelling scent. The trichomes, present on completely unrolled petals, often

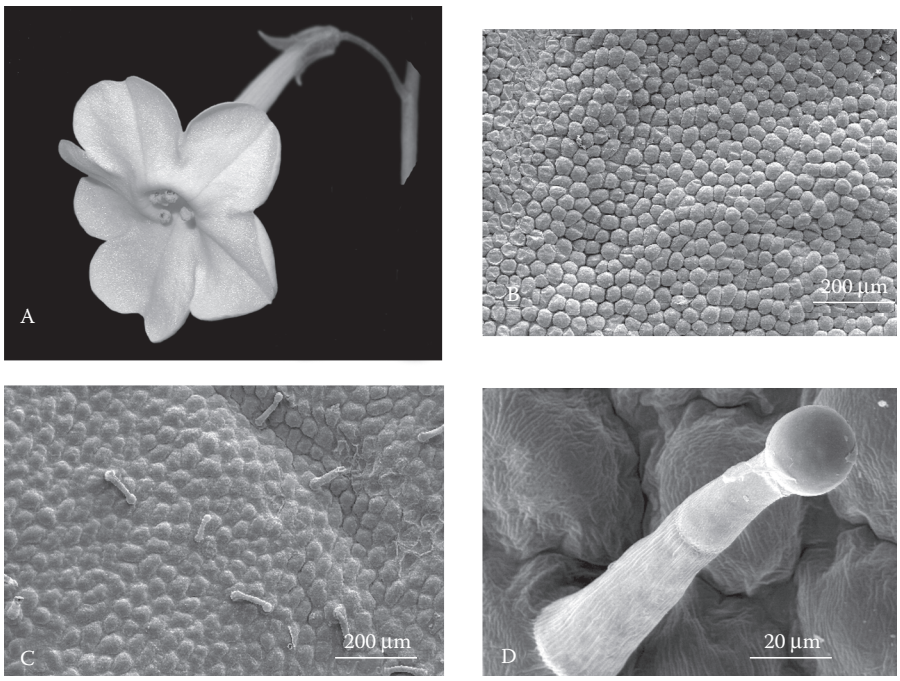

FIGURE 6.5 Petal epidermis of *N. suaveolens*. (A) An individual *N. suaveolens* flower. (B) The adaxial epidermis of the lobe exhibiting bullate epidermis cells (SEM). (C) The abaxial epidermis with flat epidermis cells and several capitate trichomes (SEM). (D) Higher magnification of the capitate trichome (SEM).

exhibit one or two dead brown cells at the tip, while the other cells remain turgescent and vital. The latter contain a large nucleus, a dispersed vacuome, and small starch grains. During flowering, when the apical cells become necrotic, terpenoid secretions can be observed. This is one of the few floral trichomes that have excretion ability and a typical osmophore function.

Trichomes on the osmophore of *Stanhopea saccata* (Orchidaceae) have also been observed, however, their functions remain unknown.[8] Secretory glands with oil-filled subcuticular space are observed on florets and developing ovules of *Chamaemelum nobile* (chamomile, Compositae), and endogenous oil glands are present in petals of *Syzygium aromaticum* (clove, Myrtaceae).[45] Vegetative excretory structures integrated into floral tissue are found in *Dictamnus albus* (burning bush, Rutaceae), having oil glands at the filaments, and in *Boronia megastigma* (Rutaceae), having lysigenous oil glands on the petal epidermis and in the mesophyll of bracts, sepals, petals, the receptacle, ovary, and nectary.[3,20,25,26] The latter glands are the site of α- and β-pinene and limonene production, monoterpenes which are found in the volatile blend of *B. megastigma*.

In the flower tube of *Antirrhinum majus*, two stripes of yellow hairs are located on the boundaries between the ventral and lateral petals. These hairs are on the adaxial epidermal surface. Environmental scanning and light microscopy revealed that these hairs are unicellular, consisting of a long stalk and a head. Cross sections incubated with antibodies against an enzyme involved in scent production (benzoic

acid methyltransferase [BAMT]) stain the head of these hairs, indicating the presence of this biosynthetic enzyme.[29]

Microscopic studies of *M. jalapa* showed that a large number of uniseriate and multicellular capitate trichomes are present on the abaxial site of the petaloid lobe (Figure 6.6A).[31] Scanning electron microscopic investigations implied a glandular character of the trichome because of the enlarged head-like cell. After electron beam disruption, organic matter leaking from the trichome was observed. Trichomes from *M. jalapa* were collected and analyzed by GC-MS. Surprisingly, not *trans*-β-ocimene as expected, but β-farnesene was found, a substance that is well known as a chemical defense compound.[68–70]

Anthers, which present the pollen, are important for the reproductive success of the plant. To limit destruction of the pollen or stamen by herbivores and pathogens, it is therefore not surprising that glandular trichomes are frequently found on the anthers. The anthers of the four fertile stamens of *Leonorus sibiricus* bear small glandular scales that rupture at the slightest touch and release a sticky substance. Their location strongly suggests a role in the production of adhesive substances, but release of volatiles has not been shown.[71] A few other reports also demonstrate that such anther glands provide an accessory pollenkitt (*Cyclanthera*, Curcubitaceae;[72] *Hedyhinum*, Zingiberaceae;[73] *Drymonia*, Gesneriaceae[74]). Anther glands present on the connectives found in *Cyphomandra* (tamarillo, Solanaceae) produce a reward for euglossine bees. Sazima et al.[21] analyzed the volatiles and the flower morphology and showed that the dorsal papillate epidermis is attached to glandular mesophyll cell layers that are responsible for production and secretion of 1,8-cineol, *trans*-β-ocimene, and germacrene D, and resembling typical osmophore characteristics. Anther glands are also quite common in Mimosoideae. A comprehensive survey of anther glands in this tribe showed that among four gland types, the unusual conical type on the ventral side of the gland just above the anther sacs was only found in *Pentaclethra macroloba* (Parkieae, Mimosoieae, Fabaceae).[19] Although the function is still unknown, the authors speculate that this structure may be involved in volatile production and emission.

Glandular trichomes present on floral organs may be a source of floral volatiles. Many investigations have shown a correlation between trichome appearance on floral tissue and scent emission, however, to our knowledge, a definite volatile presence in floral trichomes has only been demonstrated with *M. jalapa*. Since the *Mirabilis* floral trichomes contain a compound with a biological defense function, the idea is put forward that typical floral pollinator-attracting volatiles are emitted differently compared to the characteristic glandular trichome-based vegetative defense compounds. Further investigations are needed to support or reject this hypothesis.

6.6 CELLULAR AND SUBCELLULAR LOCALIZATION OF SCENT BIOSYNTHESIS

Osmophores, epidermis, and different floral organs are the structures that are the sources of fragrance emission. Recent progress in elucidating the biosynthetic pathways of volatiles has provided further tools for detailed localization of scent synthesis and emission. In the past 10 years, several enzymes involved in volatile biosynthesis

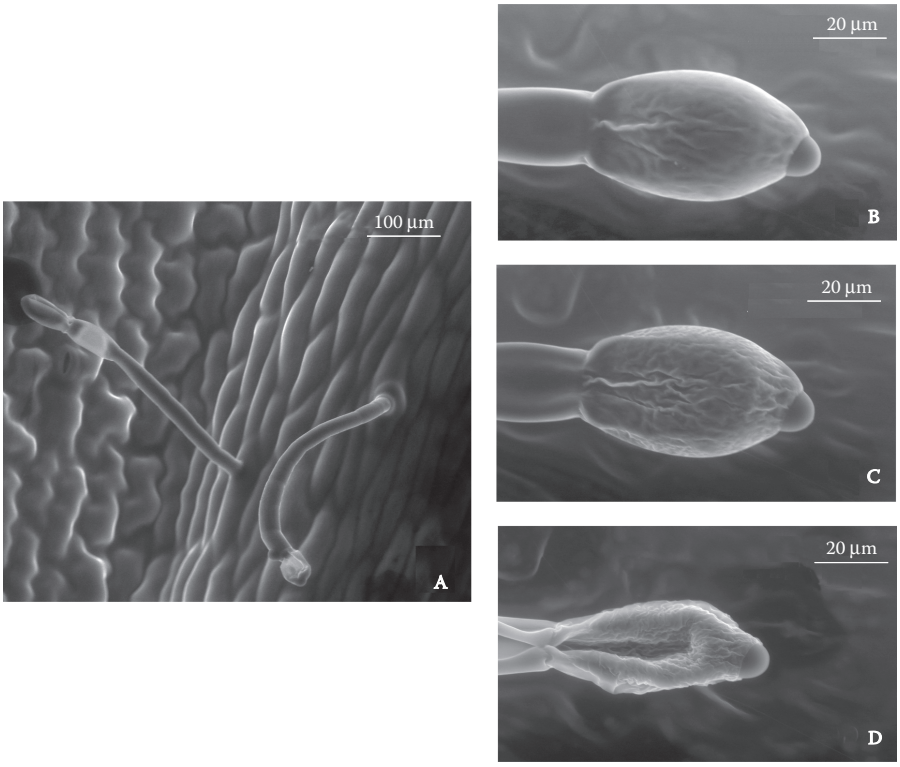

FIGURE 6.6 Trichomes of *M. jalapa*. (A) Trichomes of the abaxial epidermis of *M. jalapa*. Trichomes are localized on the veins (environmental scanning electron microscope [ESEM]). (B) Higher magnification of the trichome head (ESEM). (C, D) Collapsing trichome head after electron beam disruption (ESEM).

of terpenoids, benzenoids, and fatty acid derivatives have been isolated and characterized. In most cases, the respective genes have been cloned and analyzed, and now are available as molecular tools.

The presence of biosynthetic enzymes or their respective transcripts in particular floral organs and tissues is presently the best indicator for volatile synthesis. Northern blots performed with a probe against the benzenoid carboxyl methyltransferase (BSMT) from *N. suaveolens* show that the respective messenger RNAs (mRNAs) primarily accumulate in petal tissue and only to very small extent in other floral parts (Figure 6.7). Such organ-specific accumulation patterns are also found in snapdragon (*A. majus*) and petunia.[75–77] The expression of acetyl-CoA:benzyl alcohol acetyltransferase (BEAT) and linalool synthase (LIS) from *C. breweri* is not so organ specific, since transcripts are detectable in petals, sepals, stamen, stigma, and style to different degrees.[33,78] Separation of the perianth of snapdragon revealed significantly higher transcript levels of the ocimene synthase in lower lobe tissue.[76] In addition to the transcript appearance in extracts of floral tissue, the presence of enzyme activities in flower extracts is taken as an indicator of localized scent synthesis (e.g., in snapdragon and *S. floribunda*).[63,75] Furthermore, the molecular tools can be

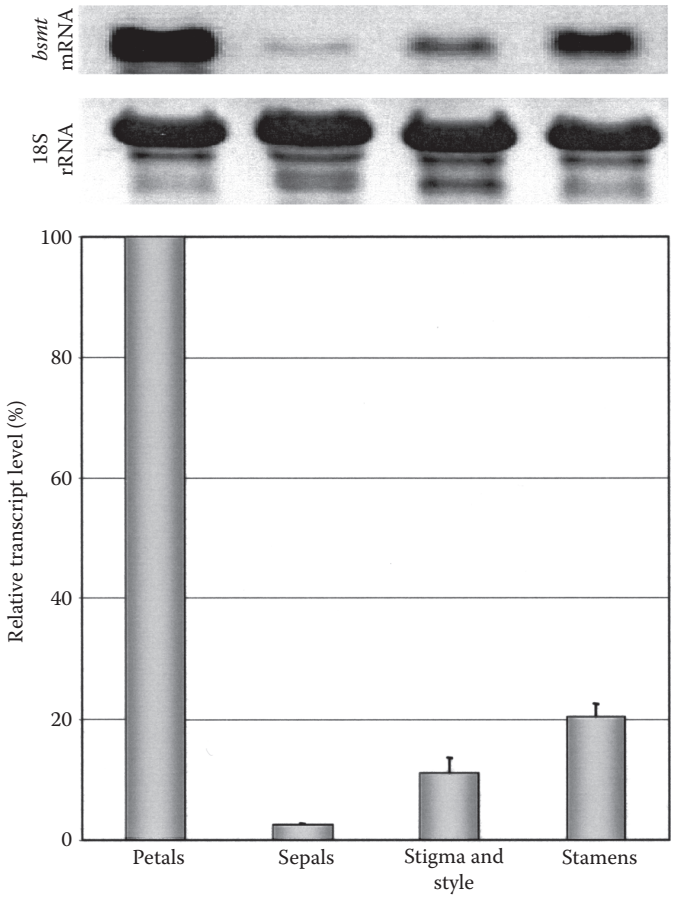

FIGURE 6.7 Expression of benzoic/salicylic acid methyltransferase (BSMT) in floral organs of *N. suaveolens*. Total RNA isolated from different floral organs was hybridized with a *BSMT* gene-specific probe. Differential mRNA accumulation is observed in the different tissues relative to 18S rRNA levels. Relative transcript levels are calculated. The highest level in petals was 100%.

used to trace respective transcripts and enzymes at the cellular and subcellular level. The spatial distribution of the LIS transcripts in *C. breweri* buds, pistils, and petals was performed by in situ hybridization using sense and antisense RNA probes.[33] In cross sections of the flower, it can be seen that LIS mRNA transcripts are mainly concentrated in the secretory zone of the four-lobed stigma and also in the epidermal layers of the petals. Since up to 70% of the total LIS activity of *C. breweri* flowers is found in the petals, they are regarded as the major source of emitted linalool.[33,34,79] The petal epidermal cells produce water-insoluble linalool on the surface, from which it can most easily escape into the atmosphere. Thus it appears that *C. breweri* has evolved its ability to emit large amounts of linalool simply by highly expressing LIS

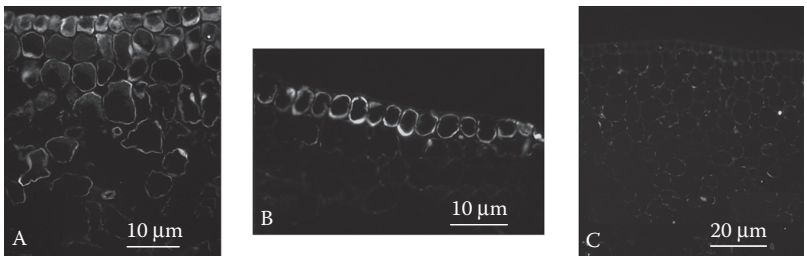

FIGURE 6.8 Immunofluorescence localization of SAMT in *S. floribunda* petals. Cross sections of the (A) *S. floribunda* lobe, (B) transition zone (upper part of the tube), and (C) lower part of the tube were incubated with antibodies against the salicylic acid methyltransferase (SAMT) and FITC-labeled secondary antibodies.

in the epidermal cells of the petals, without the concomitant development of specialized scent glands.[33]

The distribution of volatile synthesizing enzymes in floral tissue has only been investigated with *S. floribunda* (Figure 6.8) and *A. majus*.[29] Salicylic acid methyltransferase (SAMT), the enzyme that methylates salicylic acid using S-adenosyl-L-methionine as a methyl donor, was found in the petals of *S. floribunda* when specific antibodies against the SAMT enzyme and fluorescein-labeled (FITC) secondary antibodies were incubated with thin sections from various petal regions (petal lobe, upper part of the tube, lower part of the tube). The SAMT enzyme is primarily present in the epidermal cells of the petal lobe, but underlying cell layers also stain to some extent (Figure 6.8A). Since epidermis as well as subjacent cell layers express the SAMT enzyme, it is likely that a typical osmophore, in the sense of Vogel's definition, exists in *S. floribunda*, although only parts of the petals are involved. Interestingly, in the upper part of the flower tube, the SAMT is exclusively restricted to the adaxial epidermal cells (Figure 6.8B), and no enzyme could be detected in the lower part of the petal tube (Figure 6.8C). These experiments clearly define the petal lobes of the *Stephanotis* flower as the area where methyl salicylate synthesis occurs, which correlates well with GC-MS analysis (Piechulla B. et al., unpublished results). A similar enzyme from snapdragon flowers to the one mentioned above, benzoic acid methyltransferase (BAMT), turns out to be epidermis specific. Both the adaxial and abaxial epidermal petal cells are differentially involved in scent biosynthesis.[29] Methyl benzoate is predominantly produced in the conical cells of the adaxial epidermis of the lower lobe and tube. Apparently the cells between both epidermata do not contain much BAMT enzyme. They form a very loose structure with large intercellular spaces. BAMT expression was also found in the yellow hairs within the tube located on the bee's way to the nectar. Subcellular localization with immunogold-labeled antibodies localizes the BAMT in the cytoplasm of the epidermal cells, adjacent to the primary cell wall.[29]

As more enzymes and genes of the biosynthetic pathways of floral scent compounds become available, the more in situ hybridization and immunofluorescence experiments can be performed that will help to clarify our understanding of floral scent synthesis and emission on the cellular level.

6.7 CONCLUSION

It is imprecise to identify the flower as the source of scent emission. For many insects, defined guidance and orientation within the flower is absolutely necessary. Therefore it is not surprising that many investigations clearly demonstrate that not only flower organs (e.g., classical osmophores), but certain areas or parts of a floral organ emit distinct scents or scent compositions. Modern techniques allow us to obtain detailed information about which floral organs and tissues, and in which cells or cell layers scent synthesizing enzymes or transcripts are present. The precise localization of scent synthesis in many plant species will provide helpful information to further understand transport and transport mechanisms, as well as the process of scent emission in floral tissues. Furthermore, such investigations might support and clarify the present view that glandular trichomes on vegetative as well as floral tissue produce defensive volatiles, while the pollinator attracting volatiles are synthesized and emitted from classical osmophores or from other distinct floral tissues.

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