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Ultrastructure of the corona of scented and scentless flowers of *Passiflora* spp. (Passifloraceae)

María T. Amela García*, Beatriz G. Galati, Patricia S. Hoc

Depto. Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina

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Abstract

To know thoroughly the structure and function of the corona of *Passiflora*, the anatomy and ultrastructure of two species were analysed in relation to the emission of odour perceivable by humans: a scented one, *P. caerulea* L., and a scentless one, *P. suberosa* L.

Both species exhibited secretory tissue, whose cells were characterised by dense cytoplasm, numerous mitochondria and vacuoles. Evidence of granulocrine secretion was detected. Nevertheless, there were differences concerning some cytological structures: *P. suberosa* lacked smooth endoplasmic reticulum (sER) and starch but had large and many lipidic globules, while *P. caerulea* had few dictyosomes, scarce lipidic content, a greater proportion of sER/rough endoplasmic reticulum (rER) and amyloplasts. The cellular features of *P. caerulea* correspond with those of fragrance tissues. The secretion appearance and quantity were also different between both species: *P. caerulea* exhibited sparse drops on the cuticle in contrast to *P. suberosa*, which secrets a wax-like material. If this is the final product of the secretory process or just a vehicle that contributes to the emission of volatile compounds, as occurs in certain osmophores, needs further confirmation with chemical analysis.

Results are discussed in the context of the pollination syndromes of each species and their florivores.

Keywords: Osmophores; Submicroscopic structure; TEM; *Passiflora*; Scent guides; Wax

Introduction

Since the definition of osmophores by Vogel in 1962 (English translation of 1990), and even Vogel's earlier works (Weber and Sonntag, 2006), the histology of these special localised plant tissues that emit odour has been studied by means of optical microscopy (Stern et al., 1986; Weryszko-Chmielewska and Stpiczynska, 1995) and by transmission electron microscopy (TEM) (Curry, 1987; Curry et al., 1991; Pridgeon and Stern, 1983, 1985; Stern et al., 1987; Stpiczynska, 1993, 2001). Strikingly, the majority of the works deal with Orchidaceae; the rest involve Asclepiadaceae, Aristolochiaceae, Araceae and Burmanniaceae (Vogel, 1990).

The floral biology of only 23 species of *Passiflora* has been studied (Amela García, 1999, and citations therein), although more than 525 species have been described (MacDougal and Feuillet, 2004). At least 26 species produce aroma apparent to us (Amela García, 1999; Aponte and Jáuregui, 2004; Frankie and Vinson, 1977; Girón van der Huck, 1984; Kay, 2001; Koschnitzke and Sazima, 1997; Lindberg et al., 2000; Neff and Rozen, 1995; Sazima and Sazima, 1978; Varassin et al., 2001).

*Corresponding author.
E-mail address: amela@bg.fcen.uba.ar (M.T. Amela García).

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The volatile compounds have been captured and identified in 12 of them (Lindberg et al., 2000; Varassin et al., 2001). Olfactory tests to the dissected cycles of the flowers in hermetic vials performed in nine species evidenced that the odour is emitted by the different pieces of the corona, which in most species is stronger in the radii (Amela García, 1999; MacDougal, 1994). Epidermal papillae cover mainly the operculum filaments, the pali apex and the radii at least in *P. mooreana* (Amela García, 1994), *P. caerulea* (Amela García and Hoc, 1997) and *P. chrysophylla* (unpublished data); these cells react positively for lipids when stained with Sudan IV in the three species (Amela García, 1999).

The absence of odour has been reported in seven species: *P. manicata* (Girón van der Huck, 1984), *P. quadrifaria* (Vanderplank, 1996), *P. suberosa* (Koschnitzke and Sazima, 1997; Amela García, 1999), *P. andina*, *P. sanguinolenta, P. sexflora* and, doubtfully, *P. edulis* (Lindberg et al., 2000). The last four were analysed for the volatile compounds, and no substance was captured (Lindberg et al., 2000). Although the corona pieces are present in six of the scentless species, except, apparently, in *P. andina* (Killip, 1938), the anatomy and histology of this floral cycle in those species have not been described yet.

With the aim to search for the presence of secretory tissue, or at least some differences between the corona tissues in scented and scentless flowers of *Passiflora*, two species were analysed: one with odour, *P. caerulea* L., and one without odour, *P. suberosa* L.

Materials and methods

The flowers of both species last 1 day and pass through three floral phases. Odour is more strongly perceived during the second phase in *P. caerulea*. This stage was chosen in anthetic flowers for both species in the whole study. For the detailed description of the floral biology, see Amela García (1999).

Flowers of *P. suberosa* were obtained from plants collected at San Ignacio, Misiones province, Argentina, and grown in the greenhouse of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Flowers from *P. caerulea* were taken from the natural population that grows at the campus of this institution.

To ascertain the floral active secreting tissues, flowers in anthesis were immersed in neutral red solution following Vogel (1990) for 4 h and the resultant staining was analysed according to the recommendations of Vogel and Hadacek (2004).

The rest of the study was conducted in the corona pieces in which odour is more strongly perceived in the scented flowers (the radii), in both species.

Histochemical tests on freehand slices were performed, using flowers in anthesis. Three sets of sections of fresh material were placed on microscope slides, stained with neutral red, Sudan IV and picric acid, and observed with an optical microscope to detect secretory activity (Vogel, 1990), lipophilic and proteic substances (Johansen, 1940), respectively.

Sections of 1 μm thickness were stained with toluidine blue 0.1% for light microscopy studies.

Observations with TEM were carried out in anthetic and in recently closed flowers. For this purpose, fresh material was fixed in glutaraldehyde 3% in phosphate buffer pH 7.2 and postfixed in osmium tetroxide 1.5% at 2 °C in the same buffer for 3 h. This material was dehydrated in an ethanol series and embedded in Spurr's resin. Ultrathin sections of 750–900 nm thickness were produced on a Sorvall ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds) (O'Brien and McCully, 1981), observed and photographed with a Jeol-Jem 1200 EXII transmission electron microscope at 85.0 kV.

The terminology employed to denote the corona elements follows Tillet (1988), and to describe the shapes Stearn (1963) was followed.

Results

Corona morphology and detection of secretory tissues ad oculus

The radii are arranged in two series in *P. caerulea* (Fig. 1A) and only in a single series in *P. suberosa* (Fig. 1B).

P. suberosa is greenish, so there was no problem to distinguish positive staining with neutral red solution. Although *P. caerulea* is bluish, staining with neutral red solution was not masked by the flower pigments, and clearly differed from control flowers (Fig. 1). Although other floral pieces reacted positively with the neutral red solution (Table 1, Fig. 1), the radii absorbed the stain stronger in both species, more uniformly along their length in *P. caerulea* (Fig. 1).

Histology and ultrastructure of the radii

Histochemical reactions

The staining with neutral red was positive and homogeneous in all the tissues of both species.

The reaction with Sudan IV was positive in the epidermal and subepidermal layers of *P. caerulea* and in the whole tissues of *P. suberosa*; it was more intense in *P. caerulea* (strong red) and weaker in *P. suberosa* (strong orange).

The reaction with picric acid was homogeneous and weak in the whole tissues of both species.

Fig. 1. Neutral red staining of the different floral parts to detect presumptive secretory tissue. (A, C) *P. caerulea* (scented); (B, D) *P. suberosa* (scentless). (A, B) Unstained flowers; (C, D) stained flowers. o = operculum, p = palus, pe = petal, r = radius, s = sepal. Bars: A, C = 20 mm; B, D = 7 mm.

Table 1. Staining of the different parts of *P. caerulea* and *P. suberosa* flowers with neutral red solution

Floral part	*P. caerulea*	*P. suberosa*
Sepals	Dots in adaxial surface: + +	Edge, adaxial surface: + +
Petals	Dots in adaxial surface: +	Absent
Radii	Uniformly: + + + +	Stronger in base: + + +
Pali	+ + +	+ + +
Operculum	+	+
Anthers	+	+
Styles	+	Base: +
Stigmata	+	+
Staminal filaments	—	+
Androgynophore	—	—
Ovary	—	—

Note: When the whole piece did not react, the part that reacted is stated; the number of crosses represents the intensity of the reaction.

General anatomy

P. caerulea

Anthesis. Each radius was circular to slightly elliptic in cross section, depending on the height at which the section was performed, with an undulating outline (Fig. 2A). The epidermis was formed by unicellular papillae, which had a central vacuole, a dense cytoplasm and quite a voluminous nucleus (Fig. 2B). The outer tangential wall of each papilla was thicker in the apex (Fig. 2B, arrows).

The subepidermal cells comprised 3–5 layers, with relatively dense cytoplasm, a large central vacuole and a big nucleus, but smaller than the ones of the epidermal cells (Fig. 2B). The remaining parenchyma had higher vacuolated cells with small nucleus and abundant, big air spaces (Fig. 2B).

There were 4–8 collateral vascular bundles with well-developed phloem: 2–4 central ones and 4 in the periphery (Fig. 2B).

Post-anthesis. The wall thickness of each papilla was uniform in this stage. Increased vacuolation and less cytoplasm density were perceived both in the epidermal and in the subepidermal cells (Fig. 2C); the last ones had lost turgescence.

P. suberosa

Anthesis. Each radius was elliptic to ovate in cross section, depending on the height of the cut (Fig. 2D–E). The epidermis was formed by unicellular papillae (shorter than the ones of *P. caerulea*), more or less

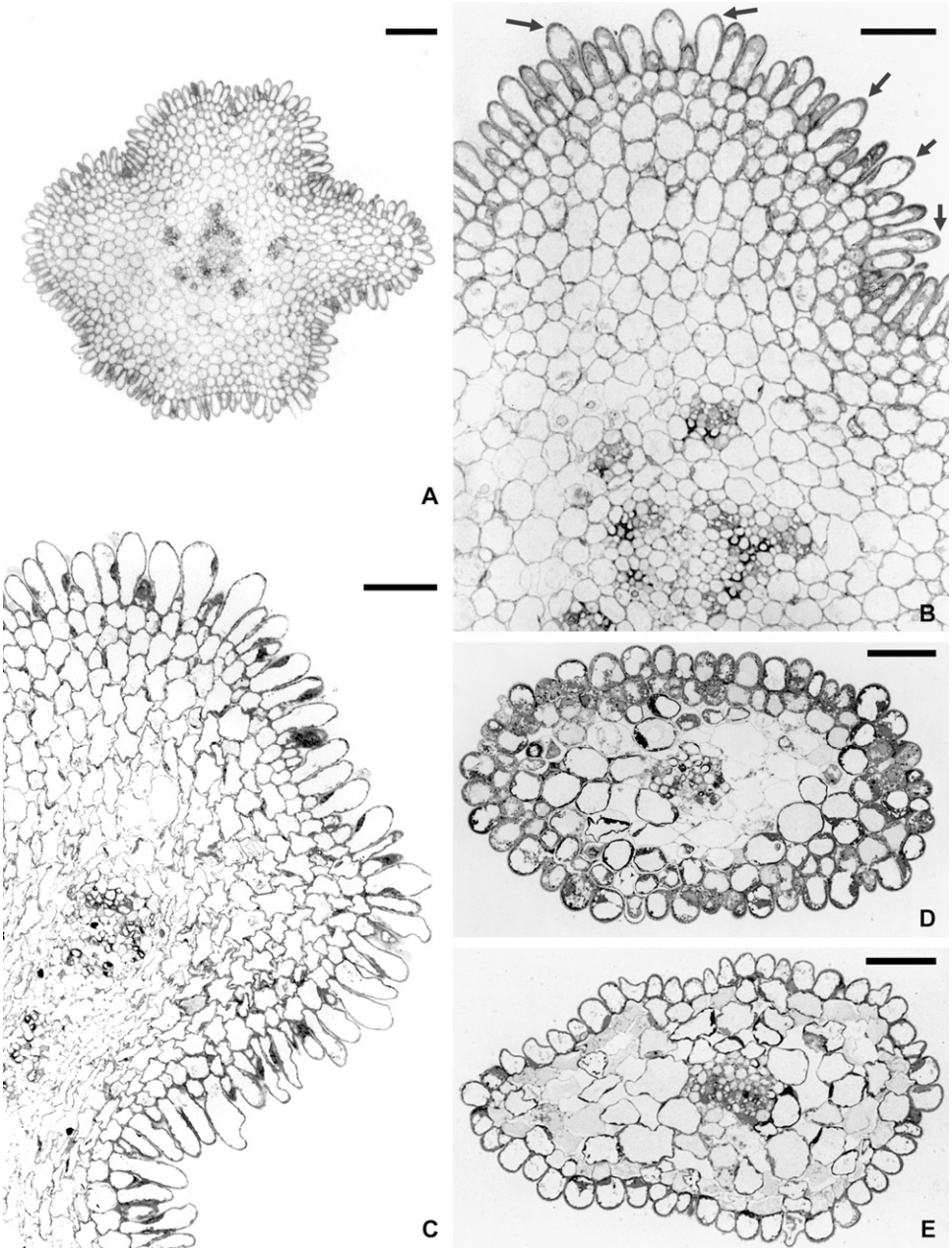

Fig. 2. Photomicrographs of transverse sections of the radii. A–C, *P. caerulea*. (A, B) Anthesis: (A) general aspect; (B) detail. Note thickened wall at papillar tips (arrows). (C) Post-anthesis, detail; (D,E) *P. suberosa*, general aspect; (D) anthesis; (E) post-anthesis. Bars: A = 100 μ; B–E = 50 μ.

vacuolated, with dense cytoplasm and conspicuous nucleus (Fig. 2D). The papillae wall was uniform in thickness.

The subepidermal cells comprised one or two layers, similar to the epidermal ones in degree of vacuolation, nucleus size and cytoplasm density (Fig. 2D). The remaining parenchyma had more vacuolated cells and numerous large air spaces (Fig. 2D).

There was one collateral vascular bundle in the centre, with well-developed phloem (Fig. 2D).

Post-anthesis. The degree of vacuolation increased, and the cytoplasm density and the nucleus volume decreased, both in the epidermal and subepidermal cells (Fig. 2E); the last ones had lost turgescence.

Ultrastructure

P. caerulea

Anthesis. The epidermal cells exhibited much smooth endoplasmic reticulum (sER) with very dilated cisternae (Fig. 3B, C), little rough endoplasmic reticulum (rER) (Fig. 3C), abundant mitochondria with highly developed cristae, some of them with a slight central constriction (Fig. 3F), abundant composed amyloplasts with numerous granules in each one (Fig. 3D), few lipidic globules

in association with vacuoles (Fig. 3A), scarce dictyosomes (Fig. 3F) and some peroxisomes (Fig. 3E).

Many vesicles with granular electron-dense content appeared in close contact with the plasmalemma (Fig. 3A) and between this and the outer tangential wall (Fig. 3B, white arrowhead).

The cuticle was thin (Fig. 3B). A high electron-dense and fibrillar zone under the cuticle, in the apical portion of the papilla wall, was observed (Fig. 3A, B). Isolated zones of the same electron density were found at deeper levels of the wall (Fig. 3B, black arrowheads).

An osmiophilic substance was deposited as droplets on the cuticle (Fig. 3A, B, arrows).

Plasmodesmata connected adjacent epidermal cells, epidermal and subepidermal cells (Fig. 3H, arrow) and subepidermal cells between themselves (Fig. 3G, arrow).

Subepidermal cells also contained a great quantity of amyloplasts, scarce dictyosomes (Fig. 3G), numerous mitochondria with highly developed cristae (Fig. 3G, H), many cisternae of rER and few of sER (Fig. 3H).

Post-anthesis. Epidermal cells still maintained mitochondria with highly developed cristae (Fig. 4B, C), most of which were around the nucleus (Fig. 4D). The plastids had a homogeneous content (Fig. 4C), starch was lacking. Remnants of rER and sER remained (Fig. 4A, B). The electron-dense fibrillar zone of the wall under the cuticle was no longer visible (Fig. 4A). Electron-dense material was seen only between the plasmalemma and the wall (Fig. 4A, arrowhead, B, arrows), and traces of it were sparse on the cuticle (Fig. 4A, arrow). The middle lamella was more notorious in this stage due to its swelling and higher electron density (Fig. 4B, arrowhead). Some plasmodesmata between epidermal (Fig. 4C, arrow) and subepidermal (Fig. 4D, arrow) cells persisted. Subepidermal cells also maintained mitochondria (Fig. 4D).

P. suberosa

Anthesis. The epidermal cells possessed a striated surface (Figs. 5A and 6A). Their cytoplasm exhibited numerous, very large lipidic globules (Fig. 5A–C, E, F), mitochondria and rER (Fig. 5B–D). Plastids with tubular elements, each one ca. 2 nm wide and parallel organised, were present (Fig. 5D).

Many dictyosomes were observed beside the large lipidic globules near the plasma membrane (Fig. 5C) and at both sides of plasmodesmata (Fig. 5F).

There were numerous vesicles in contact with the plasmalemma, between the plasmalemma and the wall (Fig. 5A–C, asterisks), and close to plasmodesmata that connected epidermal cells among themselves (Fig. 5G, asterisks) and epidermal cells with subepidermal ones (Fig. 5F, asterisk). A fibrillar substance and a dotted more electron-dense material were present between the plasmalemma and the wall, both in the epidermal cells (Fig. 5A–C, G) and towards the outer tangential wall of the subepidermal cells (Fig. 5F).

The cuticle was thick (Fig. 5A); the wall microfibrils seemed to intrude inside it, resembling microchannels (Fig. 5A, black arrowhead). A high electron-dense substance was found in the wall, near the cuticle (Fig. 5A, white arrowhead). Structures similar to plates of high electron density were deposited on the cuticle (Fig. 5A, arrow).

Plasmodesmata connected the epidermal cells (Fig. 5G, arrow), the epidermal cells with the subepidermal ones (Fig. 5E, F, arrows), and also the subepidermal ones among themselves.

The subepidermal cells had many lipidic globules, scarce dictyosomes, numerous amyloplastids with lamellae and starch (Fig. 5E, H), rER (Fig. 5F) and mitochondria (Fig. 5E, F, H).

Post-anthesis. The epidermal cells maintained rER and many mitochondria (Fig. 6B). The lipidic globules were less abundant and some of them were protruding inside the vacuole (Fig. 6A, arrow).

The high electron-dense plate-like deposits on the cuticle were no longer present, they were reduced to dots on the cuticle (Fig. 6C, black arrow). The electron-dense substance between the cuticle and the wall was more evident (Fig. 6C, white arrowheads). Granules of the same electron density were embedded at different levels in the wall (Fig. 6C, white arrows). The tiny microchannels became more conspicuous due to the presence of the electron-dense substance inside them (Fig. 6B, arrows). The fibrillar substance persisted between the wall and the plasmalemma (Fig. 6B, C, asterisk), but the vesicles were not noticeable any more.

The subepidermal cells showed a cytoplasm with signs of degeneration, where there were remnants of membranes and mitochondria (Fig. 6E, F).

Scarce or no plasmodesmata were found between epidermal cells, subepidermal ones nor between both type of these cells.

Discussion

Secretory tissue

Neutral red has been used as indicator of secretory tissues, particularly presumptive osmophores (Vogel, 1983); most of the scented parts stain (Stpiczynska, 2001; Weryszko-Chmielewska and Stpiczynska, 1995), although some scentless flowers also tinge (Stern et al., 1986; Vogel, 1990). This solution also stains anthers and stigmata, even they are not glandular (Vogel and Hadacek, 2004), so the positive reaction in this case should not be considered as indicative of osmogenic tissue, until anatomical studies confirm this not discardable fact. In *P. caerulea* and *P. suberosa* the radii stained stronger than the rest of the positively reacting parts, in

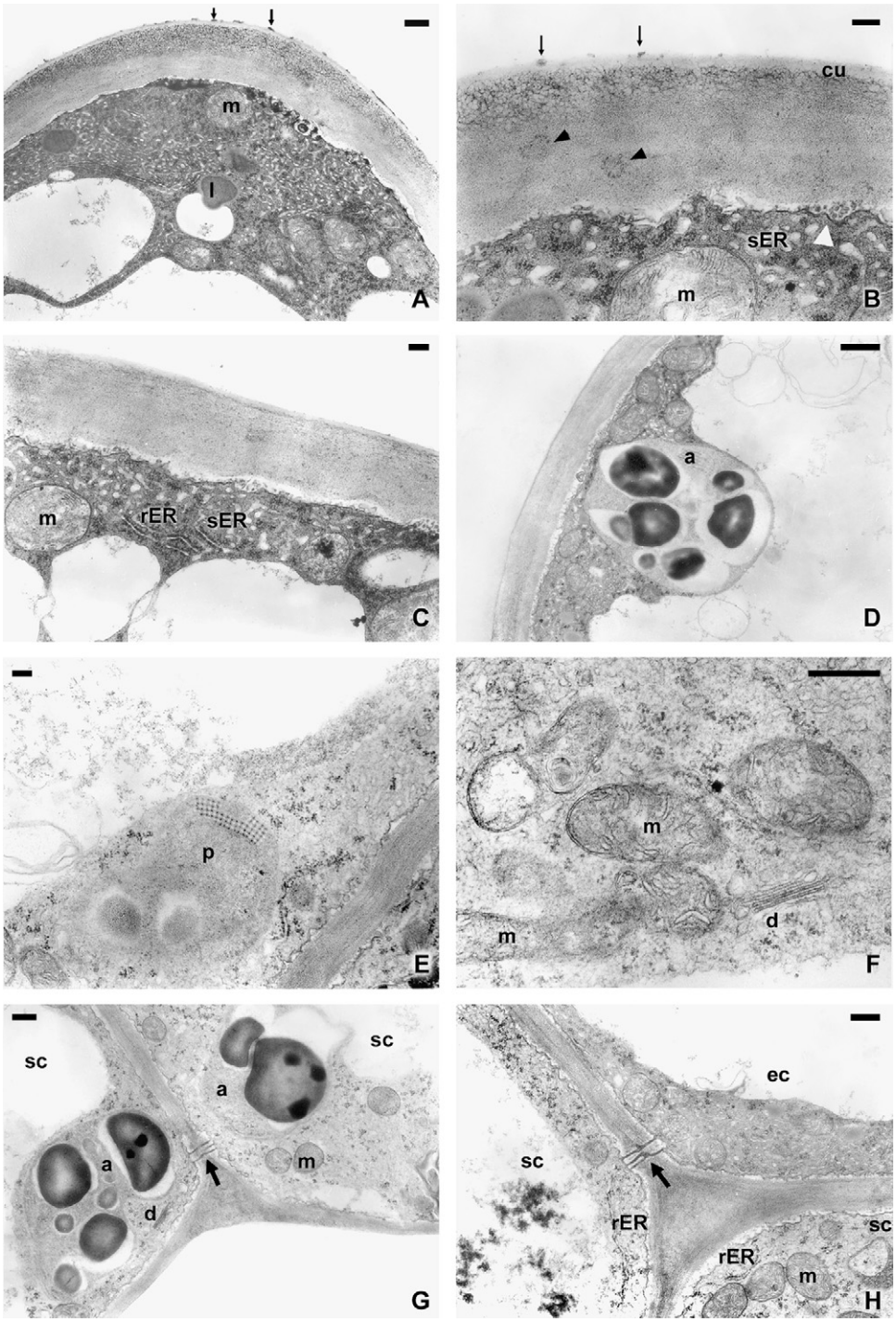

Fig. 3. TEM photographs of the radii of *P. caerulea* in anthesis. (A–F) Epidermal cells. (A, B) Papillar apex. Note: Vesicles with granular electron-density content in contact with plasmalemma (A, right side) and between this and the wall (B, white arrowhead), high electron-dense and fibrillar zone beneath the cuticle and amidst the wall (B, black arrowheads) and osmiophilic droplets on the cuticle (A, B, arrows). (C–F) Cytoplasm details of epidermal cells. (G) Subepidermal cells, connected by plasmodesmata (arrow). (H) Epidermal and subepidermal cells, connected by plasmodesmata (arrow). a = amyloplast, cu = cuticle, d = dictyosome, ec = epidermal cell, l = lipidic globule, m = mitochondrion, p = peroxisome, rER = rough endoplasmic reticulum, sc = subepidermal cell, sER = smooth endoplasmic reticulum. Bars: A, G, H = 500 nm; B, C, E = 200 nm; D = 1 μ; F = 500 nm.

accordance with the more intense fragrance perceived when olfactory tests are performed in the scented *P. caerulea* (Amela García and Hoc, 1997).

The reaction with Sudan IV in both species correlates with the high density of lipid globules in *P. suberosa* and of endoplasmic reticulum in *P. caerulea*; this organelle is

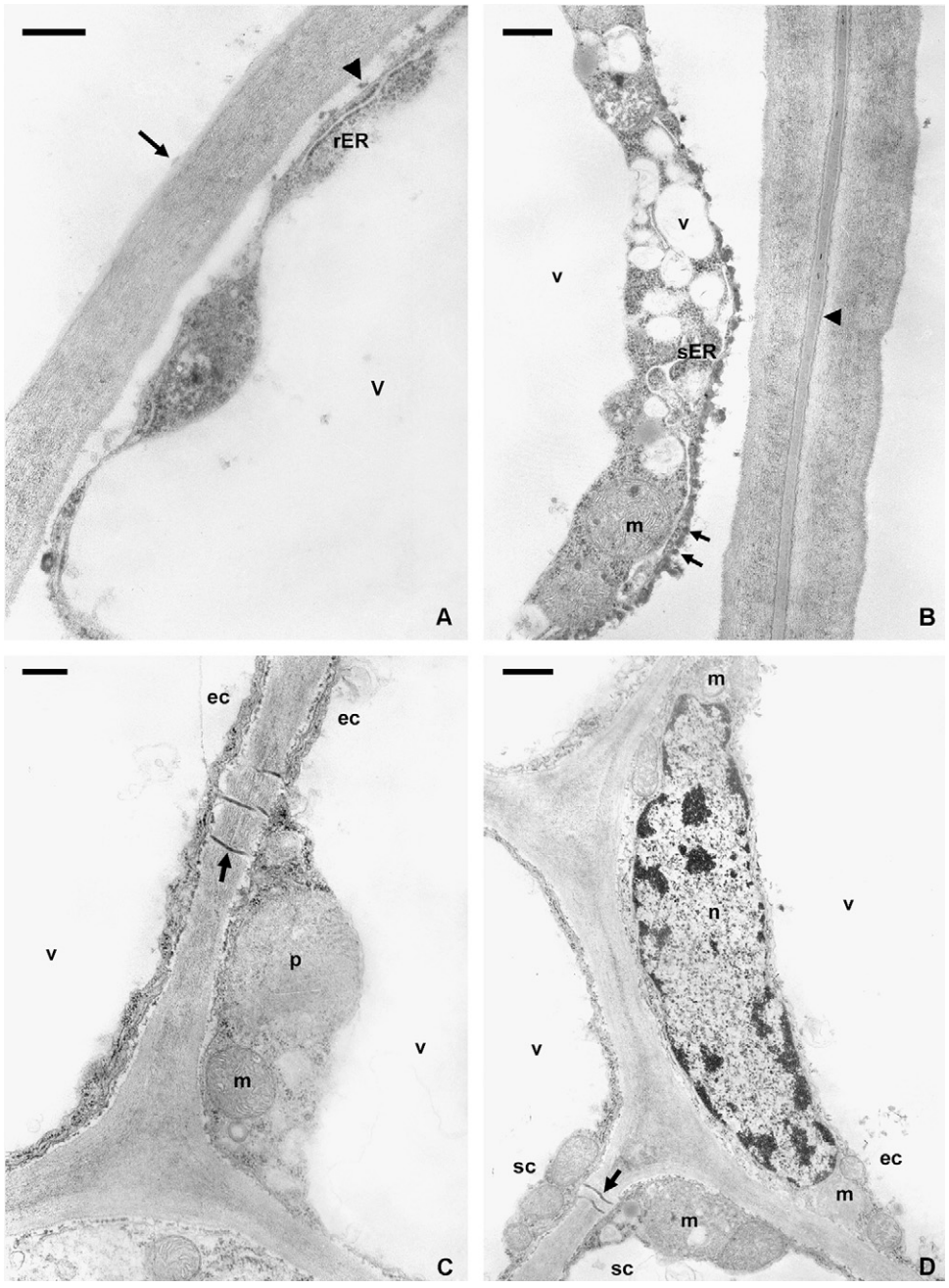

Fig. 4. TEM photographs of the radii of *P. caerulea* in post-anthesis. (A–C) Epidermal cells. (A, B) Traces of electron-dense material still visible on the cuticle (A, arrow), remnants of electron-dense granules between the plasmalemma and the wall (A, arrowhead; B, arrows) and swelled middle lamella (B, arrowhead). (C) Plasmodesmata persist between epidermal cells (arrow). (D) Plasmodesmata persist between epidermal and subepidermal cells (arrow). ec = epidermal cell, m = mitochondrion, n = nucleus, p = plastid, rER = rough endoplasmic reticulum, sc = subepidermal cell, sER = smooth endoplasmic reticulum, v = vacuole. Bars: A–C = 500 nm; D = 1 μ.

supposed to be involved in intracellular transport of lipophilic substances (Skubatz et al., 1995).

The weak reaction with picric acid reflects that only a little quantity of protein is involved in the processes that take place in these tissues. Larger amounts are expected in osmophores that emit nitrogenous compounds (Vogel, 1990).

The dense cytoplasm, numerous mitochondria and vacuoles in the epidermal and subepidermal cells of both species are typical for other glandular cells (Fahn, 1988). In particular, osmophoric cells are besides characterised by a generally large (presumed polyploid) nucleus, plasmodesmata between the participating cells and a great quantity of starch or fatty oils, specially in the

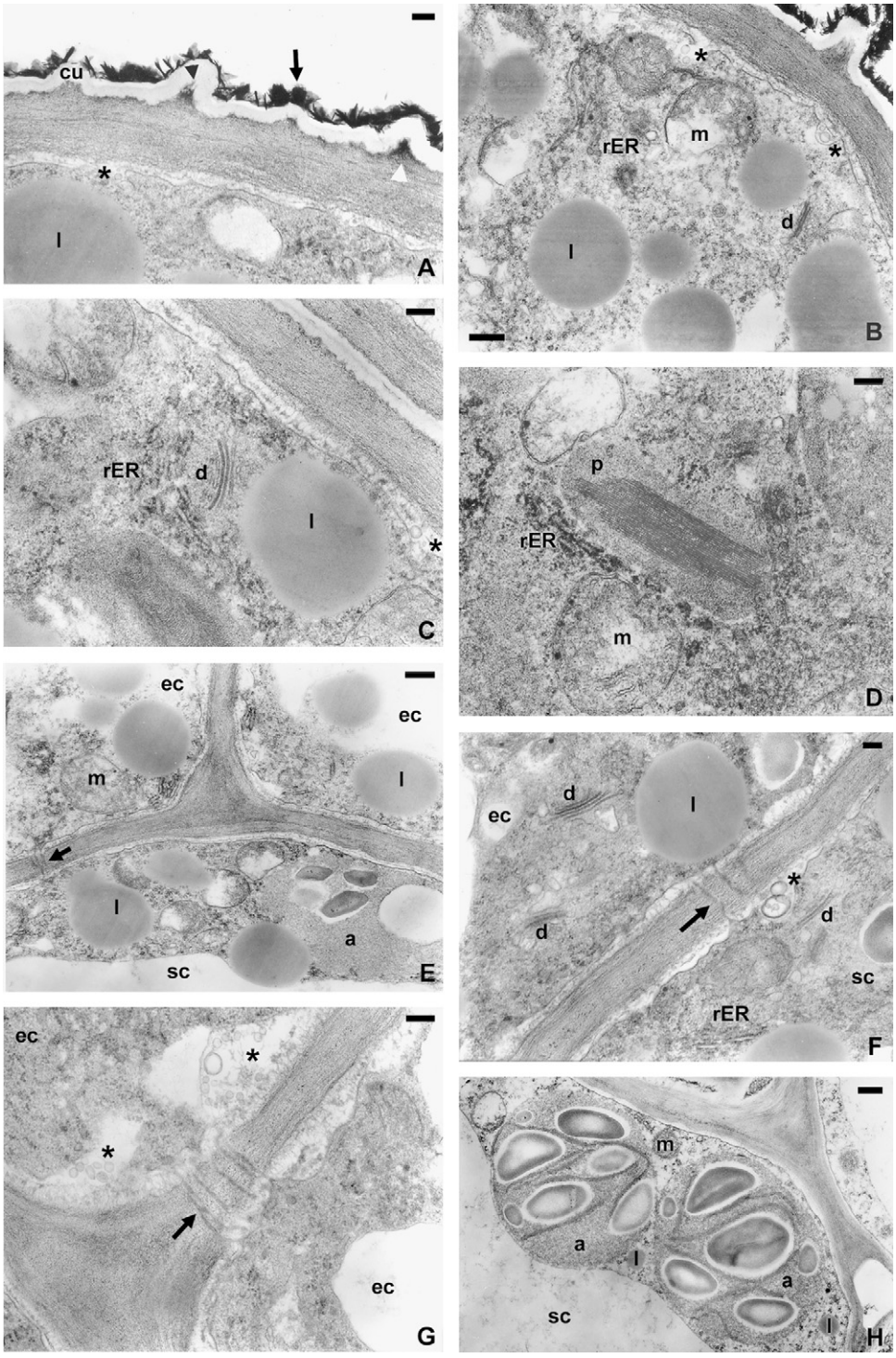

Fig. 5. TEM photographs of the radii of *P. suberosa* in anthesis. (A–D) Epidermal cells: (A) the wall microfibrills seem to penetrate the cuticle as microchannels (A, black arrowhead), a high electron-dense substance appears in the wall (white arrowhead), plate-like deposits of high electron-density cover the cuticle (arrow); (B–D) cytoplasm details. (E, F) Epidermal and subepidermal cells connected by plasmodesmata (arrows). (G) Epidermal cells connected by plasmodesmata (arrow). (H) Cytoplasm details of subepidermal cells. a = amyloplast, cu = cuticle, d = dictyosome, ec = epidermal cell, l = lipidic globule, m = mitochondrion, p = plastid, rER = rough endoplasmic reticulum, sc = subepidermal cell. Bars: A, C, D, F, G = 200 nm; B, E, H = 500 nm.

previous stages to odour emission (Vogel, 1966). All these characters were present in *P. caerulea* and *P. suberosa*. The constriction in some mitochondria in *P. caerulea* suggests division, which increases the quantity of these organelles required for the high metabolism of secretory processes (Vogel, 1990).

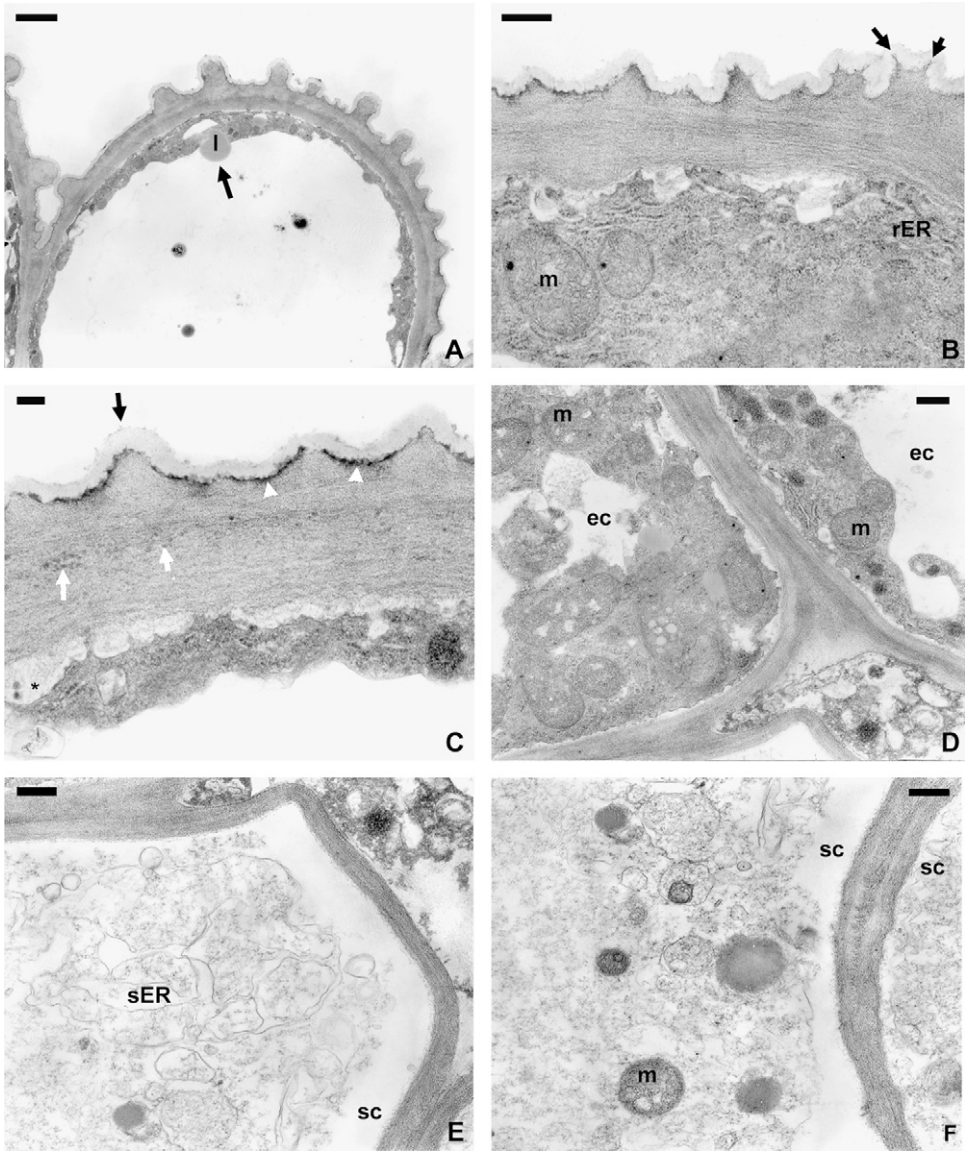

Fig. 6. TEM photographs of the radii of *P. suberosa* in post-anthesis. (A–D) Epidermal cells. (A) Lipidic globule protruding inside the vacuole (arrow). (B) The microchannels become more evident due to the electron-dense substance inside them (arrows). (C) The high electron-dense deposits are reduced to dots on the cuticle (black arrow), the electron-dense substance between the cuticle and the wall is more notorious (white arrowheads), granules of the same electron density appear in the wall (white arrows), the fibrillar substance is still observed between the plasmalemma and the wall (askerisk). (D) Cytoplasm details. (E, F) Subepidermal cells, cytoplasm. ec = epidermal cell, l = lipidic globule, m = mitochondrion, rER = rough endoplasmic reticulum, sc = subepidermal cell, sER = smooth endoplasmic reticulum. Bars: A = 2 μm; B, D–F = 500 nm; C = 200 nm.

There were only slight differences between the epidermal and subepidermal cells within each species. Pridgeon and Stern (1985) and Stern et al. (1987) state that the cytology of the few subtending layers is essentially the same to the one of the emitting epidermis. Large air spaces in the parenchyma and a well-developed phloem, both present in the studied species, are typical for osmophores (Vogel, 1990). The vascular supply of the radii varies between *P. caerulea* and *P. suberosa*, in coincidence with the considerable variety of this tissue in the corona from the species analysed by Puri (1948). The stratification of secretory tissues is common (Durkee, 1983). The osmophoric tissue frequently stratifies in layers specialised in production, storage and emission (Vogel, 1966). This arrangement has not been exactly recognised in the material studied.

One difference between both species is the thinner cuticle in *P. caerulea*. Stpiczynska (1993) describes a thin cuticle in *Cymbidium tracyanum*. Williams (1983) comments on the variability in cuticle thickness among osmophores of orchids from different genera. On the

basis of the present results, the cuticle thickness could be associated with the type of secretion emitted.

Cytology

Anthesis

An important difference between *P. caerulea* and *P. suberosa* at the subcellular level was the absence of sER in the second one and the paucity of dictyosomes in the first one. This last character and the amount of sER in the epidermal cells of *P. caerulea* was also found in the osmophores of orchids (Curry, 1987; Pridgeon and Stern, 1983; Stern et al., 1987; Stpiczynska, 1993), in the presumed osmophoric hairs of *Cypripedium* spp. (Swanson et al., 1980) and in the glandular trichomes of *Teucrium scorodonia* (Sevinate-Pinto and Antunes, 1991). The disappearance of dictyosomes takes place after different secretory processes in some species (Durkee, 1983; Vermeer and Peterson, 1978).

Another difference is the absence of amyloplasts in the epidermal cells of *P. suberosa* vs. the abundance in *P. caerulea*. In contrast, lipidic globules were large and numerous in *P. suberosa*, but small, few and restricted to the epidermal cells in *P. caerulea*. Starchless plastids were also observed in the osmophores of *Gymnadenia conopsea* (Stpiczynska, 2001) and in the supposed osmophoric hairs of *Cypripedium* (Swanson et al., 1980). Starch is the frequent energy reserve in osmophores, but lipids also occur (Vogel, 1990). Lipid substances, abundant in osmophores (Curry, 1987; Pridgeon and Stern, 1983; Stpiczynska, 1993, 2001), have been presumed to be the physical counterpart of the secreted fragrance (Curry, 1987; Pridgeon and Stern, 1983; Stern et al., 1987). This conjecture is based on the presumption that terpenes accumulate in that form (Kisser, 1958; Schnepf, 1969; cited in Pridgeon and Stern, 1983).

Intraplastidial tubules were found in *P. suberosa*. Tubular elements inside plastids were also described by Sevinate-Pinto and Antunes (1991), who related them to secretory processes. Plastids are involved in the production of enzymes of the mevalonic acid pathway (Curry, 1987), essential oil and monoterpene precursors (references in Sevinate-Pinto and Antunes, 1991), not only in osmophores but in glandular hairs too. The internal structure of plastids is correlated with the type of substances produced; tubular networks and thylakoids have been associated with the absence or very little amount of monoterpenes in the volatile extracts (Fahn, 1988). Carotenoids are deposited inside plastids as tubules, fibrils, globules or crystals (Whatley and Whatley, 1987); these compounds, closely related to terpenes, have been detected very frequently in osmophores (Vogel, 1990), which are assumed to be by-products of fragrance synthesis. The volatiles isolated from *Passiflora* spp. include fatty acid derivatives, benzenoids, isoprenoids, N-containing compounds (Lindberg et al., 2000) and phenyl propanoids (Varassin et al., 2001).

The vesicles in contact with the plasmalemma and between the plasmalemma and the wall suggest granulocrine secretion. Vesicles in such location were observed in osmophores by Pridgeon and Stern (1983), Stern et al. (1987) and Stpiczynska (1993, 2001), but eccrine secretion also occurs in fragrance tissues of other orchids (Vogel, 1990). Both epidermal and subepidermal cells seem to produce and store. The vesicles close to plasmodesmata that connect subepidermal and epidermal cells clearly show that there is a passage of substances from the subjacent layer to the emitting epidermis.

Accumulation of granular electron-dense material between the plasmalemma and the wall is common to both species. Osmiophilic deposits are visible in the osmophoric tissues analysed by Curry (1987), Stern et al. (1987) and Stpiczynska (1993), specially during anthesis.

The following facts during anthesis in *P. caerulea* are probably related with a secretion: the increased thickness of the outer tangential wall of the papillae tip, the high electron-dense fibrillar zone under the cuticle and the stronger reaction with Sudan IV than in *P. suberosa* resembling exudate accumulation (photographed in Amela García, 1999). Stpiczynska (1993) reports about a swollen surface cuticle near agglomerations of secretion and Pridgeon and Stern (1983) and Stern et al. (1987) show aggregations of osmiophilic material in the outer region of the apoplast, features comparable to the swollen wall and high electron-dense zone beneath the cuticle of *P. caerulea* during anthesis. Similar processes of the epidermal cells' outer wall extending into the cuticle in *P. suberosa* were found by Stern et al. (1987); Lyshede (1978) observed microchannels in the epidermal outer walls through which wax precursors were interpreted to pass.

No exact site of export through the cuticle of neither *P. caerulea* nor *P. suberosa* could be detected. Pridgeon and Stern (1983) and Stpiczynska (2001) documented cuticular pores. When there are no pores or ectodesmata, other pathways exist: low-molecular-weight terpenes can pass through cellulose and cutin (Vogel, 1990), certain wax-covered cuticles allow the passage of volatiles (Goodwin et al., 2003), lipids could pass through the wall as moieties and reassemble on the cuticle (Davies et al., 2003), wax precursors could be carried across the wall within the hydrophobic cavity of lipid transfer proteins or by hydrophobic domains of constitutive proteins (Kunst and Samuels, 2003). Some of these pathways may be the case in *P. caerulea* and *P. suberosa*, depending on the type of the released substances.

The appearance and quantity (perhaps the volatilisation speed) of the secretion is different comparing *P. caerulea* and *P. suberosa*. The first one exhibited sparse drops on the cuticle; the second, plate-like deposits. Extracuticular matter over osmophores' surfaces was, to date, photographed only by Pridgeon and Stern (1983), as massive accumulation of droplets, and by Stern et al. (1987), as amorphous material. Rapid volatilisation of secretory products may prevent to capture them, depending on the weather conditions when the material is fixed (Williams, 1983). The cytological evidence gathered, typical of osmophores, supports the presumption that the radii of *P. caerulea* emit fragrant compounds. It is also evident that *P. suberosa* secretes some substance; if this substance (or mixture of substances) is responsible for the odour, this is not detectable by our olfactory sense, in accordance with Knudsen et al. (2004), who captured and identified volatile compounds in flowers odourless to humans.

Post-anthesis

Vogel (1990) comments the temporary merging inside the vacuole of large oil droplets, as it was noticed with the lipidic globules in *P. caerulea* and *P. suberosa*. The storage of osmiophilic material along the tonoplast (Pridgeon and Stern, 1983) and also inside the whole vacuole (Stpiczynska, 1993) was registered during successive stages of anthesis. The amyloplasts in *P. caerulea* and the lipid globules in *P. suberosa* have almost disappeared in the post-anthetic stage. Rapid consumption of reserves is reported for fragrance emitting tissues (Vogel, 1990). The high electron-dense film under the cuticle and the granules situated at different depths in the wall during post-anthesis in *P. suberosa* might suggest a process of reabsorption, as occurs with nectar in some species, for which similar images were obtained (Radice and Galati, 2003). The increased vacuolisation after anthesis in *P. caerulea* and *P. suberosa* is also exemplified for osmogenous tissues by Pridgeon and Stern (1983) and Stern et al. (1987). The persistence of mitochondria at this stage is in accordance with the last organelles that remain intact in secretory hairs (Vermeer and Peterson, 1978).

Morphology of secretory structures, odour emission and ecological considerations

Williams (1983) comments that the structure of the osmophore region, at least in male-euglossine-pollinated orchids, is quite variable from species to species. Although generally the fragrant epidermis is located at the adaxial surface of the perianth, some Orchidaceae bear this tissue at the underside or hold fragrance hairs (Vogel, 1990). *Passiflora*'s secretory tissue encircles the whole perimeter of each radius, a case somewhat analogous to the staminal appendages of *Nelumbo nucifera* (Vogel and Hadacek, 2004). The longer papillae of *P. caerulea* and the lobulated outline of the radii in cross section, compared to the shorter papillae and even outline in *P. suberosa*, could contribute to a greater volatilisation of odour compounds due to a major surface.

The corona pieces complexity varies between the species of *Passiflora*, obviously in relation to the floral visitors received and the functions performed in each plant species (barrier against nectar robbers, landing surface and visual and/or olfactory attraction to pollinators). Lindberg et al. (2000) found a positive correlation between the amount of benzenoids and corona size. Bird-pollinated flowers usually have a short row of vertical fringes. Bee-pollinated *P. caerulea* and *P. moorena* show an operculum with a kind of a pleated ruffle at the base of its fringes. This, and the highly divided nimbus formed by numerous radii may contribute to diffuse the scents that these species emit. Bees alight on the radii. Moreover, the confluence of the radii to the centre of the flower (access to the nectar) would constitute not only a visual (Amela García, 1999) but also a scent guide. Scent guides usually coincide with nectar guides (Vogel, 1990). Scent guides are more intensely fragrant than the rest of the odoriferous pieces of a certain flower (Vogel, 1983), a fact that occurs with the radii of *P. caerulea*. In this species, all the nimbus would consist of scent guides rather than an osmophore, as it is not a tissue amidst other tissue; otherwise, it could be considered that each radius holds an osmogenic tissue in its whole periphery. Scent guides predominate in melitophiles (Vogel, 1990), a syndrome that corresponds to that of *P. caerulea*. In contrast, the operculum of *P. suberosa* lacks a ruffle and the nimbus is formed by fewer radii. This species is pollinated mainly by wasps and also by bees (Koschnitzke and Sazima, 1997). No mention was made about odour when this pollination syndrome was defined (Percival, 1965; Faegri et al., 1979). But considering that some wasp-pollinated *Passiflora* species produce an odour like musk or scatole (MacDougal, 1994) and the well-known wasp pseudocopulation in the *Ophrys* species that produce pheromone-like scent (Richards, 1986), there is no doubt that wasp-pollinated blossoms emit aroma. Wasp-pollinated flowers were not defined in such detail as the rest of the floral syndromes, perhaps because they include more varied characters, corresponding to the highly varied taxa included in "wasps" (Faegri and Pijl, 1979), so some information may be lacking with regard to those flowers' aroma. The known pollinators of scentless passion vines are wasps and birds. Nevertheless, flowers scentless to humans may emit substances detectable by their pollinators.

The floral volatile compounds may have diverse functions in the different species of *Passiflora* in relation to pollinators. Scent not only is an attractant but may also be a reward, as in entomophilous perfume flowers. Bird-pollinated blossoms were believed to be scentless for a long time, in coincidence with the less developed olfactory sense of those vertebrates. Nevertheless, Knudsen et al. (2004) reported some presumed bird-pollinated taxa different from *Passiflora* that produce smell detectable by humans and emit volatiles. In spite of this, Varassin et al. (2001) isolated only one compound from the faint scent of the ornitophilous *P. speciosa*, in contrast to the mixture of numerous constituents captured from single species with other pollinating syndromes, either from the same genus (Varassin et al., 2001) or from other genera (Lindberg et al., 2000; Skubatz et al., 1996; Vogel and Hadacek, 2004).

The epicuticular deposits of *P. suberosa* look like highly compacted and fragmented material. Vogel (1990) observed crystal platelets which later sublimate in several fragrance producing genera. Among highly varied forms, waxes may exhibit this appearance with scanning electron microscopy (Barthlott et al., 1998), and a similar image to ours was obtained by Lyshede (1978) with TEM. A temporary solution of volatiles in coatings of wax is common in certain osmophores (Vogel, 1990). Even if the secretion has no volatile, wax is collected by certain bees for nest construction (Vogel, 1983) and could constitute a reward. Equivalent assumptions were commented about the waxy products of Orchidaceae and Ericaceae flowers by Simpson and Neff (1981). No visitors have been observed scrapping the radii of *P. suberosa* (Amela García, 1999; Koschnitzke and Sazima, 1997), but *Plebeia* bees, of which one species was recorded as pollinator by Koschnitzke and Sazima (1997), use waxes for their nests (Roig Alsina, pers. comm.). Koschnitzke and Sazima (1997) reported that *Eulaema nigrita* male bees collect odour substances from the shorter pieces of the corona of *P. amethystina*, although they did not clarify if the floral scent is perceived by humans. Beetles can feed on wax (Griffiths et al., 2000); chrysomelids eat the radii of *P. caerulea* and *P. mooreana* (Amela García and Hoc, 1997, 1998), but they were only seen eating pollen on *P. suberosa* so far (Amela García, 1999). Another possibility, not necessarily excluding the fragrance fact, might be that the secretion of *P. suberosa* gives a certain texture or visual appearance to the radii during anthesis. Papillate surfaces exhibit a velvety gloss, while smoothness, which effects a reflecting lustre, may be produced, although rarely, by an oily film (Endress, 1996). In spite of their papillae, the radii of *P. suberosa* appear moderately brilliant and uniformly smooth when observed with a dissecting microscope.

Future work employing other techniques involving the overall corona and floral parts will be carried out to bring more light to these facts.

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