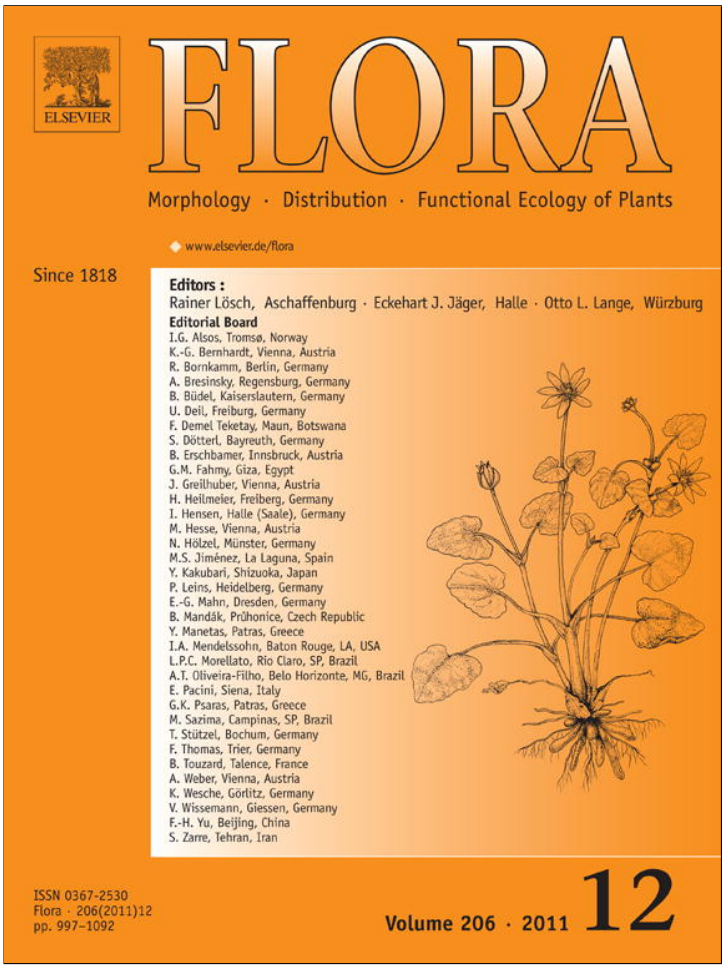

Flora 206 (2011) 1063–1068

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Anatomical studies of the secretory structures: Glandular trichomes and ducts, in *Grindelia pulchella* Dunal (Astereae, Asteraceae)

Adriana Bartoli[a,*], Beatriz G. Galati[a], Roberto D. Tortosa[a,b]

[a] *Cátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C.P. 1037, Ciudad Autónoma de Buenos Aires, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina*

ARTICLE INFO

Article history:
Received 15 February 2011
Accepted 26 May 2011

Keywords:
Anatomy
Glandular trichomes
Grindelia pulchella
Secretory ducts
Transfer cells
Ultrastructure

ABSTRACT

The ultrastructure of the glandular trichomes and secretory ducts of *Grindelia pulchella* was studied. Plastids, mitochondria and endoplasmic reticulum are involved in the secretory process of both, trichomes and ducts. A special tissue with "transfer cells" is associated with the duct epithelial cells. The secretion is produced in the transfer cells and then is transferred to the duct epithelial cells where it accumulates in the vacuoles. The occurrence of cavities within the cell walls of the trichome cells and duct epithelial cells is described. The secretion is accumulated between the cell wall and the cuticle of these cells. When the cuticle is broken the secretion is released. We conclude that granulocrine secretion operates in this species.

Introduction

In Asteraceae two secreting systems occur, glandular trichomes at the surfaces of organs and secretory ducts inside the organs. Glandular trichomes are usually formed of a biseriate peduncle and a head of one to many cells. In the most common type the peduncle is formed by five pairs of cells (Ciccarelli et al., 2007). Secretory ducts are found in leaf mesophyll and in stem cortex (Hoffmann et al., 1984; Ponce, 1986), and are often part of the bundle sheath. Most often one duct exists for a bundle, rarely two. Nothing is known about the mechanisms of secretion into these ducts.

The secretion of ducts and hairs is composed of essential oils, lipids, resins, sesquiterpene lactones, alkaloids, pectin-like substances, tannins and flavonoids (Andreucci et al., 2008; Pagni et al., 2003). In the genus *Grindelia*, the substances secreted are resins, composed of a mixture of labdane diterpene resin acids (Timmermann and Hoffmann, 1985).

Secretory structures have been investigated in several genera of Asteraceae (Ascensão and Pais, 1988; Cornara et al., 2001; Corsi and Nencioni, 1995; Del Fueyo, 1986; Guillet et al., 1997; Pagni and Masini, 1999; Pagni et al., 2003; Poli et al., 1995; Rossi Monteiro et al., 1999). However, there are few ultra-structurally studies; only the secretory cavities of *Porophyllum lanceolatum* (Rossi Monteiro et al., 1999) and *Artemisia campestris* (Ascensão and Pais, 1988) were studied.

In some cases authors attributed to these secretions defense functions against pathogens, insects or herbivores (Mayekiso, 2009; Mayekiso et al., 2006). In many genera of Asteraceae the potential use in medicine or industry of the secreted substances have been pointed out: *Artemisia* L. (Ascensão and Pais, 1988; Corsi and Nencioni, 1995; Duke and Paul, 1993; Hayat et al., 2009), *Eupatorium* L. (Ragonese, 1988), *Flourensia* DC. (Delbón et al., 2007), *Grindelia* Willd. (Timmermann and Hoffmann, 1985), *Helichrysum* Mill. (Perrini et al., 2009), *Matricaria* L. (Andreucci et al., 2008), *Montanoa* Cerv. (Robles-Zepeda et al., 2009), *Pluchea* Cass. (Cambi et al., 2006), *Porophyllum* DC. (Guillet et al., 1997; Rossi Monteiro et al., 1999), *Santolina* Tourn. (Pagni and Masini, 1999; Pagni et al., 2003), *Stevia* Cav. (Cornara et al., 2001), *Tagetes* L. (Poli et al., 1995), and *Viguiera* Kunth (Da Costa et al., 2001).

The aim of the present work is to characterize anatomical and ultrastructurally glandular trichomes and ducts of a South American species of *Grindelia*, *G. pulchella* Dunal, and also to explain the mechanism of secretion.

Materials and methods

The materials used in this study were stems, roots, leaves, and heads, obtained from plants collected at San Antonio de Areco, Province of Buenos Aires, Argentina. A reference specimen was deposited in the Herbarium Gaspar Xuarez (BAA).

Materials were fixed in FAA and embedded in paraffin. Sections (7–10 μm thick) were cut and stained with safranin combined with fast green (D'Ambrogio, 1986) and observed with a Wild M20

* Corresponding author.
E-mail address: cbartoli@agro.uba.ar (A. Bartoli).

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doi:10.1016/j.flora.2011.07.012

microscope. The photomicrographs were taken with a digital camera Canon PowerShot A650 IS.

For transmission electron microscopy (TEM) studies, materials were pre-fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) overnight and then post-fixed in OsO_4 at 2 °C in the same buffer for 3 h. Following dehydration in ethanol series, the material was embedded in Spurr's resin. Thin sections (1 μm) were made with a Reichert-Jung ultramicrotome and stained with toluidine blue for observation with light microscope. Ultrathin sections (750–900 nm) were made on the same ultramicrotome and then stained with uranyl acetate and lead citrate (O'Brien and McCully, 1981). These sections were observed and photographed with a Philips EM 301 at 60.0 kV.

Results

In *Grindelia pulchella* there are two secreting systems: glandular trichomes at the leaf, involucral bracts, and stems surfaces and secreting ducts inside the organs (Table 1).

Glandular trichomes

Glandular trichomes (Figs. 1–3) are constituted by four basal epidermal cells and a multicellular head (Fig. 6). In the leaves, the glands are associated with colorless parenchyma localized subepidermically (Table 1 and Fig. 2).

The secretory cells of the trichome head have conspicuous nuclei, a dense cytoplasm and some vacuoles (Fig. 6). The cytoplasm is characterized by the presence of abundant mitochondria, endoplasmic reticulum, dictyosomes with numerous associated vesicles – some of them in contact with the plasmalemma –, and many plastids (Figs. 7 and 8). These plastids have an elaborate system of inner membranes and numerous osmiophilic globules (Figs. 7 and 8).

The outer tangential wall of the peripheral cells of the trichome head is thicker than the other cell walls (Figs. 7, 9–11). In young glands this wall is covered by a conspicuous cuticle (Fig. 7). During active secretion, the outer tangential wall of the peripheral cells presents cavities (Fig. 9), filled with substances of moderate to high electron-density. The same substances were observed between the outer tangential wall and the detached cuticle (Figs. 10 and 11).

In post-secretion stage glandular trichomes show the cells with the cytoplasm very vacuolated and poor in organelles (Fig. 12, compare with Fig. 11), the cuticle absent and the outer tangential wall of the peripheral cells with an alveolar aspect (Fig. 12).

Ducts

Ducts are present in stems, leaves and involucral bracts of *Grindelia pulchella*, localized near the vascular bundles and associated with the phloem (Fig. 4). They are lacking in roots and floral organs (Table 1).

The duct lumen is lined with epithelial cells (Fig. 5). These cells have thin walls, conspicuous nuclei, big vacuoles (Fig. 16), abundant mitochondria and endoplasmic reticulum. The vacuoles have diverse contents, some of them with an electron-density similar to the lipid globules, and others more electron-dense (Fig. 16). The outer tangential wall of the epithelial cells is thicker than the other walls and, in young ducts, is covered by a conspicuous cuticle. When epithelial cells are in active secretion, the outer tangential wall has the cuticle detached (Fig. 16) and presents cavities that are filled with substances of moderate to high electron-density.

A special tissue is always associated with ducts. This tissue presents abundant intercellular pectin substances, especially in the intercellular corners (Fig. 13). Its cells have numerous and conspicuous wall ingrowths and plasmodesmata are abundant (Figs. 14 and 15). The cytoplasm presents numerous mitochondria with well developed cristae, endoplasmic reticulum and plastids with an extensive system of inner membranes (Fig. 15). These membranes contain many osmiophilic globules in the stroma, within the thylakoids and in the intermembranal space of the plastidal envelope (Fig. 15).

Discussion

Glandular trichomes are present in the epidermis of stems, leaves and involucral bracts of *Grindelia pulchella*. These type of trichomes, both in vegetative and in reproductive organs, were anatomically described in other Asteraceae, *Tagetes minuta* (Del Fueyo, 1986), *Artemisia campestris* (Ascensão and Pais, 1988), *Tagetes patula* (Poli et al., 1995), *Artemisia nitida* (Corsi and Nencioni, 1995), *Porophyllum* spp. (Guillet et al., 1997; Rossi Monteiro et al., 1999), *Santolina leucantha* (Pagni and Masini, 1999), *Stevia rebaudiana* (Cornara et al., 2001), *Santolina ligustica* (Pagni et al., 2003), *Achillea maritima*, *Bellis perennis*, *Dittrichia viscosa*, *Eupatorium cannabinum*, *Matricaria chamomilla*, *Pulicaria dysenterica*, and *Tanacetum parthenium* (Ciccarelli et al., 2007). These trichomes are similar in the function but present anatomical differences and are formed by a variable number of cells in the different species.

In the glandular trichomes of *G. pulchella* the outer tangential wall of the peripheral cells contains conspicuous cavities which are evident during and after the secretion process. The occurrence of cavities in the cell wall of plants is unusual, but they were observed by Stpiczynska et al. (2007) in the elaiophores of some members of Oncidiinae. According to these authors, it is possible that these cavities facilitate the transport of hydrophobic components across the largely hydrophilic cell wall, much in the same way that pores within the cell wall of certain plant species facilitate the passage of hydrophobic compounds during the formation of the cuticle.

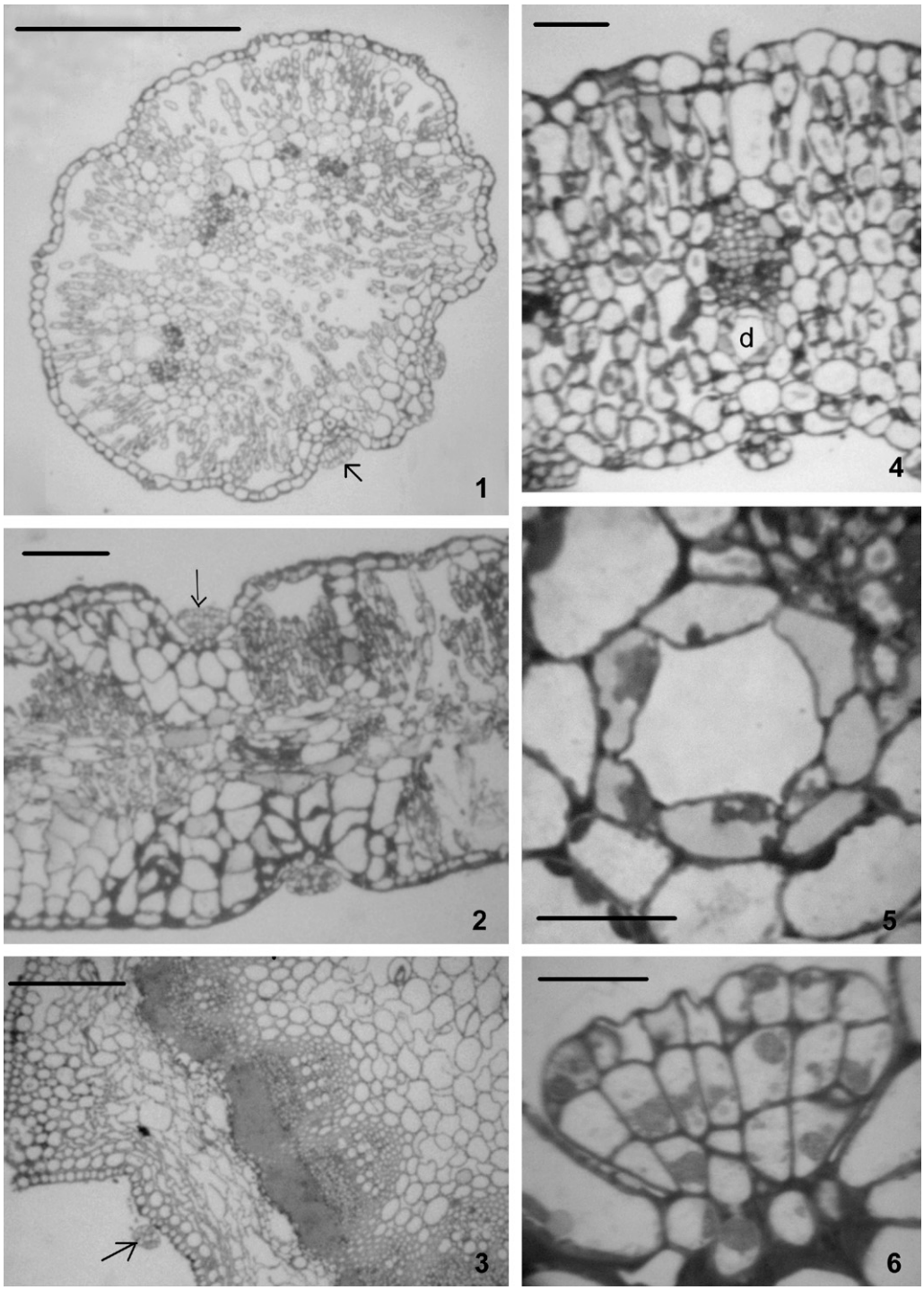
The secretion is accumulated between the cell wall and the cuticle, and then, when the cuticle is broken, the secretion is released. Rupture of the cuticular sheath along a line of weakness in the middle of the gland head was observed in *Stevia rebaudiana* (Cornara et al., 2001). This type of secretion release was described also in elaiophores of species with cell wall cavities (Stpiczynska et al., 2007). TEM revels that the trichome cells of *G. pulchella* have numerous plastids with lipidic globules or oil bodies, similar to those found in species with lipid-secreting glands (Davis et al., 2003; Stpiczynska et al., 2007).

In secretory ducts, the presence of a special tissue associated with the ducts was not described previously. In *G. pulchella* the cells of this tissue present wall ingrowths characteristic of transfer cells (Gunning and Pate, 1969), and many cytoplasm connections were observed between these cells. According to Herrero and Dickinson (1979), cell wall ingrowths associated with the plasmodesmata assure a great efficiency in the cellular exchange. The cells of this tissue have an active cytoplasm with abundant mitochondria with well developed cristae, and many plastids with osmiophilic globules inside. In the glands of *Porphyllum* (Rossi Monteiro et al., 1999) and in the secretory ducts of *Artemisia campestris* ssp. *maritima* (Ascensão and Pais, 1988) plastids show, during the secretion period, dark material in the thylacoids, between the envelope membranes, and in stroma. According to Rossi Monteiro et al. (1999) the secretion material may be synthesized in the interior of the plastids, migrate outwards to the space between the envelope membranes and subsequently is transferred to the periplastidal endoplasmic reticulum.

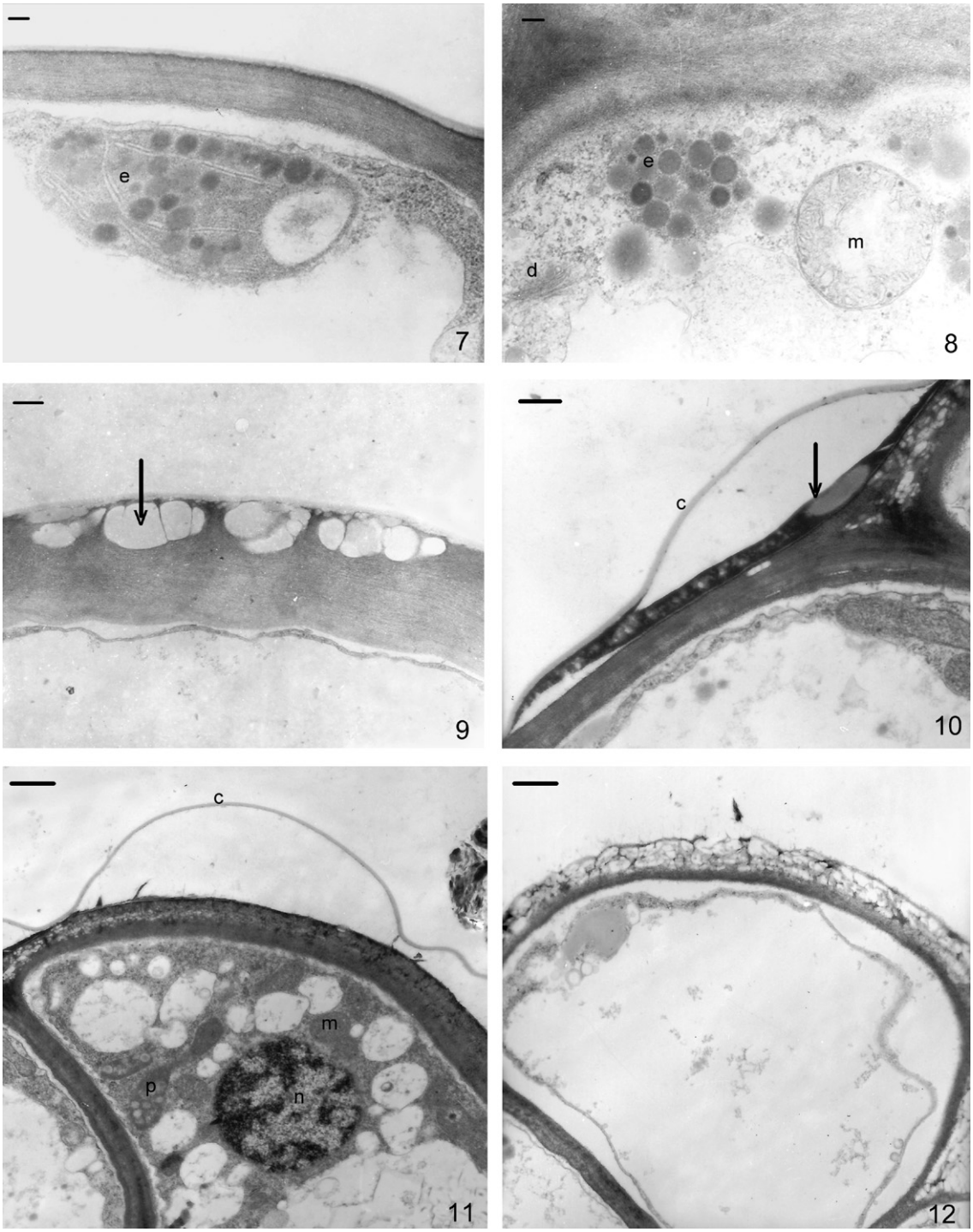
The material observed in the vacuoles of the epithelial cells of the ducts in *G. pulchella* can be related to the secretion material. Therefore, we can presume that the secretion is produced in the transfer cells and then transferred to the epithelial cells of the duct,

Table 1
Localization of secretory structures in *Grindelia pulchella* Dunal (+ presence, – absence).

Secretory structures	Roots	Stems	Leaves	Receptacle	Involucral bracts	Flower organs
Glandular hairs	–	+	+	–	+	–
Secretory ducts	–	+	+	+	+	–



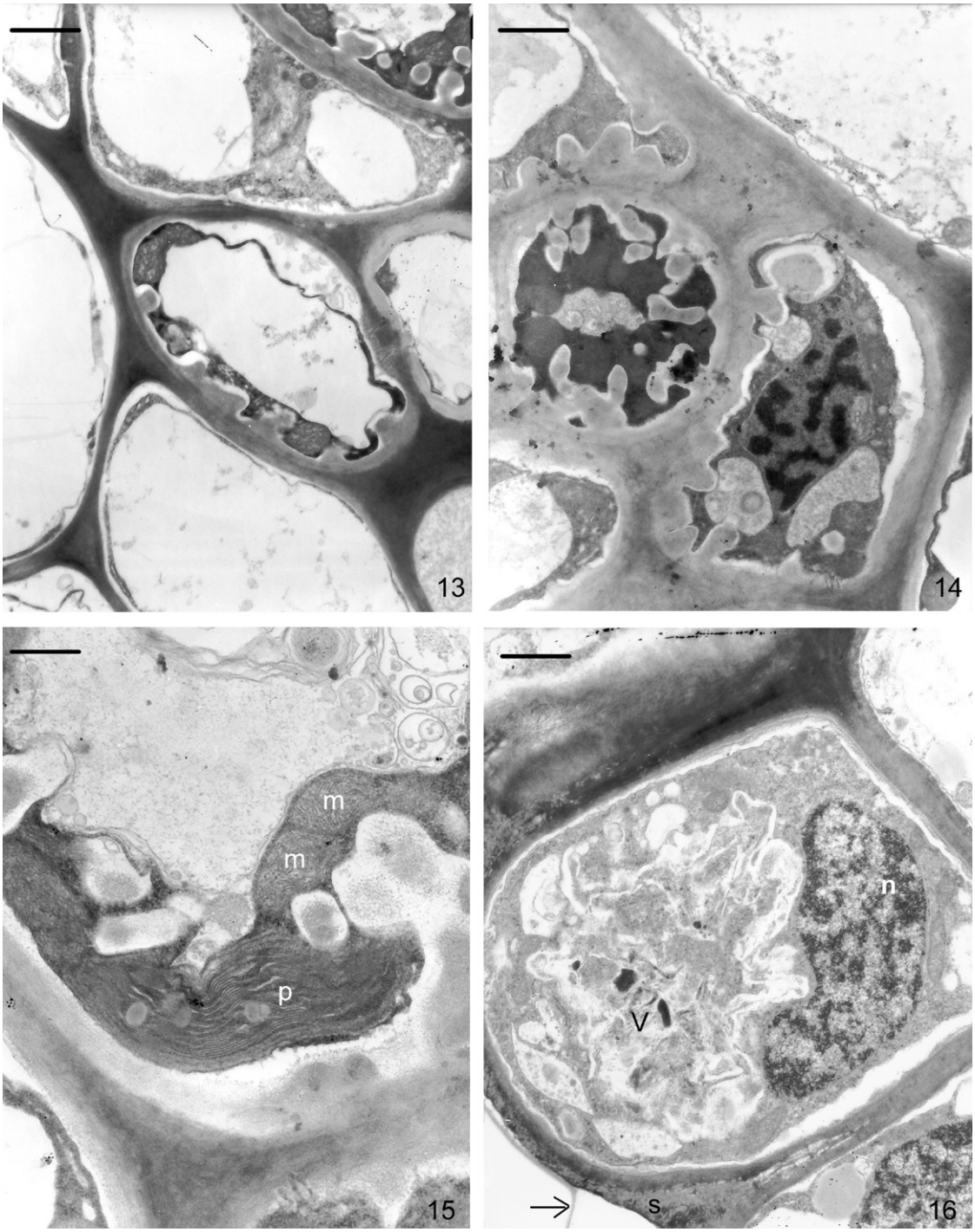
Figs. 1–6. *Grindelia pulchella*. **1**. Transversal section (TS) of involucral bract. Scale bar = 500 μm. **2**. TS of leaf. Scale bar = 100 μm. **3**. Detail of a TS of stem. The arrows show the trichomes. Scale bar = 300 μm. **4**. TS of leaf showing a vascular bundle and a duct (d). Scale bar = 40 μm. **5**. Detail of a duct in leaf. Scale bar = 20 μm. **6.** Detail of a trichome in leaf. Scale bar = 30 μm.



Figs. 7–12. *Grindelia pulchella*. TEM of the trichomes of leaf. **7–8**. Details of secretory cell showing elaioplasts (e), mitochondria (m) and dictyosomes (d). Scale bars = 0.2 μm. **9**. Detail of outer tangential cell wall, the arrow indicates the cavities. Scale bar = 0.5 μm. **10**. Detail of outer tangential cell wall, the arrow indicates the secretion between the cell wall and the cuticle (c). Scale bar = 1 μm. **11**. Cell in active secretion with nucleus (n), plastids (p) mitochondria (m) and the cuticle (c) detached. Scale bar = 1 μm. **12**. Cell in post-secretion stage showing the outer tangential wall with alveolar aspect. Scale bar = 1 μm.

where it accumulates in the vacuoles. The presence of cavities in the outer tangential wall of the epithelial cells and a detached cuticle at some points, indicate that the secretion is released to the duct cavity in the same way as in the trichomes. However, in the ducts, the secretion material is most probably synthesized in the transfer cells, while in the glands the secretion material seems to be synthesized in the same trichome cells.

From the present observations, we can say that plastids, mitochondria and endoplasmic reticulum are involved in the secretory process of both, trichomes and ducts of *G. pulchella*. We can conclude that the granulocrine mechanism is the prevalent one which enables the passage of secretion material between the cells of the trichome and from the "transfer cells" to the duct epithelial cells, as well as from the epithelial cells to the duct cavity.



Figs. 13–16. *Grindelia pulchella*. TEM of the duct cells of leaf. **13–15**. Associated tissue with "transfer cells". **13**. Abundant osmiophilic intercellular substances; a cell with wall ingrowths can be observed. Scale bar = 1 μm. **14**. Detail of two cells with wall ingrowths. Scale bar = 1 μm. **15**. Detail of a "transfer cell" with plastids (p) and mitochondria (m). Scale bar = 0.5 μm. **16**. Detail of a duct epithelial cell showing the osmiophilic material inside the vacuole (v) and conspicuous nucleus (n), the cuticle (arrow) detached and the secretion (s) in the wall cavities. Scale bar = 1 μm.

Acknowledgements

The authors gratefully acknowledge assistance of Francisco Ratto who provided us the plants for this study, and support provided by the Universidad de Buenos Aires (Grants AG806 y X823) and by the Consejo Nacional de Investigaciones Científicas y Técnicas.

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